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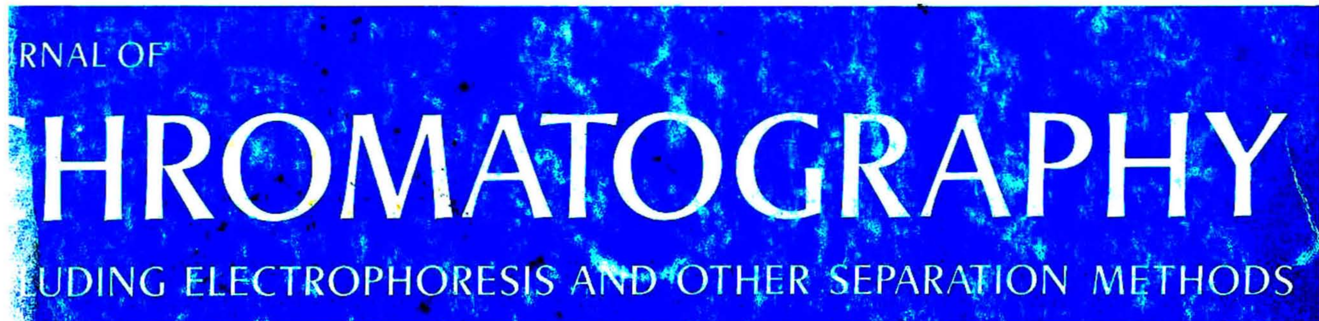

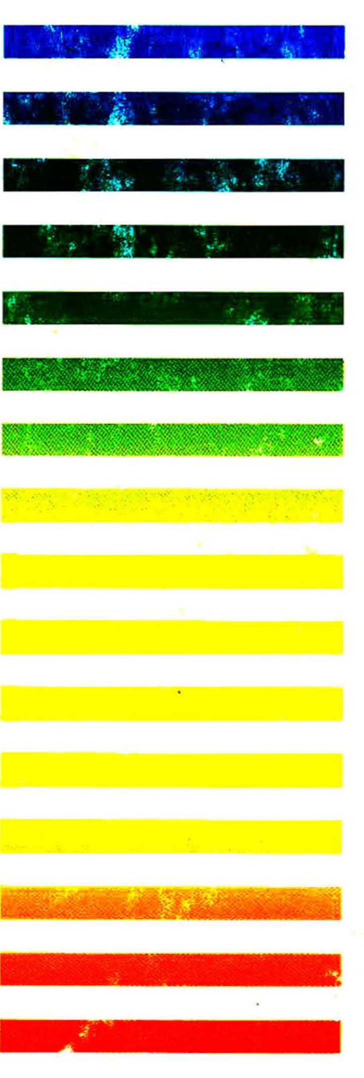

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VOL. 642 (1993)

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SPECIAL VOLUME

CHROMATOGRAPHY IN ENVIRONMENTAL ANALYSIS

PART I

The papers submitted for the Special Volume on *Chromatography in Environmental Analysis* are published in two consequtive volumes of the *Journal of Chromatography*: Vols. 642 and 643 (1993). The Preface only appears in Vol. 642. A combined Author Index to both Vol. 642 and 643 only appears in Vol. 643.

VOL. 642 NOS. 1+2 JOURNAL OF CHROMATOGRAPHY JULY 16, 1993

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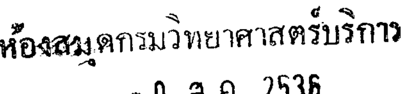

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Preface

Protection of the environment has become a pressing issue world-wide. Human activities are increasingly polluting air and water, and toxic residues on soil and food threaten our health. Gorvernments of all civilized countries have responded to this global threat by passing and enforcing regulations for environmental protection.

Dependable analytical data are the backbone of regulatory activities in the control of the environment. The specificity, sensitivity, and versatility of chromatography make it the premier method for assessing atmospheric, aquatic, and residual pollution. While the chromatographic techniques used in environmental analysis are essentially the same as those used in other applications, ingenious methods have been developed for collecting, concentrating, and preparing samples for chromatographic analysis.

It has been my privilege to invite scientists from all over the world to contribute articles to these special volumes of the *Journal of Chromatography* and to edit their work. Many of these authors are directly involved with the regulatory agencies in their country. This accounts for the differences among their concerns and approaches. If mutual comparisons and discussions are stimulated by bringing their experiences together in these volumes, its greatest aspiration will be fulfilled.

Orinda, CA (USA) Erich Heftmann

Journal of Chromatography, 642 (1993) 3–14
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Review

Partition coefficients (*n*-octanol–water) for pesticides

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ABSTRACT

Data for the partition coefficients (*n*-octanol–water) (P_{OW}) of 221 pesticides and pesticide metabolites are presented with their method of derivation and source. The methods of measurement and calculating log P_{OW} are reviewed. Octanol–water partition coefficients are measured by shake-flask methods, reversed-phase HPLC, reversed-phase TLC, slow-stirring partition methods and column generator method. Octanol–water partition coefficients are calculated from substituent constants, molecular fragment summation and solubilities. It has been proposed that the HPLC operating conditions for any compound should be applicable to other compounds which have the same log P_{OW}. The log P_{OW} may be used in estimating the environmental behaviour of pesticides. A classification of pesticides as fat-soluble has been proposed for compounds with a log P_{OW} > 4.

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1. INTRODUCTION

The partition coefficient (*P*) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents [1], in this case *n*-octanol and water. Octanol represents a substitute for biotic lipid and hence gives an approximation to a biotic lipid–water partition coefficient [2]. The ratio is reported as a logarithm usually as log P_{OW} or log K_{OW}.

Comprehensive data on partition coefficients have been published [3–5] however these cover a wide range of organic compounds and searching is required to find any pesticide of interest. The partition coefficients for some pesticides are given with other parameters in a published list of environmental data [6]. Because of the importance of the environmental effects of pesticides a detailed list of important parameters such as partition coefficients is necessary.

A number of techniques have been used to mea-

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sure or calculate log P_{OW}. Because of the range of methods used there is a wide discrepancy in the values reported [7]. To make decisions on the usefulness of these values it is necessary to have access to as many as possible and also to know their derivation.

The purpose of this review is to set out a comprehensive list of pesticides with their partition coefficients and the methods of derivation. Information on some metabolites is also included. Some uses of partition coefficients are also examined.

2. DIFFERENT METHODS OF MEASURING OR CALCULATING LOG P_{OW}

2.1. Experimental measurement

The classic measurement for log P_{OW} is the shake-flask method [1] where a compound is shaken in *n*-octanol–water and after equilibrium the concentration is measured in one or both phases. It can measure a range of log P_{OW} from −2.5 to 4.5, is applicable to most classes of compounds but is affected by impurities and difficult for compounds of low solubility [8]. Variations of the shake flask method include inversion [9–16], stirring [17], dissolved in octanol and water added before shaking [18] and shaking in a separating funnel [19].

Octanol–water partition coefficients have applications in many areas. Much research involves the development of new methods, to measure log P_{OW}, which are easier, more accurate, more reproducible and less time consuming.

Reversed-phase high-performance liquid chromatography (HPLC) has been used extensively [2,8,20–30]. It is an indirect method. To find an unknown log P_{OW}, a set of reference compounds of known log P_{OW} needs to be run under the same conditions. Calibration curves of log P_{OW} against retention time or capacity factors are used. The method preferably should be used for chemicals and reference compounds whose chemical structures do not differ significantly [31]. It can be used for a range of log P_{OW} from 0 to 6, is convenient, relatively fast, reproducible and less sensitive to impurities [8]. The disadvantages are that it is an indirect method, requires calibration and that occasional outliers occur [8].

Reversed-phase thin-layer chromatography (RP-TLC), also an indirect method, has been used [2,13,32,33] and again reference compounds of known log P_{OW} must be run. It has a range of 0 to 12 and is fast but less reproducible than HPLC, has inferior resolution and is less accurate than the shake flask method [8].

In the slow stirring method [2,23,31,34] the water and octanol phases are equilibrated under conditions of slow stirring. The formation of emulsions can be prevented and a very high log P_{OW} can be measured [31]. The method is slow, 2–4 days for equilibration to be achieved but has high accuracy and good repoducibility.

Another technique used is the column generator method where log P_{OW} up to 8.5 can be measured [2]. It is a complex method which includes a generator column for the preparation of an equilibrated solution, an extractor column for the collection and concentration of these solutes and a HPLC system for the measurement of the collected material [35]. Although the column generator method is suitable for hydrophobic chemicals, the labour required in the method is an important disadvantage [31]. Centrifugal partition chromatography, a form of counter current chromatography has also been used [36,37]. It allows the direct measurement of log P_{OW} and the ease of automation and accuracy may make this a useful measurement technique in the future.

2.2. Calculation

The calculation of log P_{OW} based on substituent constants (π) has been described [3,4]. These constants can be used only when the log P_{OW} of a structurally similar parent compound is known and they are dependent on the positions of the substituents in the molecule [31].

Another method of calculation is based on the summation of molecular fragments [4,38]. It is limited by applicability of additivity and interaction rules and availability of fragment constants and is less accurate than the shake flask method [8].

These methods of calculation do not have a limit on the range of log P_{OW} values which can be found. They can give a useful approximation of the log P_{OW}.

A relationship between log P_{OW} and solubilities has been shown [39]. Solubility has been used to calculated log P_{OW} [23,33]. It is limited to com-

pounds with low solubilities in both solvents, is affected by impurities and has low accuracy. The solubilities of a compound in both solvents are often not available [8].

Detailed reviews of the methods for the determination of log P_{OW} have been written by several authors [2,8,31].

3. APPLICATION OF LOG P_{ow} FOR CHOOSING HPLC CONDITIONS FOR ANALYSIS

Details of the use of reversed-phase HPLC for the determination of low P_{OW} have been given by several authors [8,21–23,25–27,29,30,33,40]. Methanol–water has been used for the mobile phase with one use of ethanol–water [33] and one laboratory of 22 in an inter-laboratory comparison [30] used acetonitrile–water. Only two laboratories used elevated temperature [22,33] and solvent programming was used by two authors [22,26] as well as by 9 out of 22 laboratories in an inter-laboratory comparison [30]. Commercial reversed-phase C_{18} columns (10 μm, 250 mm length) have been used by most authors. The most common mobile phase was methanol–water (3:1) at a flow-rate of 1 ml/min at ambient temperature.

Two inter-laboratory comparisons have been carried out [8,30]. The reproducibility of HPLC for log P_{OW} was found to be slightly superior or equivalent to the shake-flask method while the log P_{OW} could be estimated to within ± 1 log unit of the shake-flask value [8]. With the use of 22 collaborators [30] a validated list of reference compounds was established for use in the HPLC determination of unknown log P_{OW} values and it was found that the log P_{OW} could usually be determined with a deviation of less than 0.5 from the shake-flask value.

With the detailed information available for HPLC determinations of log P_{OW} values for particular compounds these conditions would also be applied for any compound with the same or similar log P_{OW}. Where HPLC conditions for a compound with a well established log P_{OW} are known but not necessarily used for log P_{OW} determinations, these conditions should also be suitable for other compounds with the same or similar log P_{OW}. Hence log P_{OW} could be used to predict useful operating HPLC conditions. It would be applicable to hydrophobic compounds but it may not apply to hydrophilic compounds with a log P_{OW} less than two. In these cases the retention times are short and hydrogen bonding effects would play an important part.

4. APPLICATION OF LOG P_{ow} IN ESTIMATING ENVIRONMENTAL BEHAVIOUR OF PESTICIDES

Since the use of log P_{OW} as a measure of hydrophobicity was developed [3] correlations between various combinations of partition coefficients have been published. The log P_{OW} value has come to represent the tendency of a chemical to partition itself between the organic and aqueous environmental compartments. It has been found to be related to water solubility, organic carbon–water partition coefficient and bioconcentration factors (BCF) [21].

Correlations have been found between log P_{OW} and the BCF in aquatic and terrestrial organisms [41], with the BCF in fish [23,28,42,43], and bioconcentration kinetics in fish [34,44]. Relationships have been found between log P_{OW}, soil sorption, water solubilities, BCF and the parachor [13]. The uptake of pesticides in worms [14] and also the BCF of chemicals by alga [45] have relationships with log P_{OW}. Solubilities have been correlated with log P_{OW} [7,39,46,47] as have solubilities and BCF [19,48,49]. A relationship has been established between log P_{OW} and toxicity [18] for six organophosphates and their corresponding oxygen analogs in adult, male mice.

5. DECIDING IF A PESTICIDE SHOULD BE CLASSIFIED AS FAT-SOLUBLE

Partition coefficients have been used to classify compounds. They have been used with the classification of chemical mobility in soil [21]. The relationship between various properties of neutral molecules has been classified according to their log P_{OW} values [13].

A connection between hydrophobicity or fat-solubility and partition coefficients has been developed [3] and log P_{OW} can be considered to be a quantitative measure of the hydrophobicity of a compound [35]. Compounds with high log P_{OW} such as DDT and dieldrin have been classified as lipophilic [43].

When residues of fat-soluble pesticides are present in animal commodities such as meat and milk, they exist almost exclusively in the fat fraction. In

TABLE 1

PARTITION COEFFICIENTS (*n*-OCTANOL–WATER) OF PESTICIDES

Compound	Log P_{ow}	Derivation	Ref.
Acrolein	0.90	Cited	39
Aldicarb	1.08	Measured, inversion	9
	1.57	Measured, inversion	13
	1.13	Measured, shake flask	7
Aldicarb sulfone	−0.57	Measured, inversion	13
Aldicarb sulphoxide	−1.0	?	32
Aldoxycarb	−0.57	Measured, RP-TLC	9
Aldrin	5.66	Calculated	41
	7.4	Measured, RP-TLC	13
	6.50	Measured, shake flask	31
Allethrin	5.0	?	32
Ametryn	3.07	Measured, HPLC	27
	3.07	Calculated	27
Aminocarb	1.73	Measured, shake flask	7
Amitrole	−0.87	Measured, inversion	11
Atraton	2.69	Measured, HPLC	27
	2.69	Calculated	27
Atrazine	2.40	Measured, HPLC	40
	2.21	Measured, HPLC	40
	2.64	Measured, shake flask	
	2.68	or	45
	2.75	Cited	
	2.61	Measured, HPLC	27
	2.61	Calculated	27
	2.75	Measured, RP-TLC	33
	2.47	Measured, HPLC	30
Azinphos ethyl	3.40	Measured, shake flask	7
Azinphos ethyl O-analogue	1.63	Measured, shake flask	7
Azinphos methyl	2.69	Measured, shake flask	7
Azinphos methyl O-analog	0.78	Measured, shake flask	7
Benalaxyl	3.4	Cited	53
Benomyl	2.12	Cited	15
α-BHC	3.81	Measured, shake flask	54
	3.78	Measured, slow stirring	31
β-BHC	3.80	Measured, shake flask	54
	3.84	Measured, slow stirring	31
γ-BHC (lindane)	3.72	Measured, shake flask	54
	3.72	Measured, shake flask	25
	3.66	Measured, shake flask	43
δ-BHC	4.14	Measured, shake flask	54
Bifenthrin	6.00	Cited	55
Bromophos	4.88	Measured, shake flask	7
	5.21	Measured, slow stirring	31
Bromophos ethyl	5.68	Measured, shake flask	7
	6.15	Measured, slow stirring	31
Camphechlor	5.50	Cited	39
Captafol	3.83	Measured, inversion	13
Captan	2.54	Measured, inversion	13
	2.35	Measured, inversion	13
Carbanolate	2.3	Measured, inversion	13
Carbaryl	2.34	Measured, shake flask	56
	2.32	Measured, inversion	13
	2.36	Measured, inversion	13
	2.31	Measured, shake flask	7
	2.29	Measured, shake flask	43

TABLE 1 *(continued)*

Compound	Log P_{ow}	Derivation	Ref.
Carbendazim	1.40	Measured, inversion	14
	1.52	Measured, inversion	15
Carbofuran	1.63	Measured, shake flask	7
Carbophenothion	5.12	Measured, shake flask	7
	5.66	Measured, slow stirring	31
Carbophenothion methyl	4.82	Measured, shake flask	7
Carboxin	2.14	Cited	4-536[a]
Chloramben methyl ester	2.8	Measured, inversion	13
Chlorbromuron	3.09	Measured, inversion	13
Chlordane	5.16	Calculated	41
	6.00	Measured, HPLC	28
α-Chlordane	6.00	Cited	39
	6.0	Cited	44
γ-Chlordane	6.0	Cited	44
Chlordimeform	2.89	Measured, shake flask	7
Chlorfenac methyl	3.8	Measured, inversion	13
Chlorfenvinphos	3.10	Measured, inversion	13
	3.23	Measured, inversion	14
	3.81	Measured, shake flask	7
	3.80	Measured, shake flask	25
	3.82	Measured, shake flask	8
Chloridazon	1.14	Measured, shake flask	27
	1.50	Measured, inversion	13
Chlornitrofen	3.67	Measured, shake flask	43
Chlorotoluron	2.41	measured, inversion	13
Chloroxon	1.83	Measured, shaking	18
Chloroxuron	3.7	Measured, inversion	13
Chlorpyrifos	5.11	Measured, shake flask	48
	4.96	Measured, shake flask	7
	5.2	Measured, shaking	19
	5.27	Measured, slow stirring	31
Chlorpyrifos methyl	4.31	Measured, shake flask	48
	4.30	Measured, shake flask	7
Chlorsulfuron	1.09 to −0.41 at pH 4.5-12.0	Measured, stirring	17
Chlorthion	3.45	Measured, shaking	18
	3.63	Measured, slow stirring	34
Clofentezine	2.18	Cited	55
	3.1	Cited	53
Clopyralid	1.76	Calculated	41
Cyanazine	1.8	Measured, HPLC	27
	1.66	Calculated	27
Cyanophos	2.71	Measured, slow stirring	34
Cycloheximide	0.55	Measured, inversion	13
Cyhexatin	5.39	Cited	41
Cypermethrin	4.47	Measured, shake flask	59
2,4 Dichlorophenoxyacetic acid (2,4-D)	2.90 (undissociated)	Measured, inversion	10
	−0.24 (dissociated)	Measured, inversion	10
	2.81	Measured, shake flask	16
2,4-D dimethylamine	0.65	Measured, HPLC	24
2,4-D octyl ester	5.86	Measured, HPLC	40
	6.71	Calculated	40
	6.89	Calculated	40

(Continued on p. 8)

TABLE 1 *(continued)*

Compound	Log P_{ow}	Derivation	Ref.
DDE	5.63	Measured, HPLC	21
p,p'-DDE	5.69	Measured, HPLC	22
	5.89	Measured, HPLC	40
	6.96	Measured, slow stirring	31
	5.69	Measured, HPLC	28
	6.09	Measured, HPLC	29
DDT	4.64	Measured, HPLC	21
	3.98	Measured, shake flask	60
	5.90	Measured, shake flask	8
	6.12	Measured, HPLC	8
	5.89	Measured, HPLC	30
o,p'-DDT	5.75	Measured, HPLC	28
p,p'-DDT	6.38	Measured, shake flask	46
	6.2	Measured, slow stirring	23
	6.19	Measured, shake flask	25
	6.91	Measured, slow stirring	31
Deet	2.02	Measured, HPLC	24
Demetonthiol	1.93	Measured, shake flask	58
Desethylatrazine	1.53	Calculated	27
	1.51	Measured, HPLC	27
Desisopropylatrazine	1.12	Calculated	27
	1.15	Measured, HPLC	27
Dialifos	4.69	Measured, shake flask	48
Diazinon	3.11	Measured, inversion	13
	3.81	Measured, shake flask	7
	3.14	Measured, shake flask	43
Diazoxon	2.07	Measured, shake flask	7
Dicapthon	3.44	Measured, shaking	18
	3.58	Measured, shake flask	48
	3.62	Measured, shake flask	7
	3.72	Measured, slow stirring	34
Dicapthoxon	1.84	Measured, shaking	18
Dichlofenthion	5.14	Measured, shake flask	48
3,4 Dichloroaniline	2.78	Measured, inversion	13
p-Dichlorobenzene	3.42	Measured, shake flask	2
	3.38	Measured, generator column	2
	3.44	Measured, slow stirring	2
2,4-Dichlorophenol	2.8	Measured, inversion	13
Dichlorvos	1.47	Measured, shake flask	7
Dieldrin	4.54	Measured, slow stirring	23
	4.32	Measured, shake flask	43
	5.40	Measured, slow stirring	31
Dimethoate	0.50	Measured, shake flask	58
	0.79	Measured, inversion	13
	0.78	Measured, shake flask	7
Diphenyl	3.63	Measured, shake flask	25
	4.00	Measured, slow stirring	31
	4.00	Measured, shake flask	2
	3.83	Measured, generator column	2
	4.01	Measured, slow stirring	2
	3.91	Measured, shake flask	8
Diquat dichloride	−3.55	Cited	4-921[a]
Disulfoton	4.02	Measured, shake flask	7
Disulfoton sulfone	1.87	Measured, shake flask	7
Disulfoton sulfoxide	1.73	Measured, shake flask	7
Diuron	2.68	Measured, inversion	13

TABLE 1 *(continued)*

Compound	Log P_{ow}	Derivation	Ref.
Dowco 275	3.51	Measured, inversion	13
2,2-DPA (Dalapon, 2,2-dichloropropionic acid)	0.78	Calculated or cited	41
Endrin	4.56	Measured, shake flask	25
	5.20	Measured, slow stirring	31
EPN (O-ethyl O-4-nitrophenyl phenylphosphonothioate)	3.85	Measured, shake flask	43
Ethion	5.07	Measured, shake flask	7
ETU (ethylene thiourea)	−0.66	Cited	6
Fenamiphos	3.18	Measured, inversion	13
	3.23	Measured, shake flask	7
Fenchlorphos	4.88	Measured, shake flask	48
	4.81	Measured, shake flask	7
	5.07	Measured, slow stirring	31
Fenitrooxon	1.69	Measured, shaking	18
Fenitrothion	3.38	Measured, shake flask	48
	3.30	Measured, shaking	18
	3.40	Measured, shake flask	7
	3.47	Measured, slow stirring	34
	3.44	Measured, shake flask	43
Fenobucarb	3.18	Measured, shake flask	43
Fenoprop	2.44	Cited	41
	3.86	Cited	39
Fenpropathrin	3.03	Measured, shake flask	59
Fensulfothion	2.23	Measured, shake flask	7
Fensulfothion sulfide	4.16	Measured, shake flask	7
Fensulfothion sulfone	2.56	Measured, shake flask	7
Fenthion	4.09	Measured, shake flask	7
	4.17	Measured, slow stirring	34
Fenuron	0.96	Measured, inversion	13
Fenvalerate	4.42	Measured, shake flask	59
	6.2	Measured, shaking	19
Flamprop	2.90 (undissociated)	Measured, inversion	10
	−0.40 (dissociated)	Measured, inversion	10
Flucythrinate	6.2	Measured, shaking	19
Fluometuron	2.42	Measured, inversion	13
Fluorodifen	4.4	Measured, inversion	13
Fluvalinate	>3.85	Cited	55
Fonofos	3.89	Measured, shake flask	7
Fonofos O-analogue	2.11	Measured, shake flask	7
Guazatine	−1.15 at pH 3	Cited	55
Haloxyfop	4.47	Cited	55
	3.52	Cited	61
Haloxyfop methyl ester	4.07	Cited	55
Hexachlorobenzene (HCB)	5.50	Measured, shake flask	46
	5.44	Measured, inversion	13
	6.18	Measured, shake flask	25
	5.47	Measured, shake flask	2
	5.47	Measured, generator column	2
	5.73	Measured, slow stirring	2
	5.66	Measured, shake flask	8
Heptachlor	5.38	Calculated	41
	5.27	Measured, HPLC	40
	6.06	Calculated	40
	5.44	Measured, HPLC	28

(Continued on p. 10)

TABLE 1 *(continued)*

Compound	Log P_{ow}	Derivation	Ref.
	5.5	Calculated	29
	5.58	Measured, HPLC	29
Heptachlor epoxide	5.40	Measured, HPLC	28
Hexythiazox	2.53	Cited	55
Hydramethylnone	2.31	Cited	55
Imazapyr	0.11	Cited	55
Imazaquin	0.34	Cited	55
3-Indoleacetic acid	1.41	Cited	4-505[a]
Iodofenphos	5.16	Measured, shake flask	7
Iprobenfos	3.21	Measured, shake flask	43
Isazofos	3.82	Calculated from solubilities	33
	3.82	Measured, RP-TLC and HPLC	33
Isofenphos	4.12	Measured, shake flask	7
Leptophos	6.31	Measured, shake flask	48
	5.88	Measured, shake flask	7
	4.32	Measured, shake flask	43
Leptophos O-analogue	4.58	Measured, shake flask	7
Linuron	2.76	Measured, inversion	13
Malathion	2.89	Measured, shake flask	48
	2.84	Measured, shake flask	7
	2.94	Measured, slow stirring	34
Maleic hydrazide	-0.63	Measured, inversion	10
Metalaxyl	1.27	Calculated from solubilities	33
	1.65	Measured, RP-TLC and HPLC	33
Metflurazon	2.67	Measured, shake flask	57
Methidathion	2.42	Measured, shake flask	7
Methiocarb	2.92	Measured, inversion	13
Methomyl	0.13	Measured, shake flask	7
Methoxychlor	3.31	Measured, shake flask	60
Metobromuron	2.38	Measured, inversion	13
Metolachlor	3.28	Calculated from solubilities	33
	3.13	Measured, RP-TLC and HPLC	33
Metoxuron	1.64	Measured, inversion	13
Metribuzin	1.70	Cited	45
Mirex	6.89	Measured, HPLC	28
Molinate	3.21	Measured, shake flask	43
Monolinuron	2.30	Measured, inversion	13
Monuron	1.98	Measured, inversion	13
Naled	1.38	Measured, shake flask	58
Naphthalene	3.36	Measured, inversion	13
	3.25	Measured, slow stirring	23
	3.28	Measured, shake flask	25
	3.31	Measured, shake flask	8
NIA 24 110 (5-benzylfur-3-ylmethyl-*trans*-(+)-3-cyclopentylidenemethyl-2,2-dimethylcyclopropanecarboxylate) (RU 11679)	7.14	?	32
Nitrapyrin	3.02	Measured, inversion	13
Norflurazon	2.30	Measured, shake flask	57
Oxamyl	−0.47	Measured, inversion	13
Oxycarboxin	0.9	Measured. inversion	13
Paclobutrazol	3.2	Cited	62
Paraquat di-iodide	−5.00	Cited	4-921[a]
Paraoxon	1.59	Measured, shake flask	58
	1.98	Measured, shake flask	7
Paraoxon methyl	1.28	Measured, shake flask	58
	1.21	Measured, shaking	18

TABLE 1 *(continued)*

Compound	Log P_{ow}	Derivation	Ref.
Parathion	2.15	Measured, shake flask	63
	3.93	Measured, inversion	13
	3.81	Measured, shake flask	48
	3.76	Measured, shake flask	7
Parathion amino	2.60	Measured, shake flask	7
Parathion methyl	2.04	Measured, shake flask	65
	2.99	Measured, shaking	18
	2.94	Measured, shake flask	7
	1.8	Measured, shaking	19
	3.04	Measured, slow stirring	34
Pentachlorophenol	5.01	Measured, HPLC	22
	3.69	Measured, shake flask or cited	45
	5.01	Measured, HPLC	28
Pentanochlor	3.7	Measured, inversion	13
Permethrin	6.6	Measured, calculated	13
	3.49	Measured, shake flask	59
	6.5	Measured, shaking	19
	5.84 (*trans*)	Measured, HPLC	26
	6.24 (*cis*)	Measured, HPLC	26
Phenothiazine	4.15	Cited	3
Phenoxyacetic acid	1.47	Calculated from solubilities	47
	1.52	Measured, HPLC	30
Phenthoate	3.96	Measured, slow stirring	34
	2.89	Measured, shake flask	43
o-Phenyl phenol	3.09	Cited	4-558[a]
Phorate	4.26	Measured, inversion	13
	3.83	Measured, shake flask	7
Phorate sulfone	1.99	Measured, shake flask	7
Phorate sulfoxide	1.78	Measured, shake flask	7
Phosalone	4.30	Measured, shake flask	48
	4.38	Measured, shake flask	7
Phosmet	2.83	Measured, shake flask	48
	2.78	Measured, shake flask	7
	2.81	Measured, slow stirring	34
Phoxim	4.39	Measured, shake flask	7
Picloram	0.30	Calculated	41
Picloram methyl ester	2.3	Measured, inversion	13
Pirimiphos ethyl	4.85	Measured, shake flask	7
Pirimiphos methyl	4.20	Measured, shake flask	7
PP450 (Flutriafol)	2.29	Measured, inversion	12
Profenofos	4.70	Calculated from solubilities	33
	4.70	Measured, RP-TLC and HPLC	33
Profluralin	6.34	Calculated from solubilities	33
	5.58	Measured, RP-TLC and HPLC	33
Prometon	3.1	Calculated	27
	2.99	Measured, HPLC	27
Prometryn	3.48	Calculated	27
	3.34	Measured, HPLC	27
Propanil	2.8	Measured, inversion	13
Propazine	3.02	Calculated	27
	2.91	Measured, HPLC	27
Propham	2.60	Measured, inversion	13
Propoxur	1.58	Measured, shake flask	56
	1.55	Measured, shake flask	7
Quintozene	4.22	Measured, shake flask	43
Resmethrin	6.14	?	32

(Continued on p. 12)

TABLE 1 (continued)

Compound	Log P_{OW}	Derivation	Ref.
Simazine	1.51	Measured, inversion	13
	2.06	Measured, HPLC	40
	1.96	Measured, HPLC	40
	2.2	Calculated	27
	2.26	Measured, HPLC	27
Simetryn	2.66	Calculated	27
	2.8	Measured, HPLC	26
Strychnine	1.93	Cited	4-505[a]
Swep	2.80	Cited	4-273[a]
2,4,5-Trichorophenoxyacetic acid (2,4,5-T)	0.60	Calculated	41
Trichloroacetic acid	1.33	Cited	64
	0.10	Cited	6
	1.96	Calculated	6
p,p-TDE	6.22	Measured, slow stirring	31
Temephos	5.96	Measured, shake flask	7
Terbufos	4.48	Measured, shake flask	7
Terbufos sulfone	2.48	Measured, shake flask	7
Terbufos sulfoxide	2.21	Measured, shake flask	7
Terbumeton	3.1	Calculated	27
	3.1	Measured, HPLC	27
Terbuthylazine	3.02	Calculated	27
	3.06	Measured, HPLC	27
Terbutryn	3.72	Calculated from solubilities	33
	3.74	Measured, RP-TLC and HPLC	33
	3.48	Calculated	27
	3.43	Measured, HPLC	27
Tetrachlorvinphos	3.53	Measured, shake flask	8
Tetramethrin	4.7	?	32
Thiazfluron	1.46	Calculated from solubilities	33
	1.85	Measured, RP-TLC and HPLC	33
Thiobencarb	3.4	Measured, shaking	19
	3.42	Measured, shake flask	43
Tolylfluanid	3.90	Cited	62
Triazophos	3.55	Measured, shake flask	7
Triadimefon	2.77	Measured, inversion	12
Trichlorfon	0.43	Measured, shake flask	7
Trichloronate	5.23	Measured, shake flask	7
2,4,6-Trichlorophenol	2.97	Measured, shake flask or calculated	45
Tridiphane	4.34	Cited	55
Trietazine	3.15	Calculated	27
	3.07	Measured, HPLC	27
Trifluralin	3.97	Measured, shake flask	43
Vinclozolin	3.0	Cited	53
Warfarin	0.05	Cited	4-883[a]
	2.72	Cited	4-883[a]

[a] The additional number with ref. 4 gives the actual number of the reference in that source.

the case of meat, the residues of pesticides defined as fat-soluble are reported in terms of their concentration in the fat, not in the whole meat.

Extending the use of log P_{OW} to classify pesticides as fat-soluble has been proposed [50]. The scheme put forward suggests for log P_{OW} <3 pesticides would not be fat-soluble, log P_{OW} 3–4 is an overlapping region and for log P_{OW} >4 pesticides would

be fat-soluble.

The organochlorine pesticides are defined as fat-soluble. Most have a log P_{OW} >5 (aldrin, chlordane, DDE, DDT, dieldrin, endrin, heptachlor). The isomers of 1,2,3,4,5,6-hexachlorocyclohexane (BHC) have a log P_{OW} in the range of 3–4 and methoxychlor has a value of 3.31. The pyrethroids, also defined as fat-soluble, cyfluthrin, fenvalerate and permethrin have log P_{OW} values >5 while cypermethrin has values >4.

Organophosphates such as chlorfenvinphos (log P_{OW} 3.10–3.82), diazinon (log P_{OW} 3.11–3.81), fenitrothion (log P_{OW} 3.30–3.47) and phenthoate (log P_{OW} 2.89–3.96) are designated as fat-soluble [51]. Methidathion (log P_{OW} 2.42) and phosmet (log P_{OW} 2.83, 2.75, 2.81) are also designated as fat-soluble but these are the only such examples. It is compounds such as the organophosphates, the BHC isomers and methoxychlor, which are designated as fat-soluble and have log P_{OW} values from 3 to 4, which cause an overlapping region instead of a definite boundary.

Some compounds have log P_{OW} >4 but are not designated as fat-soluble. Cyhexatin (log P_{OW} 5.39), disulfoton (log P_{OW} 4.02), phorate (log P_{OW} 3.83, 4.26), phosalone (log P_{OW} 4.30, 4.38), profenofos (log P_{OW} 4.70) and terbufos (log P_{OW} 4.88) come in this category. There are some compounds such as the polymeric dithiocarbamates which are poorly soluble in both octanol and water. In this case if the log P_{OW} is greater than four these compounds could not be considered as fat-soluble and the use of log P_{OW} to classify these pesticides as fat-soluble would not be suitable.

6. COMPILATION OF THE DATA

The log P_{OW} values of pesticides and some breakdown products, metabolites and analogs are summarised in Table I. For consistency the common names used have come from one source [52]. The method of determination has been given (HPLC, RP-TLC, shake-flask and also slow stirring, inversion and shaking where there has been a variation on the shake flask method). Where the values have been calculated from substituent constants or fragment constants they are noted as calculated; however, where they have been calculated from solubilities this has been stated. The additional number with ref. 4 gives the actual number of the reference in that source.

The shake-flask method or a variation of it has been the preferred source of the values in an attempt to give the most accurate and useful data. In some cases close agreement was not found in the values for particular compounds even with this method. When values derived from a direct method were not available values from all other sources have been given. It has been indicated [8] that the useful range of the shake-flask method is −2.5 to 4.5. Where shake-flask values above this level have been found those from the slow stirring method are also included and if these were not available HPLC values have also been given.

Values from the RP-HPLC and RP-TLC methods are not as accurate as those from the shake-flask method. The accuracy of the HPLC method was considered not to be satisfactory according to the ±10% of reliably measured log P_{OW} values criteria adopted by one author [23]. Calculation by summation of molecular fragments is less accurate than the shake-flask method [8] and calculation from solubilities in water and octanol has low accuracy [8].

Data from the HPLC and calculation methods would not be accurate enough for use in bioactivity modling using partition coefficients because of the potential compounding of errors. Some values have been cited without the original method but only where no other values were found.

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Review

Rapid high-performance liquid chromatographic methods that screen for aromatic compounds in environmental samples

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ABSTRACT

In order to monitor the quality of coastal waters that provide habitats for living marine resources, samples of sediment and biota must be analyzed to assess the degree and distribution of anthropogenic contamination. Analytical time and costs can be greatly reduced by first employing methods that screen for contaminants before selecting samples for rigorous analyses. In this paper, we review the applications of rapid high-performance liquid chromatographic (HPLC) methods to screen for aromatic compounds in sediment, bile and tissue samples. These methods have been used to assess damage to natural resources after the Exxon Valdez oil spill. In addition, the bile screening method has also been used to evaluate contaminants in fish sampled for a national monitoring program. The rapid screening of sediment or bile provides an estimate of contaminant concentrations that can then be confirmed in selected samples by more complicated and expensive analyses by gas chromatography–mass spectrometry (GC–MS). Furthermore, HPLC and GC–MS chromatographic patterns from sediment and bile can provide information about the source of contamination, *e.g.*, crude oil, diesel fuel or pyrogenic contaminants. We also discuss the important role screening methods will play in the future in assessing the quality of aquatic habitats, the safety of seafood, and other important issues related to anthropogenic contamination.

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1. INTRODUCTION

Maintaining the quality of our coastal waters is essential to providing a habitat in which important living marine resources can live and reproduce. However, in many urban estuaries, anthropogenic contaminants have degraded the quality of the water, sediment and biota. A major challenge confronting environmental scientists is developing accurate and cost-effective analyses for contaminants in marine biota and their habitats. In particular, reliable methods are needed to determine concentrations of toxic contaminants, *e.g.*, polynuclear and heterocyclic aromatic compounds (ACs) and their metabolites or polychlorinated biphenyls (PCBs), in a variety of environmental matrices. The data provided by these analyses can then be used by managers to evaluate problems as diverse as oil spills, marine mammal strandings, oil well fires, fish kills and chemical contamination in commercial seafood products. Often, analytical time and costs can be greatly reduced by first employing screening methods to rapidly estimate contaminant concentrations. Based on the results of the screening analyses, a subset of samples can then be selected for detailed analyses, *e.g.*, gas chromatography–mass spectrometry (GC–MS), that can confirm the presence and identity of the contaminants.

Both non-chromatographic [1–4] and chromatographic procedures [5,6] have been used to screen sediments for aromatic contaminants. Non-chromatographic procedures, such as normal [1] or synchronous scan [2–4] fluorescence spectrometry are of limited value because they do not separate out possible interfering compounds before measuring the analytes [7,8]. Even some chromatographic procedures, such as flash evaporation–pyrolysis–GC–MS, failed to eliminate all interferents [6]. Recently, however, Krahn *et al.* [9] reported a sensitive method that uses high-performance size-exclusion chromatography (HPSEC) to remove interferents before analyzing for AC analytes in sediments.

Choices of methods to screen for ACs in biota are more limited. Fish and marine mammals rapidly take up ACs (*e.g.*, naphthalene, benzo[*a*]pyrene) present in their environment, extensively metabolize most ACs in their livers, and then transfer a large proportion of the polar metabolites to bile for elimination [10–12]. As a result, the metabolic products, rather than the parent ACs, must be determined in these animals. Two methods that employ reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection have been developed in our laboratories to screen for metabolites of ACs in fish. The first, a bile screening method was developed to evaluate anthropogenic contamination by ACs in fish sampled from urban estuaries [13,14]. In addition, a method that estimates the concentrations of AC metabolites in fish tissues, *e.g.*, liver and muscle, has been developed recently to address the issue of seafood contamination [15], but has not yet undergone extensive field testing.

In this paper, we review applications of the sediment, bile and tissue screening methods to problem in the marine environment. These methods have been applied to establishing the extent of damage to natural resources after the 1989 Exxon Valdez oil spill in Prince William Sound, Alaska, USA. In addition, the bile screening method has been used ex-

tensively in the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends (NS&T) Monitoring Program to compare concentrations of AC metabolites in fish captured from sites in USA coastal waters. These applications have demonstrated that sediment or bile samples can be rapidly screened to select samples for confirmation of AC concentrations by the more complicated and expensive GC–MS analyses. In addition, HPLC and GC–MS chromatographic patterns from sediment and bile can often provide information about a possible source of contamination, *e.g.*, crude oil, diesel fuel or pyrogenic contaminants. We also describe analytical problems that need to be addressed in the future: rapidly determining the concentrations and toxicities of chemicals such as the coplanar PCB congeners or polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs); developing screening analyses that can readily be used in "real-time" in field applications; and improving immunoassay methods that test for specific chemicals or groups of chemicals.

2. METHODS TO SCREEN FOR AROMATIC CONTAMINANTS

2.1. *Screening for metabolites of aromatic compounds in tissues*

Enzymatic hydrolysis, methylene chloride extraction and hexane–potassium hydroxide partitioning were used with HPLC–fluorescence analysis to screen tissue samples for metabolites of ACs [15]. Briefly, tissues were homogenized and then treated with β-glucuronidase and aryl sulfatase to hydrolyze glucuronide and sulfate conjugates of AC metabolites. The hydrolysate was extracted with methylene chloride and this solvent was replaced with methanol. A portion of this methanol extract was diluted with hexane and the organic solution was partitioned with aqueous potassium hydroxide to extract the metabolites. The neutralized aqueous phase, containing AC metabolites, was analyzed by reversed-phase HPLC chromatography as described below for bile screening analyses.

TABLE 1

CONCENTRATIONS OF METABOLITES OF AROMATIC COMPOUNDS IN BILE DETERMINED BY THE HPLC SCREENING METHOD AT PHENANTHRENE WAVELENGTHS AND SUMS OF CONCENTRATIONS OF PHENANTHROLS DETERMINED BY GC–MS [16]

Bile sample	Concentration (ng/g wet mass)	
	Phenanthrene equivalents from HPLC	Sum of phenanthrols from GC–MS
Salmon	380 000	14 000
Pollock	90 000	2100
Reference pollock	5000	410

2.2. *Screening for metabolites of aromatic compounds in bile*

The method for bile analysis by HPLC with fluorescence detection is described in detail by Krahn and co-workers [13,14]. Briefly, bile was injected directly onto a reversed-phase C_{18} HPLC column and eluted with a linear gradient from 100% water (containing a small amount of acetic acid) to 100% methanol at a flow of 1.0 ml/min. Chromatograms were recorded at two or more fluorescence wavelength pairs where the parent compounds and metabolites fluoresce: naphthalenes and dibenzothiophenes at 290/335 nm (excitation/emission), phenanthrenes at 260/380 nm and benzo[*a*]pyrenes at 380/430 nm. The metabolite-containing portion of the chromatogram (> 7 min) was integrated and concentrations were calculated by normalization to an AC standard, *e.g.*, a phenanthrene standard was used to calculate concentrations of "phenanthrene equivalents" for chromatograms recorded at 260/380 nm (Table 1).

2.3. *Screening for aromatic compounds in sediment*

The method used to screen sediments for ACs features sonic extraction and HPSEC with fluorescence detection as described by Krahn *et al.* [9]. Briefly, sediment, sodium sulfate, activated copper and methylene chloride were mixed together in a centrifuge tube. The tubes were placed into a sonic

TABLE 2

CONCENTRATIONS OF AROMATIC COMPOUNDS IN SEDIMENT DETERMINED BY THE HPLC SCREENING METHOD AT BENZO[*a*]PYRENE (BaP) WAVELENGTHS AND SUMS OF CONCENTRATIONS OF HIGH-MOLECULAR-MASS AROMATIC HYDROCARBONS (HAHs) DETERMINED BY GC–MS [9]

	Concentration (ng/g wet mass)	
	BaP equivalents from HPLC	Sum of HAHs from GC–MS
South San Diego Bay	790	2300
Hunters Point	530	2400
Bodega Bay	6	5

bath and sonicated. The tubes were then centrifuged and the extracts were decanted into concentrator tubes. These steps were repeated twice and the combined extracts were concentrated by evaporation. A portion of the concentrated extract was injected onto a 100 Å HPSEC column, was eluted isocratically with methylene chloride at a flow of 2.5 ml/min for 12 min, and fluorescence was recorded at two or more of the wavelengths used for the bile analyses (above). The fraction containing ACs (> 8.2 min) was integrated and concentrations of AC equivalents were calculated in a manner similar to the bile screening method (Table 2).

2.4. Selecting the chromatographic mode

Although the bile, sediment and tissue screening methods are similar in many respects, HPLC columns and conditions were selected to best separate and detect the particular analytes to be determined by each method. For example, sediments often contain both pyrogenic and petrogenic ACs, possibly including petroleum-related components of high molecular mass (*e.g.*, asphaltenes), so a HPSEC column that separates analytes according to molecular size and shape was chosen for sediment screening. In contrast, ACs in bile are present almost entirely as polar metabolites due to the facile ability of many vertebrates to metabolize ACs in their livers and eliminate the metabolites via bile [10–12]. The ACs or their metabolites can also be transported to other tissues (*e.g.*, muscle) within the animal. The polar metabolites are best determined using a gradient elution from water to methanol on a reversed-phase C_{18} HPLC column, conditions under which the most polar compounds elute first. However, this reversed-phase column is not well-suited to the screening of sediment extracts, because many of the non-polar, high-molecular-mass compounds in the sediments can become irreversibly bound to the packing. Although the HPSEC column does not resolve AC metabolites well and, as a result, would not be a good choice for bile screening, this column can be used for separating endogenous substances in bile from the AC metabolites prior to GC–MS analysis [16].

3. VALIDATING THE SCREENING METHODS

3.1. Tissue screening method

To test the tissue screening method, rock sole (*Lepidopsetta bilineata*) were injected with Prudhoe Bay crude oil (PBCO) and samples of liver and muscle were screened for AC metabolites [15]. The concentrations of AC metabolites in both liver and muscle of these PBCO-exposed fish were linearly proportional to dose. In addition, when tissues from English sole (*Parophrys vetulus*) sampled from the Duwamish Waterway (an urban site in Seattle, WA, USA) and President Point (a non-urban reference site in Puget Sound, WA, USA) were screened, concentrations of polar metabolites of ACs were found to be about 15 times higher in livers of fish from the urban site compared to the reference site. Furthermore, concentrations of AC metabolites in muscle of fish from both sites were very low —*i.e.*, near the limit of detection. As demonstrated in laboratory studies with radiolabeled ACs, the parent compounds are extensively metabolized in liver and most of the metabolites are excreted into bile, thus limiting the accumulation of parent ACs or metabolites in extrahepatic tissues [12,17,18]. As a result, AC metabolites are not transported to edible tissues unless the animal is exposed to high concentrations of ACs, as demonstrated in dose–response studies with PBCO [15]. In the future, the tissue screening method needs to be further field-tested and validated for several fish and crustacean species, so that its application to important environmental issues, such as seafood contamination, can be assessed.

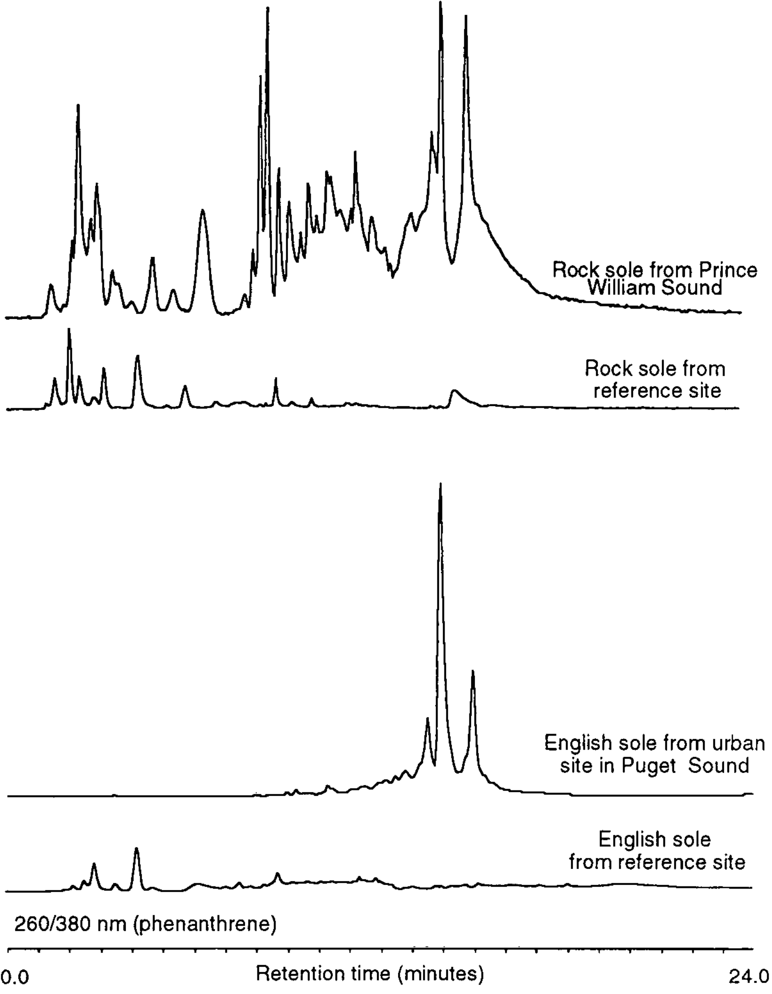

Fig. 1. Chromatograms from the HPLC–fluorescence screening of bile at phenanthrene wavelengths: from a rock sole from Prince William Sound, AK, USA, after the Exxon Valdez oil spill; from a rock sole from a reference (non-oiled) site; and from English sole captured from an urban site and from a non-urban reference site.

3.2. Bile screening method

Metabolites of ACs in bile elute from the reversed-phase HPLC column according to polarity and form a chromatographic pattern containing several sharp fused peaks (Fig. 1) [19]. Concentrations of AC metabolites were estimated by integration of the area (> 7 min) in the chromatograms where the metabolites of ACs elute (examples in Table 1) [13,14]. Detailed GC–MS analyses for individual AC metabolites were conducted on bile samples from fish captured from urban sites [19] and from Prince William Sound after the Exxon Valdez oil spill [16]. The concentrations of metabolites were then summed by classes (*e.g.*, the phenanthrols) for comparison to HPLC screening results from the same samples (examples in Table 1). Statistical comparison of the HPLC screening and GC–MS results from fish from the oil spill study showed an excellent correlation ($p \leqslant 0.0001$) between these methods [16]. Thus, bile screening was validated as a rapid method for determining the exposure of fish to ACs.

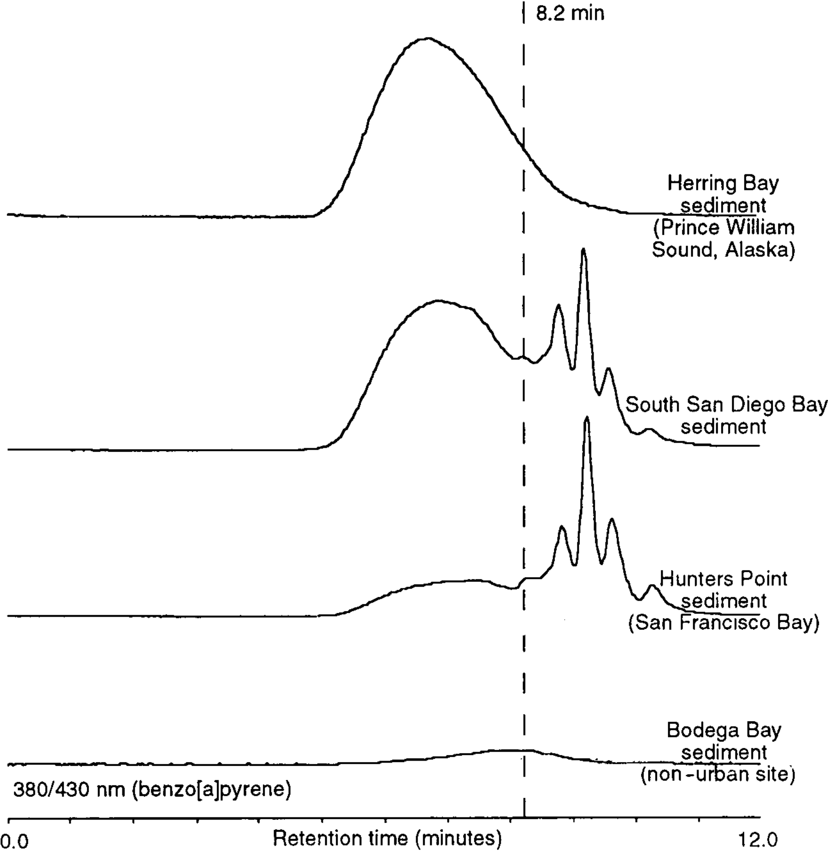

Fig. 2. Chromatograms from the HPLC–fluorescence screening of sediment at benzo[*a*]pyrene wavelengths: from Herring Bay, a site in the path of the Exxon Valdez spill; from the contaminated urban areas of South San Diego Bay and Hunters Point, CA, USA; and from the non-urban reference site of Bodega Bay, CA, USA. The retention time (8.2 min) at which integration of the AC fraction was begun is marked on the chromatograms and the peak.

3.3. Sediment screening method

Aromatic compounds elute from the HPSEC column according to molecular "volume" (largest first), forming a chromatographic pattern that is a continuum of broad peaks (Fig. 2) [9]. This pattern is in contrast to the sharp fused peaks found in chromatograms of AC metabolites in bile (Fig. 1). Concentrations of ACs were estimated by integrating that portion of the chromatogram ($>$ 8.2 min) containing the ACs (examples in Table 2). To compare the results of screening with those from rigorous GC–MS analyses for ACs, a number of sediments from relatively uncontaminated sites and from sites in urban [9] or in oil spill areas [20] were screened and individual ACs were also determined by GC–MS in the same sediments (examples in Table 2). The AC concentrations from HPLC screening were highly correlated ($p \leqslant 0.0001$) with the sums of individual ACs determined by the GC–MS analysis of the same sediments [9,20]. Thus, the HPLC screening method was validated for use in estimating concentrations of ACs in contaminated sediments.

4. IDENTIFYING CONTAMINANT SOURCES

4.1. HPLC patterns from bile

Although HPLC chromatographic patterns from bile can be variable and somewhat difficult to interpret, these patterns can sometimes be used to

suggest a possible source of contamination, *e.g.*, pyrogenic or petrogenic contamination. For example, the chromatographic pattern from bile of a rock sole captured from Prince William Sound after the Exxon Valdez spill appeared very dissimilar to that of an English sole exposed to contaminants from an

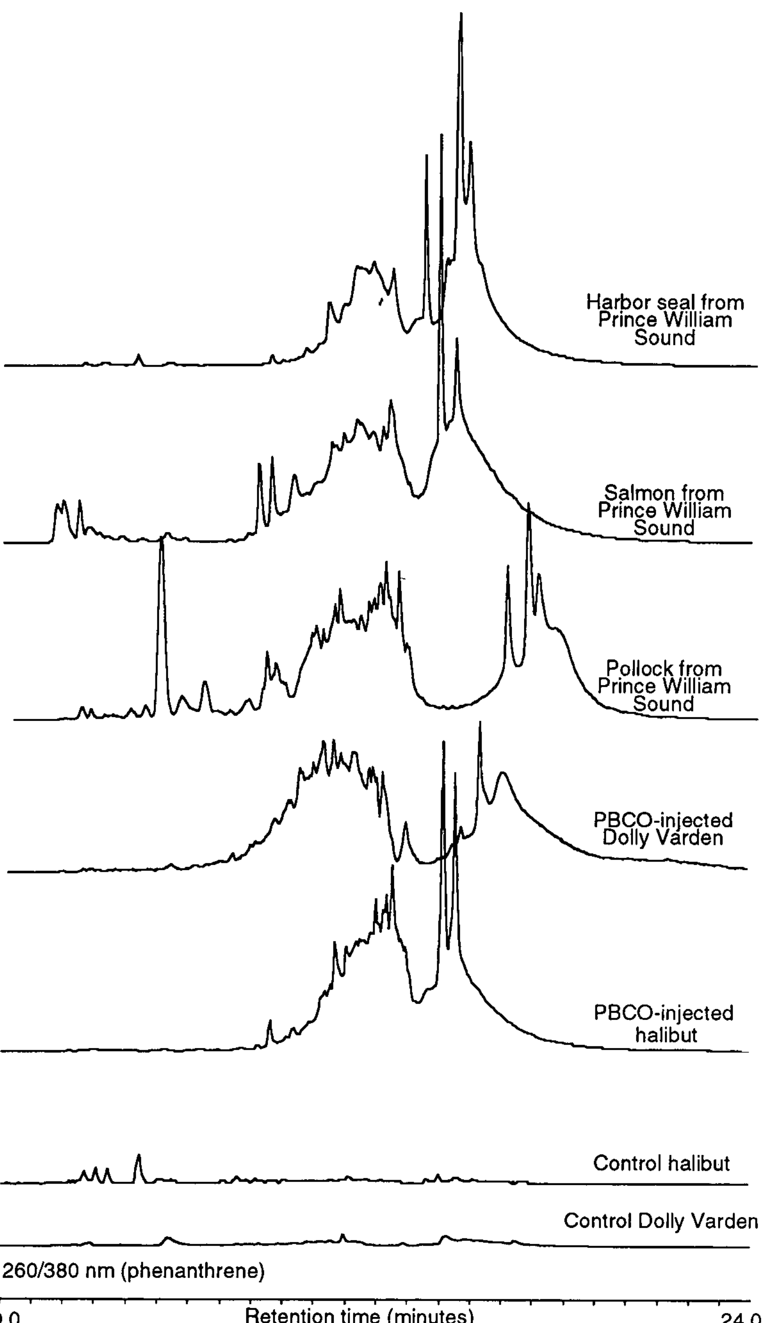

Fig. 3. Chromatograms from the HPLC–fluorescence screening of bile at phenanthrene wavelengths: from a harbor seal, a salmon and a pollock captured from Prince William Sound after the Exxon Valdez oil spill; from a Dolly Varden and a halibut injected with Prudhoe Bay crude oil (PBCO); and from a halibut and a Dolly Varden injected with carrier solvent (controls).

urban site (Fig. 1). However, even when the source of contamination in the fish was the same, *e.g.*, exposure to or injection with PBCO, some differences were apparent in the chromatographic patterns (Fig. 3). Chromatograms of bile from the PBCO-exposed fish exhibited two groups of peaks with a valley between them at *ca.* 13 min, but the retention times and intensities of individual peaks varied (Fig. 3). Variations of these sorts can occur in bile chromatographic patterns, possibly due to variations in the degree of exposure of individual animals to the oil or to species-specific differences in metabolism of the petroleum ACs [16]. For example, the same relative amount of weathered PBCO injected into two species of fish (halibut and Dolly Varden) resulted in HPLC chromatographic patterns of bile (Fig. 3) and proportions of each metabolite [16] that were similar, but not identical. In addition, physical factors from the chromatographic process itself, such as the chromatography column used, the condition of the column or the acidity of the mobile phase, can affect the appearance of a chromatogram in reversed-phase HPLC. For example, without the addition of a small quantity of acetic acid to the water in the mobile phase, the conjugated metabolites will elute earlier [13]. Therefore, due to both the variability of the metabolic process in various fish species and to the variability of the reversed-phase chromatography of the metabolites, the HPLC chromatographic pattern of bile can only suggest the source of contamination.

4.2. Relative proportions of bile metabolites by GC–MS

When bile samples were enzymatically hydrolyzed and their extracts were subjected to GC–MS analysis [16,19], the source of contamination suggested by HPLC screening could often be confirmed from the identities and the proportions of the individual metabolites determined by GC–MS. For example, bile of fish that were exposed to ACs from urban sites contained higher proportions of metabolites of the 4–6-ring ACs from pyrogenic sources [19] than did fish exposed to weathered PBCO [16]. Conversely, the bile of fish exposed to weathered PBCO [16] contained much larger proportions of metabolites of alkylated naphthalenes, phenanthrenes and dibenzothiophenes than were present in the urban fish [19]. Furthermore, the identification in bile of relatively large proportions of metabolites of the alkylated dibenzothiophenes that are recognized as marker compounds for PBCO [16,21] provided evidence for the exposure of the fish to PBCO [16]. Thus, the source of contamination suggested from the HPLC chromatogram can often be substantiated by examining the relative proportions of AC metabolites determined by the GC–MS analysis of bile.

4.3. HPLC patterns in sediment

HPLC chromatographic patterns of sediment extracts can also be used to suggest a possible source of AC contamination, *e.g.*, crude oil or pyrogenic ACs from urban sites. These chromatographic patterns are less variable than those from bile, because sediment screening measures the ACs themselves, with none of the confounding factors found in the bile chromatograms due to species-specific differences in degree of metabolism or excretion of metabolites. In addition, retention times in size-exclusion chromatography are stable over extended periods of time [20]. Thus, HPLC chromatograms from sediment are easier to interpret than those from bile. For example, a chromatogram of sediment from Herring Bay, a site in Prince William Sound that was in the path of the Exxon Valdez spill, showed a pattern very different from those of sediments from urban sites (South San Diego Bay or Hunters Point in San Francisco Bay) or the nonurban reference site of Bodega Bay (Fig. 2). The chromatogram of the Herring Bay sediment showed a continuous broad peak at BaP wavelengths and only a small portion of this peak was in the AC fraction (fraction after 8.2 min in which BaP and other ACs elute). In contrast, the chromatograms of the urban sediments exhibited the continuous peak before 8.2 min, but also had several well-defined peaks in the AC fraction (Fig. 2). In addition, the nonurban Bodega Bay sediment showed only low concentrations of contaminants by HPLC screening (Fig. 2).

When a particular contaminant source is suspected, *e.g.*, weathered PBCO in the sediment from Herring Bay, chromatograms can be recorded at several wavelengths and compared to those from the suspected source (Fig. 4) [20]. For example, the chro-

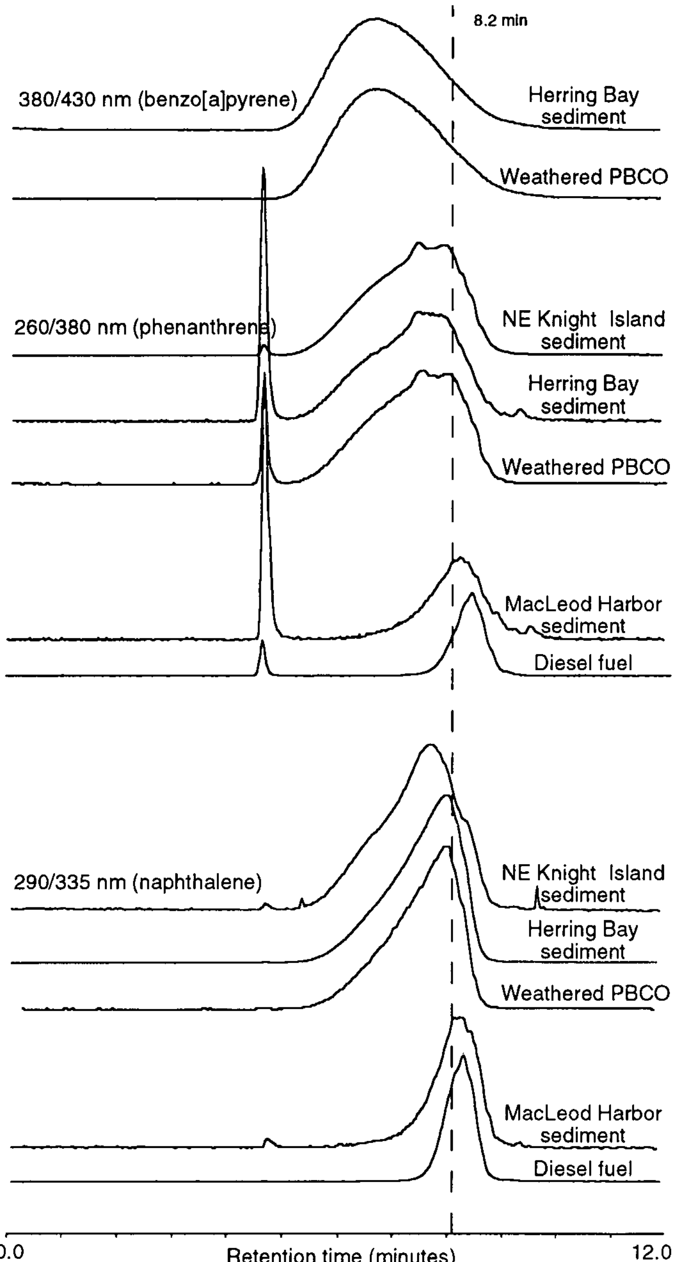

Fig. 4. Chromatograms from the HPLC–fluorescence screening of intertidal sediment from Prince William Sound: from two oiled sites (Herring Bay and Knight Island); from an unoiled site (MacLeod Harbor); and from some possible sources of contamination in the marine environment (diesel fuel and weathered Prudhoe Bay crude oil). Fluorescence was recorded at benzo[*a*]pyrene, phenanthrene and naphthalene wavelengths. To facilitate visual comparisons between chromatographic patterns of the Prince William Sound sediments and possible contaminant sources, the chromatograms in this figure have been electronically adjusted to the same height. Integration mark is as described in Fig. 2. The peak at 4.5 min (phenanthrene wavelengths) is the internal standard.

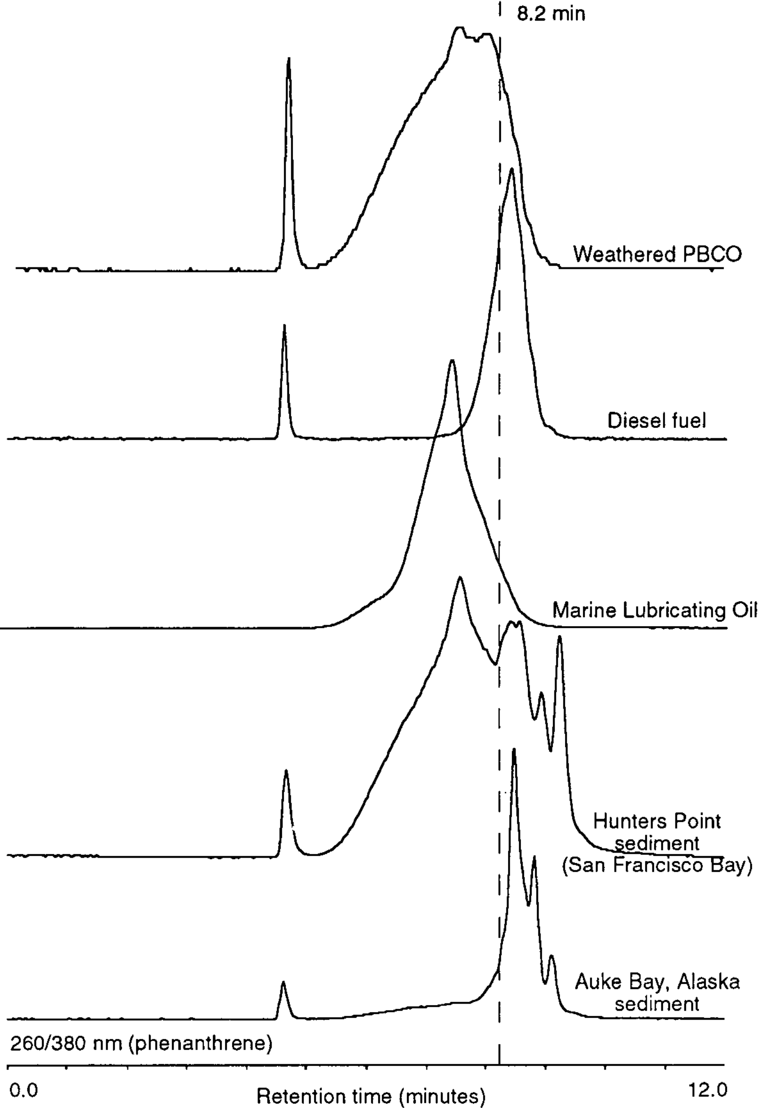

Fig. 5. Chromatograms from the HPLC–fluorescence screening of possible sources of contamination in the marine environment: weathered Prudhoe Bay crude oil (PBCO); diesel fuel; marine lubricating oil; and "urban" contamination in sediments from Hunters Point in San Francisco Bay, CA, USA and from Auke Bay, AK, USA. Fluorescence was recorded at phenanthrene wavelengths. Integration mark and internal standard peak are as described in Fig. 3.

matograms of Herring Bay and Knight Island sediments were nearly superimposable with those from weathered PBCO at all recorded wavelengths (Fig. 4). Furthermore, the chromatographic patterns (molecular size distributions) from the Herring Bay and Knight Island sediments were very different from those of other sources of contamination (*e.g.*, diesel fuel or marine lubricating oil) that might be found in Alaskan sediments (Fig. 5) [20]. Therefore, the HPLC chromatographic pattern provided evidence that PBCO was the likely contaminant source in certain Alaskan sediments. In contrast, results from screening sediment from MacLeod Harbor, a site not in the direct path of the spill, revealed low

concentrations of ACs and an HPLC chromatographic pattern that resembled that of diesel fuel (Fig. 4). Furthermore, chromatograms of some urban sediments showed evidence of both pyrogenic and petrogenic contaminants. For example, in the chromatogram of sediment from South San Diego Bay (BaP wavelengths; Fig. 2), the portion eluting before the AC fraction (<8.2 min) was similar to that from Herring Bay (Fig. 2) and weathered PBCO (Fig. 4), whereas several large peaks were detected in the fraction (> 8.2 min) where ACs, such as the 4–6-ring pyrogenic ACs, elute [9]. This chromatographic pattern is consistent with more than one source of contamination. Thus, contamination from multiple sources in urban estuaries may be more common than contamination from a single source. Contamination from a single source is likely only when that source (*e.g.*, crude oil or diesel fuel) is spilled into a relatively pristine environment, such as Alaska.

4.4. *Relative proportions of aromatic compounds in sediment by GC–MS*

To substantiate the identification of a contaminant source suggested by HPLC screening of sediment, individual ACs can be determined by GC–MS and the identities and proportions of the ACs in the sediment can be compared to similar characteristics of the probable sources. For example, when the AC fraction (> 8.2 min; Fig. 4) of the Herring Bay sediment was collected and analyzed by GC–MS, high proportions of 2–3-ring ACs typical of petroleum contamination, *i.e.*, alkylated naphthalenes, phenanthrenes and dibenzothiophenes were found [20]. The alkylated dibenzothiophenes have been identified as marker compounds for North Slope crude oils, such as PBCO [20,21]; hence, their presence in relatively large proportions in the Herring Bay sediment is an important factor in confirming PBCO as the contaminant source [20]. Furthermore, the pyrogenic 4–6-ring ACs were present in low or non-detectable amounts in this sediment, due to the low proportions of these ACs in PBCO [21]. In addition, the results from screening the MacLeod Harbor sediment were confirmed by GC–MS analysis. Although measurable concentrations of alkylated naphthalenes and phenanthrenes were found in MacLeod Harbor sediments, the concentrations of the alkylated dibenzothiophenes that are marker compounds for PBCO were below detectable limits [20]. These results are consistent with a diesel fuel —one refined from a crude oil low in dibenzothiophenes— as the contaminant source, not surprising in an area with commercial and recreational vessel traffic.

The AC fractions from sediments that exhibit "urban" HPLC chromatographic patterns, *e.g.*, South San Diego Bay and Hunters Point in San Francisco Bay, were also characterized by GC–MS [9]. High concentrations of several pyrogenic ACs (4–6 rings, *e.g.*, fluoranthene, pyrene and benzo[*a*]pyrene) that fluoresce at BaP wavelengths were found, as expected from the large peaks comprising the AC fraction of the chromatograms at BaP wavelengths (Fig. 2). In the reference sediment from Bodega Bay (north of San Francisco), GC–MS analyses found only low or non-detectable concentrations of ACs [9]. Thus, the presence of high proportions of pyrogenic ACs in the South San Diego Bay and Hunters Point sediments helped to distinguish these urban sediments from those of sediments contaminated primarily by petrogenic sources, such as crude oil or diesel fuel.

5. HPLC SCREENING OF BILE: URBAN SITES

5.1. *Determining the exposure of adult fish to aromatic compounds*

Bile screening was originally developed as a tool to rapidly estimate concentrations of metabolites resulting from the uptake and transformation of ACs by English sole exposed to environmental contaminants in Puget Sound [13,14]. These studies also revealed significant correlations between concentrations of AC metabolites in bile and prevalences of hepatic diseases (*e.g.*, neoplasms and megalocytic hepatosis) in fish. Because of this link between AC metabolites in bile and possible biological effects to the fish, bile screening has been used as a monitoring tool in the NS&T Program. This multiyear program is designed to measure concentrations of chemical contaminants in sediments and in tissues of bottom-dwelling fish species at selected sites in USA coastal areas and to determine prevalences of diseases as related to chemical contaminants in these fish. For example, tissues of white croaker

(*Genyonemus lineatus*) from several sites in the Los Angeles area contained much higher concentrations of organic contaminants, including metabolites in bile, than did fish from a non-urban reference site [22]. In addition, a number of liver lesions (*e.g.*, neoplasms, basophilic foci of hepatocellular alteration and megalocytic hepatosis) were detected in croaker from the Los Angeles sites, but only a low prevalence of a single lesion type (nuclear pleomorphism) was found at the reference site [22]. Other NS&T sites, including certain sites in San Diego Bay, San Francisco Bay and Puget Sound, were also found to have a variety of fish species with high concentrations of AC metabolites in bile [23,24] and with various pollution-associated pathological conditions (*e.g.*, liver lesions or fin erosion) [23].

Many of the ACs detected in the NS&T fish are known to be carcinogenic in fish and mammals, but these compounds exert their effect only after metabolic activation [12]. Hence, the presence of metabolites of ACs in fish bile can be used as an indicator of exposure of these animals to potential carcinogens that may have been activated through metabolism. As a result, the statistical correlation between AC metabolites in bile of the fish and tumors and other diseases in their livers demonstrates that bile screening is an indicator of potential risk factors for environmentally caused diseases. Moreover, the simplicity of the bile screening method is particularly important, because as Melancon *et al.* [25] conclude, "Techniques that measure important metabolites simply and at low cost are obviously desirable in monitoring programs."

5.2. *Determining the exposure of juvenile salmon to aromatic compounds*

In a pilot study conducted to assess the potential for the uptake of toxic chemicals by downstream migrant juvenile chinook salmon (*Oncorhynchus tshawytscha*) in the Duwamish Waterway (an urban estuary in Seattle, WA, USA), concentrations of AC metabolites in bile were estimated by screening to determine the uptake of ACs by the salmon [26]. Although the potential exposure of benthic and sedentary species to contaminants can be estimated from concentrations of contaminants in sediments, for migratory species, *e.g.*, salmon or marine mammals, bile screening is the only means of rapidly determining exposure to ACs. The mean concentrations in bile of AC metabolites that fluoresce at BaP wavelengths were significantly higher in salmon from the Duwamish Waterway than in salmon from a reference estuary or the hatchery (Table 3). Concentrations of other toxic compounds, such as PCBs, were also determined in the fish and also found to be higher in the salmon from the urban estuary [26]. This study demonstrated that the juvenile salmon bioaccumulated substantial concentrations of ACs and other toxic chemicals during their brief residency in the urban estuary. However, possible effects of exposure to these chemicals on the health and survival of the salmon are unknown. Consequently, a more detailed, multiyear study is currently underway.

TABLE 3

CONCENTRATIONS OF FLUORESCENT AROMATIC COMPOUNDS, MEASURED AT 380/430 nm (BENZO[*a*]PYRENE WAVELENGTHS), IN BILE OF JUVENILE SALMON CAPTURED FROM A HATCHERY AND FROM URBAN AND NON-URBAN WATERWAYS [26]

Site	Concentrations of fluorescent aromatic compounds, mean ± S.D. (ng/g bile)
Duwamish Waterway	1300 ± 430[a], 6 composites (n = 17, 17, 18, 19, 22, 27)
Kalama Creek Hatchery	150 ± 90, 3 composites (n = 10, 10, 10)
Nisqually River (reference)	50 ± 10, 3 composites (n = 20, 21, 21)

[a] Significantly different ($p < 0.05$) from the hatchery or reference fish.

6. HPLC SCREENING OF BILE: OIL SPILL SITES

6.1. Columbia River oil spill

The spill of a relatively small amount of high-density residual and industrial oil into the Columbia River provided an opportunity to test the bile screening method with fish exposed to oil in the field. Within five days after the spill, mean concentrations of metabolites in the bile of white sturgeon (*Acipenser transmontanus*) captured 57 miles (1 mile = 1609 m) downstream from the spill were significantly higher than those of sturgeon captured upriver from the spill site [27]. The bile screening method provided a rapid and cost-effective means of evaluating, in part, the impact of an oil spill on fish. Thus, this study provided a basis for using the bile screening method in assessing the exposure of fish and marine mammals to oil from the Exxon Valdez spill in Prince William Sound, Alaska in March, 1989.

6.2. Monitoring for PBCO in commercial fish species after the Exxon Valdez spill

Bile and tissue samples from many species of fish were collected and analyzed after the Exxon Valdez oil spill [28,29]. Clearly, the spill raised immediate concerns about the possible exposure of commercially important fish species to petroleum hydrocarbons. The halibut season was due to open on May 15, 1989, less than two months post spill, so NOAA and the International Halibut Commission immediately conducted a joint survey to determine if Pacific halibut (*Hippoglossus stenolepsis*) were being exposed to the petroleum hydrocarbons from the oil spill [30]. Bile from halibut (n = 81), screened for metabolites of petroleum-related ACs, had mean concentrations of AC metabolites that were similar to those from fish sampled before the spill from a pristine site. In addition, GC–MS analyses of edible tissue from these fish revealed concentrations of ACs that were below detection limits. Thus, the finding from the analyses of both bile and edible flesh indicated that these halibut were not exposed to appreciable concentrations of crude oil.

Native Alaskans were also concerned that their seafood might be contaminated by the crude oil after the Exxon Valdez spill. NOAA, in cooperation with Exxon and the Alaska Department of Fish and Game, analyzed edible flesh from fish and shellfish collected from native fishing grounds [31]. The principal use of bile screening for this study was to assess exposure of the fish to ACs and to set priorities for GC–MS analyses of the corresponding edible flesh. Screening analyses found elevated concentrations of metabolites of petroleum ACs in the bile of several fish species from sites in the path of the spill [31]. In contrast, results from tissue screening analyses, conducted on edible flesh from several of the fish having the highest concentrations of bile metabolites, found that concentrations of AC metabolites were near or below detectable limits [28]. Similarly, GC–MS analyses of the edible flesh showed relatively low concentrations of ACs (the majority of the samples had concentrations < 30 ng/g), even in the fish that had the highest concentrations of AC metabolites in bile [31]. These relatively low concentrations of petroleum-related ACs in edible tissue are consistent with the efficient metabolism by the fish of most ACs and the elimination of metabolites, predominantly via the bile [17].

Information about the exposure to PBCO of another commercially important fish species was obtained about one year after the spill. Samples from pollock, a species that feeds in the water column, were collected during an annual survey conducted by the National Marine Fisheries Service in February and March 1990 at sites along the path of the spill [28,29]. Pollock were tested for exposure to petroleum-related contaminants by screening for AC metabolites in their bile. The PBCO concentrations in bile of pollock from 9 of the 14 sites surveyed, including all the sites within Prince William Sound, were significantly different (higher) from those in bile of pollock captured from a reference site in Southeastern Alaska (Table 4).

Because bile is a major excretory route in fish for the elimination of metabolites of lipophilic contaminants, metabolites of ACs in bile are considered a short-term indicator of exposure [12,32]. Therefore, the AC metabolites found in bile of the pollock were an indication that these fish had been exposed to ACs near the time of their capture, suggesting that crude oil was still present in the Alaskan waters where the pollock resided nearly one year after the spill. Moreover, when mean PBCO concentrations in pollock from each site sampled in

TABLE 4

CONCENTRATIONS OF FLUORESCENT AROMATIC COMPOUNDS, MEASURED AT 260/380 nm (PHENANTHRENE WAVELENGTHS), IN BILE OF POLLOCK CAPTURED IN PRINCE WILLIAM SOUND AND SHELIKOF STRAIT IN 1990 AND 1991.

Site[a]	Concentrations of fluorescent aromatic compounds, mean ± S.D. (ng/mg protein[b])				Distance from spill site (nautical miles, 1 nautic mile = 1852 m)
	1990	*n*	1991	*n*	
Mummy Island	7600 ± 4700[c]	20	1200 ± 350	13	54
Naked Island	6400 ± 3500[c]	17	1200 ± 700	5	20
Point Bazil	3100 ± 1800[c]	20	1700 ± 600	14	64
Goose Island	2800 ± 1900[c]	20	NS[d]		9
Cape Ugat	2300 ± 1200[c]	10	NS		295
Cape Uyak	2300 ± 920[c]	10	NS		315
Cape Paramanof	2300 ± 870[c]	10	NS		260
Tugidak Island	2100 ± 760[c]	8	NS		440
Kinak Bay	2000 ± 600[c]	13	NS		300
Cape Kekurnoi	2000 ± 770	9	NS		340
Portage Bay	1800 ± 550	10	1200 ± 410	10	380
Malina Point	1800 ± 810	14	NS		270
Sanak Island	1600 ± 620	20	1400 ± 400	10	710
Chirikof Island	1300 ± 530	10	NS		500
Port Gravina	NS		2300 ± 670[c]	15	NA[e]
Bay of Isles	NS		2200 ± 1100[c]	8	NA
Hogan Bay	NS		2100 ± 980[c]	14	NA
Port Fidalgo	NS		2100 ± 740[c]	10	NA
Naked Island East	NS		1400 ± 770	6	NA
Bogoslof	NS		1400 ± 140	10	NA
Sutwick Island	NS		1300 ± 790	11	NA
Trinity Islands	NS		1300 ± 300	10	NA
Kuliak Bay	NS		1200 ± 550	12	NA
Cape Ikolik	NS		1200 ± 300	11	NA
Katmai Bay	NS		1100 ± 270	10	NA
Sturgeon Head	NS		1100 ± 290	11	NA
Uganik Island	NS		1000 ± 290	10	NA
Reference site	1300 ± 550	14	1500 ± 240	13	out of spill area

[a] Sites located in Prince William Sound are shown in italics.
[b] The concentrations of fluorescent aromatic compounds have been normalized to the amount of bile protein.
[c] Significantly different by analysis of variance from the reference site.
[d] NS = Not sampled.
[e] NA = Not applicable.

1990 were compared to the distance of each site from the origin of the spill (Table 4), a very good inverse correlation resulted ($p \leqslant 0.001$). This correlation may be interpreted like a dose-response relationship, *i.e.*, as the distance from the origin of the spill increased, the concentrations of PBCO in the pollock decreased proportionally.

HPLC patterns indicative of metabolites of petroleum-related ACs were found in pollock with elevated concentrations of AC metabolites in bile (*e.g.*, compare the pollock from Prince William Sound with the PBCO-injected fish, Fig. 3). Furthermore, metabolites of petroleum-related ACs, *e.g.*, alkylated naphthols, phenanthrols and dibenzothiophenol marker compounds, were found in bile of the pollock by GC–MS [16], thus confirming the exposure of these fish to crude oil. However, individual ACs measured by GC–MS in fish muscle were present

only in low or non-detectable concentrations [28]. These data for fish muscle agree with results from previous studies [15,31] (discussed above); even in those fish that had the highest concentrations of metabolites in bile, AC concentrations in muscle were very low.

Because the pollock captured at several sites in 1990 had elevated concentrations of metabolites of petroleum ACs in bile, pollock were sampled again during the 1991 annual survey to determine if exposure to PBCO was continuing. Five of the sites that were sampled in 1990 were also sampled in 1991. Concentrations of AC metabolites in bile of pollock from these sites were much lower in 1991 compared to 1990 (Table 4). Of the remaining sites sampled in 1991, pollock from four sites in Prince William Sound had concentrations of AC metabolites in bile that were significantly different (higher) from those in bile of pollock from the reference site (Table 4), but still were relatively low compared to the 1990 Prince William Sound sites [28,29]. Concentrations of AC metabolites in bile of pollock from the other 1991 sites were statistically indistinguishable from those in fish from the reference site. Thus, two years after the spill, bile from pollock from most sites had concentrations of AC metabolites at background levels, although fish from four sites within Prince William Sound still exhibited exposure to oil.

6.3. *Determining the exposure of marine mammals to PBCO*

Samples from several species of marine mammals, collected after the Exxon Valdez oil spill in June–August, 1989, were obtained from dead animals in the spill area, as well as from animals killed by native Alaskan subsistence hunters [33]. For example, harbor seals (*Phoca vitulina*) were substantially affected by exposure to petroleum-related ACs from the spilled crude oil. The harbor seals captured in Prince William Sound were found to have elevated concentrations of AC metabolites in bile (Table 5) compared to similar measurements in bile of harbor seals obtained from the spill area outside Prince William Sound and from Ketchikan, a site in Southeastern Alaska unaffected by the spill. Furthermore, the HPLC chromatograms of the seal bile were similar to those of the fish exposed to PBCO (Fig. 3). When a sample of hydrolyzed bile from a harbor seal was analyzed for individual metabolites by GC–MS, petroleum-related ACs, including the dibenzothiophenol marker compounds, were found in high proportions [33]. Therefore, harbor seals from Prince William Sound were exposed to PBCO and this exposure may have contributed to the deaths of some of these animals.

TABLE 5

CONCENTRATIONS OF FLUORESCENT AROMATIC COMPOUNDS, MEASURED AT 260/380 nm (PHENANTHRENE WAVELENGTHS) IN BILE OF HARBOR SEALS CAPTURED AFTER THE EXXON VALDEZ OIL SPILL

Site[a]	Concentrations of fluorescent aromatic compounds, mean ± S.D. (ng/mg protein[b])	
	1990	*n*
Bay of Isles	6700 ± 7900	4
Herring Bay	1400 ± 1700	6
Seal Island	1200 ± 470	2
West Amatuli Island	430	1
Agnes Island	290	1
Applegate Rocks	220	1
Perl Island	160	1
Ushagat Island	80 ± 13	2
Perenosa Bay	40	1
Big Fort Island	10	1
Ketchikan	70 ± 45	2

[a] Sites in italics are located in Prince William Sound.
[b] The concentrations of fluorescent aromatic compounds have been normalized to the amount of bile protein.

7. HPLC SCREENING OF SEDIMENTS: URBAN SITES

In a study of creosote contamination in the Elizabeth River (VA, USA), the HPLC method was used to screen sediments for ACs [34]. Concentrations of ACs in sediments were found to decrease with the distance of the sampling sites from the site of an old creosote manufacturing plant. In addition, an excellent correlation was found between summed concentrations of ACs in the sediments determined by GC–MS and those estimated by the HPLC screening method ($r = 0.92, p \leqslant 0.001, n = 6$ sites) [34], thus again demonstrating that sediment screening is a useful tool for rapidly assessing relative amounts of AC contaminants in sediments from polluted sites.

Our laboratory obtained some supposedly uncontaminated sediment from Auke Bay (near Juneau, AK, USA) for the purpose of conducting toxicity bioassays. To assure that the sediment was actually uncontaminated, replicate samples were analyzed by the HPLC screening method [20]. The HPLC pattern at phenanthrene wavelengths was suggestive of pyrogenic contamination, because the pattern was most similar to that of sediment from Hunters Point, a contaminated site in San Francisco Bay (Fig. 5). Moreover, the phenanthrene equivalents calculated for the Alaskan sediment were 17 000 ± 3300 ng/g ($n = 2$), a relatively high level of contamination compared to other sediments analyzed previously [20].

To substantiate this information, individual ACs were determined in the Alaskan sediment by GC–MS analyses. High concentrations of ACs, particularly the pyrogenic ACs, were found (sum of total ACs was 3700 ± 930 ng/g, wet mass, $n = 2$ and the sum of 4–6-ring ACs was 3500 ± 770 ng/g) [20]. Although this sediment had been collected from a site thought to be uncontaminated, the HPLC screening assay correctly predicted its high level of contaminants. As a result, another sediment sample was obtained for the bioassay study. Accordingly, the sediment screening method has proven to be a valuable and often-used tool in our research program.

8. HPLC SCREENING OF SEDIMENTS: SPILLED OIL

Following the Exxon Valdez spill, hundreds of sediment samples were collected to determine the degree and distribution of the oiling. Because analyzing all these samples by GC–MS would be excessively expensive and time-consuming, priorities for analyses needed to be set. Therefore, the HPLC screening method was used to determine concentrations of PBCO in more than 400 sediment samples from a large number of sites in the Exxon Valdez spill area and many were found to be contaminated by PBCO [20]. Formerly, information about oil contamination had to be obtained by GC–MS [35,36]. However, the expense of the GC–MS method limits the number of analyses that can be made. In contrast, sediment screening is a rapid and cost-effective tool with which to obtain data that can be used to plan the sampling of biota and to devise or evaluate site cleanup strategies. Furthermore, the screening method can increase the confidence in the results by providing sufficient data for adequate statistical analyses.

9. PROSPECTS FOR THE FUTURE OF SCREENING METHODS

One of the biggest challenges confronting environmental scientists today is to devise state-of-the-art techniques that are both accurate and practical. These methods must also be cost-effective, so that sufficient data can be collected to decrease the uncertainty in the conclusions while maintaining reasonable analytical costs. In addition, a rapid turnaround of analytical results is essential, so that environmental managers can evaluate the data and take action to solve the problem. In this regard, the utility and practicality of the HPLC bile and sediment screening analyses were recently demonstrated on board the NOAA research vessel Mt. Mitchel during the Arabian Gulf Project. Estimates of petroleum contamination in sediment and fish were available rapidly, allowing modification of the sampling strategy based on these results. In the future, miniaturization of instruments may allow such analyses to be conducted from a portable field laboratory without need for a support vessel or laboratory.

Future effort also needs to be expended on methods to rapidly determine the concentrations of coplanar PCBs, PCDDs, PCDFs and other toxic chlorinated compounds. These highly toxic compounds usually comprise only a small proportion of a total extract that also contains other, less toxic compounds, *e.g.*, the other (non-coplanar) PCBs, the DDTs, their breakdown products and chlorinated pesticides. Recent progress has been made toward developing rapid HPLC methods to separate the coplanar PCBs, PCDDs and PCDFs from coextracted compounds [37–39] and to screen for toxic PCBs in tissue samples [40]. Toxicity can then be estimated from the concentrations of these compounds via the "toxic equivalency factors" proposed by Safe [41]. However, these estimated toxicities need to be tested against a biological assay, *e.g.*, the rat hepatoma cell line bioassay, that accounts for possible interactions among the toxic chlorinated compounds and correlates with toxic

effects *in vivo*. A good correlation of the bioassay and the screening method would demonstrate the potential of the latter for providing data that rapidly and accurately estimate potential toxicity. As a result, the toxicities of a large number of samples could be determined by screening and then the results could be confirmed in selected samples by more laborious and expensive analytical or bioassay procedures.

Another expanding field —immunoassays based on monoclonal antibodies for detecting toxic substances such as the PCDDs— is in the preliminary stages of development [42]. Although the results show that monoclonal antibodies can be generated to small organic haptens such as PCDDs, the test needs to be made more specific. At present, certain of the PCDDs, PCDFs and one of the toxic PCBs are all recognized by the test. However, such an assay should be useful as a preliminary screening tool to determine those samples needing further characterization by standard analytical procedures. Ideally, future monoclonal antibodies will be selective and sensitive detectors of particular chemical contaminants and these procedures will be readily adapted as field assays.

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Review

Recent advances in the analysis of polycyclic aromatic hydrocarbons and fullerenes

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ABSTRACT

This paper highlights advances which have occurred in the last three years related to the analysis of polycyclic aromatic hydrocarbons (PAHs) and fullerenes by chromatographic and related techniques. The techniques reviewed include sample preparation, supercritical fluid extraction (SFE), gas chromatography, liquid chromatography, supercritical fluid chromatography, thin-layer chromatography and micellar electrokinetic capillary chromatography. Some of the most significant advances have occurred in the area of sample preparation, including the increased adoption of solid-phase extraction, supercritical fluid extraction, hyphenated chromatographic methods (*e.g.* LC–LC, LC–GC, etc.) and the chromatographic separation of fullerenes which did not exist on a macroscopic scale three years ago.

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1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) comprise the largest class of known chemical carcinogens, produced during the combustion, pyrolysis and pyrosynthesis of organic matter. PAHs are ubiquitous in air, water and soil, and their identification and determination continues to be an important analytical problem. No less than one hundred articles have appeared in each of the last three years relating to the analysis of PAHs, making it a formidable task to keep abreast of the current literature. In this review, we survey the recent literature relating to the chromatographic analysis of PAHs. We have attempted to highlight advances which have occurred in the last three years (*ca.* August, 1989–August, 1992) in chromatographic and related techniques and refer readers to more comprehen-

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sive reviews if more thorough background material is required [1,2]. We have also included analysis methods for the closely related family of carbon allotropes known collectively as fullerenes, which have only recently been isolated on a macroscopic scale. Due to their chemical similarities, PAHs (*e.g.* triphenylene) and fullerenes (*e.g.* C_{60}) can be analyzed by similar chromatographic methods as discussed later in this paper. To make this review more manageable, we have limited the scope to advancements in analytical methodologies related to chromatography and, therefore, studies focusing on the distribution, biotransformation, flux and spectroscopic properties of PAHs have been omitted.

2. ASTRONOMICAL ANALYSIS

PAHs are more ubiquitous than some readers may be aware of. In fact, it is now generally accepted that PAHs are present in interstellar medium and that they may significantly influence the chemistries and charge balance within interstellar environments. PAHs are believed to be responsible for so-called unidentified infrared emission features (UIRs) and possibly diffuse interstellar band (DIBs) observed in outer space. In fact, PAHs are probably more abundant than all other known interstellar polyatomic molecules combined [3]. Scores of articles have appeared recently, including studies of the possible origin of interstellar PAHs [4,5], their stability [6,7], their location [8], and some interesting chemical implications here on earth. For example, it has been suggested that interstellar PAHs on meteorites may represent the starting material for the synthesis of complex molecules including amino acids [9], and primitive pigments in the prebiotic environment [10]. A thorough discussion of this topic is beyond the scope of this paper and interested readers are referred to reviews of interstellar PAHs which have appeared recently such as ref. 11.

3. SAMPLE PREPARATION

The PAHs from solid environmental samples such as air particulates, soils and sediments are traditionally extracted by Soxhlet extraction or ultrasonication using a variety of organic solvents including acetone, benzene, toluene, methylene chloride, etc. These traditional methods are efficient for many samples; however, they often require large volumes of solvents, are time consuming, and yield incomplete recovery of higher-molecular-mass PAHs from materials on which they are strongly adsorbed (*i.e.* carbon black or coal fly ash). For these reasons, supercritical fluid extraction (SFE) has received considerable attention as an alternative to these classical methods as discussed later in this paper and in other papers in this issue. The recovery of PAHs from aqueous solutions has traditionally involved liquid–liquid extraction (LLE) methods with organics solvents (*i.e.* hexane, dichlormethane, chloroform, etc.). Since environmental samples generally contain interferents and trace amounts of PAHs of interest, concentration and clean-up procedures are usually required prior to the final chromatographic analysis. In many cases, the sample pretreatment procedure is the critical step in achieving reliable quantitative results.

PAH concentration and clean-up is increasingly being performed by solid-phase extraction (SPE). For preconcentration of PAHs from drinking water samples, best results were obtained for combined octadecylsilane (C_{18})/ammonia (NH_2) solid-phase cartridges, whereas the enrichment of PAHs from soil samples was best achieved with silica (Si)/cyano (CN) or C_{18}/CN combinations [12]. The choice of SPE sorbent type is often dictated by the chromatographic method to be subsequently used for PAH separation and identification. For example, a recent study showed that for the determination of PAHs in lake sediments, C_{18} and silica columns could be used to satisfactorily clean up extracts for subsequent HPLC analysis with fluorescence detection; however, they could not be used for gas chromatography (GC)–mass spectrometry (MS) for PAHs greater than chrysene due to interferences from aliphatic waxes. Fully activated silicic acid and neutral alumina columns were recommended [13]. A standard leaching test employing SPE with C_{18} packings has proven to be a fast reliable method for determining the PAH leachability from waste materials [14]. Florisil (SiO_2 and MgO) cartridges have yielded rapid and efficient recovery of PAHs for petroleum and sediment extracts [15]. Extraction and concentration of PAHs in oils was achieved by charge-transfer liquid chromatography on an improved tetrachlorophthalimidopropyl-bonded silica [16]. A quantitative procedure for the determina-

tion of PAHs in biomass tar has been described using SPE with aminopropylsilane packings [17]. Chromosorb T and XAD-2 have been compared for the *in situ* extraction of PAHs from fresh water and seawater. Neither sorbent was useful for PAHs with molecular masses less than that of phenanthrene due to low recoveries or PAH contaminants, and were comparable for the study of three-ring and higher PAHs [18]. Structures for some common PAHs mentioned in this review are given in Fig. 1. A convenient method for the separation and preconcentration of traces of PAHs in aqueous solutions has been achieved by adsorption on cobalt phthalocyanine and barium salts of sulphophthalocyanines followed by thermal desorption gas chromatography [19].

Numerous studies have dealt with the sampling procedure for airborne PAHs including the combination of a PTFE filter for particulates with polyurethane foam (PUF) for gaseous compounds [20]. A comparison of filter material used in high-volume sampling of PAHs revealed that glass-fibre filters yielded substantially higher concentrations of the lower-molecular-mass PAHs compared to PTFE filters [21]. A new technique for controllable vapor-phase deposition of PAHs onto particulate matter was developed to provide particle-bound radiolabeled substrate for use in metabolism and toxicological studies [22]. The sorption and desorption properties of PAH-coated ultrafine particles was studied with a photoelectric sensor revealing the following ranking for desorption temperatures: Aerosil 200 > aluminum oxide > carbon > sodium chloride [23]. The concentrations of PAHs adsorbed onto air particulates was found to correlate negatively with temperature during the sampling, due to volatilization, photodegradation and seasonal modifications of emissions from urban traffic [24]. Storage stability tests revealed that PAHs (fluoranthene/pyrene to coronene) from ambient air collected on PTFE/PUF can be stored in the dark in closed vessels at room temperature for up to 118 days without observable losses [25].

Details of the preparation and analysis of a new National Institute of Science and Technology (NIST) Standard Reference Material (SRM) 1941 have been described. SRM 1941, Organics in Marine Sediment, has been certified for concentrations

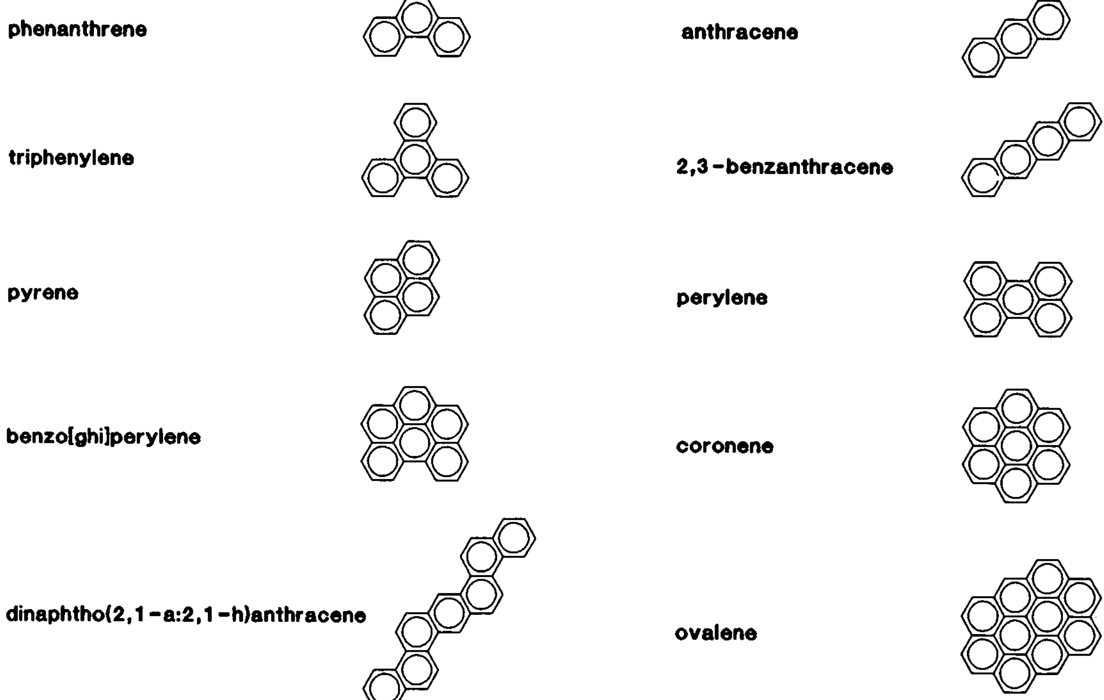

Fig. 1. Structures for some common PAHs mentioned in this review.

of 11 PAHs and provides non-certified values for 24 additional PAHs using results from GC with flame ionization detection, GC–MS and liquid chromatography with fluorescence detection [26]. Improvement in the precision and accuracy of the analytical procedures used by fourteen European laboratories should now permit the certification of coconut oil reference materials for low concentrations of PAHs [27]. A simplified version of an HPLC method has been described for the determination of PAH in suspended particles collected from small air volumes collected indoors, outdoors and in personal exposure measurements. A comparison of procedures for the determination of PAHs in low-volume samples has recently appeared [28]. A simple low-pressure liquid chromatography procedure has been developed for the isolation of PAHs from shale oil followed by GC analysis [29].

4. SUPERCRITICAL FLUID EXTRACTION AND CHROMATOGRAPHY

Supercritical fluid extraction (SFE) has proven to be a powerful alternative to conventional liquid extraction methods used in environmental analysis [30,21]. PAHs have been extracted directly from endogenous solid and liquid matrices, as well as trapped onto solid adsorbents with subsequent recovery by SFE [32,33]. One major advantage of SFE is the relative ease with which it can be coupled to chromatographic techniques, particularly GC and supercritical fluid chromatography (SFC). Hyphenated SFE–GC [34] and SFE–SFC techniques have recently been applied for the determination of PAHs from environmental samples [35,36].

Carbon dioxide is the primary fluid used in most SFE applications because it has low critical points (critical temperature 31.3°C, cricital pressure 1070 p.s.i., 1 p.s.i. = 6894.76 Pa), is non-toxic, non-flammable, odorless, readily available in high purity, inexpensive, and eliminates solvent waste disposal problems. Unfortunately, the non-polar nature of carbon dioxide has hindered its application for the recovery of higher molecular PAHs or those strongly adsorbed to (or trapped in) the environmental matrix. Workers have strived to overcome this limitation in recent years. Alternative fluids such as N_2O and $CHClF_2$ (Freon-22) yield higher recovery of PAHs from petroleum waste sludge and railroad bed soil, compared to CO_2 [37]. Alternatively, the use of organic solvent modifiers (*i.e.* methanol) or *in situ* chemical derivitization has been shown to improve the recovery of PAHs while still employing the preferred supercritical fluid, carbon dioxide [38]. Other studies have focused on optimizing the major controllable SFE variables and minimizing problems including restrictor plugging, particularly when extracting high-molecular-mass PAHs or employing samples with a high sulfur content. One approach to minimizing restrictor blocking employed a copper scavenger column placed after the sample cell. By this technique, SFE of PAHs was accomplished for high-sulfur-content samples without restrictor blocking [39]. Restrictor plugging while extracting PAHs has also been minimized by nebulizing an organic solvent with the restrictor effluent or by simply heating the restrictor from 50 to 200°C, depending on the analyte and sample matrix [40].

A model for dynamic SFE has been proposed and applied to the SFE of the PAH, phenanthrene, from railroad-bed soil with generally good agreement [41]. Models such as this are useful as they provide an extrapolation method for obtaining quantitative analytical extractions in the shortest analysis time. A dynamic tracer response technique has been applied for simultaneous measurement of equilibrium and rate parameters for the dynamic extraction of analytes from solid matrices. The technique has allowed adsorption equilibrium constants, effective diffusivities and axial dispersion coefficients to be determined for the system naphthalene–alumina–supercritical CO_2 [42]. We have reported the measurable effect of the extraction vessel dimensions (length × I.D.) on the elution of PAHs from octadecylsilane SPE sorbents [43], and the relative effect compare to the two major controllable variables; namely, temperature and density [44]. These results from SPE sorbents differ from those seen for the SFE of PAHs directly from environmental solids where no effect has been observed [45]. More thorough discussions of the effect of SFE variables and comparisons for different SPE matrix/analyte types have recently been published [46,47].

The development of SFE for extending the molecular mass range of PAHs normally separated by

GC continues. The modification of a GC–MS to SFC–MS mode has been described and used to separate PAHs with molecular masses up to 532 [48]. The retention behavior of PAHs in SFC for various stationary phases has been shown to be controlled by molecular size as in liquid chromatography, but also influenced by additional parameters including solute dipole moments, solubilities and volatility [49]. A molecular theory of chromatography based on mean-field statistical thermodynamics has been developed to describe the partitioning of blocklike molecules, such as PAHs, between an isotropic mobile phase and an anisotropic stationary phase. The theory was qualitatively applied to the interpretation and analysis of experimental data in gas, liquid and supercritical fluid chromatography [50]. The supercritical fluid retention of PAHs on a polymeric smectic phase has been compared to theoretical predictions using this molecular theory of chromatography with encouraging results [51]. The potential for predicting the utility of SFE from existing SFC retention data has recently been addressed [52]. In addition, we have found that of numerous physical and molecular descriptors studied, the molecular connectivity correlates best with SFC retention data for normal- and reversed-phase systems. Table 1 lists various physical parameters and the molecular connectivity calculated for some common PAHs. The excellent correlation between molecular connectivity and the logarithm of the capacity factor for three different SFC systems is illustrated in Fig. 2. The correlation is linear for the reversed-phase

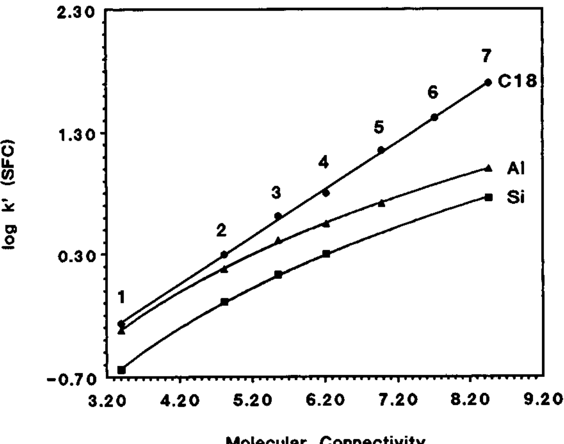

Fig. 2. Plot of log k' *versus* the molecular connectivity for PAHs. C18 = octadecyl column, 100°C, 300 atm, supercritical carbon dioxide mobile phase; Al = alumina column, 245°C, 47 atm, supercritical isopropanol mobile phase; Si = silica gel column, 260°C, 40 atm, supercritical ethanol–hexane (10:90) mobile phase. PAH identities given in Table 1.

octadecylsilane column and logarithmic for the normal-phase alumina and silica columns.

5. GAS CHROMATOGRAPHY

GC is the method of choice for high-resolution separation of complex PAH mixtures with moderate to low molecular masses. GC analysis of high-molecular mass PAHs (molecular mass exceeding 500) has been performed; however, these analyses

TABLE 1

PHYSICAL PARAMETERS FOR SOME COMMON POLYCYCLIC AROMATIC HYDROCARBONS

No.	Name	Molecular mass	Fused ring number	Melting point (°C)	Boiling point (°C)	van der Waals volume (Å^3)	Average molecular polarizability	Molecular connectivity (χ)
1	Naphthalene	128.17	2	81	218	127.1	17.48	3.40
2	Anthracene	178.23	3	217	340	170.0	25.93	4.81
3	Pyrene	202.26	4	150	404 (S)[a]	185.5	29.34	5.56
4	2,3-Benzanthracene	228.29	4	347	380 (S)	213.9	32.86	6.22
5	Perylene	252.32	5	274	340 (S)	229.0	38.84	6.98
6	Benzo[*ghi*]perylene	276.34	6	278	510 (S)	243.8	41.31	7.72
7	Coronene	300.36	7	427	525	259.2	42.50	8.46

[a] S = sublimes.

have traditionally involved very short columns with correspondingly low resolution. Improved phases for high-temperature use continue their development and will allow even higher-molecular-mass PAHs to be separated while maintaining high resolution. A recent comparison of four high-temperature GC columns illustrated their utility for the high-resolution separation of PAHs with a molecular mass of 328 with seven-ring PAHs including dinaphtho(2,1-*a*:2.1-*h*)anthracene eluting in less than 35 min [53]. The utility of selective liquid crystalline phases has been demonstrated for the determination of bioconcentration factors of PAHs in polychaete worms [54]. Another recent study praised the long column lifetime (more than six years) and short analysis time (*ca.* 15 min) of a liquid-crystalline stationary phase for the analysis of PAHs in carbochemical products [55]. A double internal standard procedure has been described to increase the precision and accuracy of PAH determinations by GC [56].

Several recent studies have focused on relationships between GC retention of PAHs and molecular properties. Regularities of GC retention behavior have been described by structural models containing Van der Waals volume and molecular connectivity indices of different levels depending on the class of PAHs [57]. The heats of adsorption for PAHs on macroporous silicas has been studied by GC in the range 80–200°C [58]. Investigation of the relationship between GC retention indexes and computer-calculated physical properties of PAHs revealed that molecular polarizability was the most important property [59]. A study of the relationship between GC retention and thermal reactivity for PAHs in coal tar pitch indicates that, generally, those retained on OV-1701 more strongly than on SE-54 stationary phase are more thermally stable [60].

Although flame ionization detection and MS remain the methods of choice for PAH detection, other methods continue to show promise. GC–matrix isolation infrared spectrometry (MI-IR) can identify PAHs difficult to distinguish by electron impact MS [61]. The GC–Fourier transform IR spectra of 33 PAHs have been measured and interpreted [62]. A highly sensitive method for determining PAHs using GC–electron-capture detection after derivatisation with bromine has been presented [63]. Other selective detectors, including the photoionization detector, continue to find application particularly when attempting to eliminate interferences such as aliphatic hydrocarbons [64]. Analysis techniques for PAHs by GC and GC/MS continue their development. A complete method for the determination of PAHs in soil by GC has been described [65]. Thermal desorption GC–MS has been applied to the analysis of PAHs in contaminated soils [66,67]. The importance of solvent choice and initial column temperature has been investigated for PAH determination by GC–MS with splitless injection [68]. The use of toluene and xylenes gave enhanced signals up to 100 times greater compared to other solvents for the higher-molecular-mass PAHs through benzo[*ghi*]perylene. Complete analytical methodologies including GC–MS analysis have recently been described for the determination of PAHs in sediments [69], in glass manufacturing oils [70], in soils [71], and in soot produced by combustion of plastics and wood [72]. A recent method for the analysis of PAHs in vegetable oils and fish includes a gel permeation chromatography clean-up step prior to final analysis by GC–MS [73].

6. LIQUID CHROMATOGRAPHY

Since its inception more than twenty years ago, high-performance liquid chromatography (HPLC) has been applied to the separation of PAHs. Although HPLC still cannot compete with GC in terms of high efficiency and short analysis times, it does offer numerous advantages, including very sensitive and selective detectors and the ability to be used as a fractionation method for other chromatographic or spectroscopic techniques. The application of HPLC for PAH fractionation has become very popular due to its high efficiency, ease of automation and potential for column switching techniques and on-line coupling with other techniques including gas chromatography. The recoveries of PAHs from a soot sample were compared for ultrasonic ether, Soxhlet toluene and Soxhlet extraction with liquid CO_2 followed by HPLC fractionation [74]. The results showed liquid CO_2 Soxhlet extraction to be superior for lower-molecular-mass PAHs (to chrysene). A routine method for the analysis of mononitro-PAHs in environmental samples has been developed based on micro-scale liquid–liquid

partition (dimethylformamide–cyclohexane) and silica column HPLC fractionation prior to GC–electron-capture detection and GC–MS [75].

Techniques incorporating HPLC fractionation have gained in popularity in recent years. HPLC fractionation followed by GC analysis has been applied to the determination of PAHs in urban street dusts with primary components found to range from phenanthrene (three aromatic rings) to benzo[*ghi*]perylene (six aromatic rings) [76]. A column-switching technique utilizing a silica gel and an aminosilane-bonded silica gel column has been used to separate PAHs in lubricating oil base stocks into compound class fractions followed by GC–MS analysis [77]. A similar HPLC column-switching technique with silica gel and aminosilane-bonded silica gel columns has been used to fractionate monomethylated PAHs from heavy oil followed by GC analysis [78]. Column-switching HPLC techniques have also been developed for the analysis of PAHs in petroleum products [79] and the group separation of PAHs and nitrogen-containing PAHs [80]. A fully automated column switching HPLC method has been developed for the determination of 1-hydroxypyrene in urine of subjects exposed to PAHs [81]. A new on-line concentrator has been developed and applied to the analysis of PAHs in soot by on-line HPLC–GC [82]. On-line HPLC fractionation–GC analysis has also been applied to the separation and identification of PAHs in heavy oil [83]. On-line LC–GC–MS has been demonstrated for the analysis of PAHs in vegetable oils with detection limits down to 1 pg with selective ion monitoring [84].

The most popular method of PAH separation is reversed-phase HPLC with octadecylsilica phases dominating. Notable exceptions include anthyrl-modified silica phases used to separate PAHs and nitro-PAHs [85], and phenyl-modified silica gel column used for improved separation of ^{32}P-labelled nucleoside 3′,5′-bisphosphate adducts of PAHs [86]. Multidentate phenyl-bonded phases have been shown to provide higher non-planarity recognition of PAHs than that typically seen for octadecylsilica phases [87]. Reversed-phase HPLC provides unique selectivity for the separation of PAH isomers and particularly alkyl-substituted PAHs. Anomalous retention behavior of methyl-substituted PAHs on polymeric C_{18} phases was found to be related to the non-planarity of the PAHs due to the presence of the methyl group in the so-called "bay-region" of the PAH structure [88]. Microcolumn C_{18} HPLC with 200 000 theoretical plates was used to separate a standard mixture containing 16 PAHs employing selective fluorescence quenching [89].

Numerous standard methods for the analysis of PAHs employing C_{18} columns have recently appeared including the determination of PAHs in air particulate samples [90], diesel soot [91], biomass emissions [92], mineral waters [93], sea mussels [94] and anthracene cake [95]. A variety of applications have involved the separation and detection of PAH metabolites. The determination of 1-hydroxypyrene in human urine has been developed as an indicator of exposure to PAHs [96,97]. Similarly, hydroxyphenanthrene has been detected after intake of PAHs [98]. Details of the metabolism [99], as well as the HPLC separation of nitro-PAHs, has been described [100]. A combination of both reversed- and normal-phase HPLC was used to provide efficient separation of the ring-oxidized derivatives of nitro-PAHs. A method using three C_{18} columns in tandem has been described for the separation of fish biliary PAH metabolites [101]. Numerous additional methods employing C_{18} HPLC separation have been developed for use in a variety of applications, including the study of PAHs originating from pan fry cooking of meat [102], woodburning [103] and sewage sludge-amended agricultural soil [104]. A method for derivatizing PAHs to quinones for C_{18} HPLC with selective electrochemical detection has been applied to the detection of selective PAHs in tap water and motor oil [105].

The retention mechanism in reversed-phase liquid chromatography for large PAHs has been investigated by Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy and differential scanning calorimetry. The results indicate that a change in mobile phase from methanol to dichloromethane induces further non-planarity in non-planar solutes; whereas, increases in column temperature drastically change the structure of the stationary phase from solid-like to liquid-like, with subsequent losses in planarity recognition [106]. The use of subambient temperatures (*ie.* 0 to -20°C) can significantly enhance shape selectivity of PAHs for polymeric C_{18} phases resulting in a phase with liquid crystal-like retention properties

[107]. Retention characteristics of nitrated PAHs on C_{18} columns demonstrated a linear dependence of logarithm of the capacity factor *versus* both the organic modifier concentration and the reciprocal of the absolute column temperature, allowing thermodynamic variables to be evaluated [108]. The retention in non-aqueous reversed-phase HPLC has been studied with the use of large PAHs and correlated to the amount of red shift in the fluorescence spectra for 11 common HPLC solvents [109]. The elution order of 10 PAHs up to coronene on C_{18} columns could not be satisfactorily explained with retention models based on molecular weight and length to breadth ratio alone, but improvement was made by taking into account the effects of intramolecular steric strain and the resulting degree of non-planarity [110]. The separation of large PAHs (benzo[*ghi*]perylene to ovalene) from a diesel particulate extract by reversed-phase HPLC with photodiode array detection revealed that all of the PAHs of six or more rings were highly fused, and no linear or non-alternate types were seen [111].

Although reversed-phase HPLC has dominated PAH separations, numerous normal-phase and specialty columns have been investigated to improve the selectivity of PAH separations. The separation of amino- and acetylamino-PAHs using six different reversed- and normal-phase columns has been compared with a Pirkle-type chiral phase exhibiting the best separation [112]. Derivatization of dihydrols of PAHs to O-methyl ethers has been shown to improve enantiomeric separation on Pirkle-type chiral stationary phases [113]. A liquid-crystal bonded phase has been shown to possess a planarity recognition power higher than that seen for typical polymeric C_{18} phases [114]. A comparison of several normal-phase packings demonstrated the advantage of a cyanopropyldimethyl-bonded silica gel packings for the group separation of chloro-added and chloro-substituted PAHs [115]. The effect of polar mobile phase modifiers on the retention of various classes of PAHs and the selectivity of their separation by normal-phase (silica gel) HPLC has been studied. The linear dependence of logarithm of capacity factor *versus* number of carbon atoms of the sorbate has been studied for unsubstituted PAHs monoalkylbenzene, and monoalkylnaphthalenes with different mobile phases [116]. The effect of the column material and mobile phase solvents on retention of PAHs in size-exclusion chromatography has been investigated. The use of sulfonated poly(divinylbenzene) packings has been shown to improve the performance of this technique whose application to PAH analysis has been problematic [117].

The incorporation of cyclodextrins has received attention recently as a way of improving the selectivity of PAH separations. The unique shape selectivity of a cyclodextrin phase towards eleven five-ring PAHs has been compared to C_{18} columns. Although the polymeric C_{18} demonstrated the highest overall selectivity for the PAHs isomers, it was suggested that the lack of retention dependence on molecular mass could be advantageous for the separation of PAHs of different molecular masses [118]. Bonded β- and γ-cyclodextrin phases showed relatively low efficiency but high selectivity, allowing the separation of different classes of isomeric compounds, including PAHs, which were difficult to separate on conventional LC stationary phases [119]. β-Cyclodextrin has also been used as a selective inclusion reagent in reverse-phase HPLC separation of PAHs. Molecular interaction of PAHs was determined to be mostly electrostatic and retention order strongly influenced by PAH molecular shape [120,121].

7. MISCELLANEOUS TECHNIQUES/APPLICATIONS

Thin-layer chromatography (TLC) is often used as a sample preparation method in PAH analysis, particularly when oil samples are involved. Standardized GC methods employing TLC separation have been developed for the determination of PAHs in petrochemical plants and oil refineries [122] and in olive oil [123]. The utility of urea-solubilized β-cyclodextrin TLC mobile phases was demonstrated by the resolution of a variety of compounds, including four PAHs, on a polyamide stationary phase [124]. The analysis of PAHs in spa waters has been accomplished by two-dimensional TLC using plates containing a mixture of aluminum oxide, silica gel and acetylated cellulose with detection by spectrofluorometry [125]. Laser mass spectrometry has been used to detect separated PAHs directly from polyamide TLC plates [126]. On-line coupled HPLC–TLC allowed for the successful separation of PAHs in marine sediment using a simple isocratic

microbore HPLC system and a conventional fluorescence spectrometer for detection [127].

The effectiveness of the anionic surfactant dodecylsulfate in solubilizing various PAHs has been studied for several different sediment and soil solid phases and aqueous phases [128]. The solubilization and partitioning of PAHs between micelle phase and aqueous phase has also been determined for non-ionic polyoxyethylene surfactants [129]. These data can be combined with additional information on surfactant and PAH sorption on soil/sediment to understand mechanisms affecting the behavior of PAHs in soil/sediment–water systems in which surfactants play a role in contaminant remediation or facilitated transport. The effect of varying sodium dodecylsulfate concentration and the introduction of γ-cyclodextrin in the micellar electrokinetic capillary chromatographic separation of PAHs investigated with an average theoretical plate number of more than 160 000 [130,131].

Direct experimental determinations of Henry's law constants for nine PAHs using a wetted-wall column technique and GC analysis compared favorably to other calculated and measured values [132]. Molecular topology has been used to model *n*-octanol–water partition coefficients of PAHs and their alkyl derivatives [133]. PAH partitioning mechanisms with activated sludge have been studied and an equation developed from thermodynamic principles to estimate lipid-waste water distribution coefficients [134]. PAH–chlorobutane and PAH–dichlorobutane equilibrium constants have been calculated from solubility data [135]. The potential health hazard from water chlorination due to formation of chlorinated PAH has been investigated. Chlorination of PAH contaminated humus poor lake water was found to result in formation of chlorinated derivative for some PAHs. However, in the presence of high concentrations of humic substances, no chlorinated PAHs were detected [136]. The extent of reaction of PAHs with hypochlorite has been shown to depend on the chlorine dose, the solution pH, the concentration of both compounds (high values studied) and the structures [137].

8. FULLERENES

Although PAHs are customarily regarded as planar structures, there are well known exceptions such as corannulene which has a bowl-shaped structure and forms one of the faces of C_{60}, buckminsterfullerene, named after Buckminster Fuller, the architect widely known for his invention and advocacy of the geodesic dome. The approximate structure of buckminsterfullerene is shown in Fig. 3 along with several PAHs (triphenylene, coronene and corannulene) for comparison. The motivation for the experiments which led to the hypothesis of the soccer ball-shapped C_{60} molecule [138] was an attempt to understand the mechanisms by which long-chain carbon molecules are formed in interstellar space and to explain spectral features of interstellar matter, including UIRs discussed earlier for PAHs [139–141]. In 1990, preparation of the first macroscopic quantities of C_{60}, the third allotropic and first molecular form of carbon, were reported [142]. Although not considered an environmental concern, fullerenes have recently been found in the geological environment [143] and may require the revision of our ideas about carbon structures, including graphite [144]. The first and most studied fullerenes, C_{60} and C_{70}, were extracted with benzene, toluene or carbon tetrachloride followed by column chromatography on alumina columns [145–

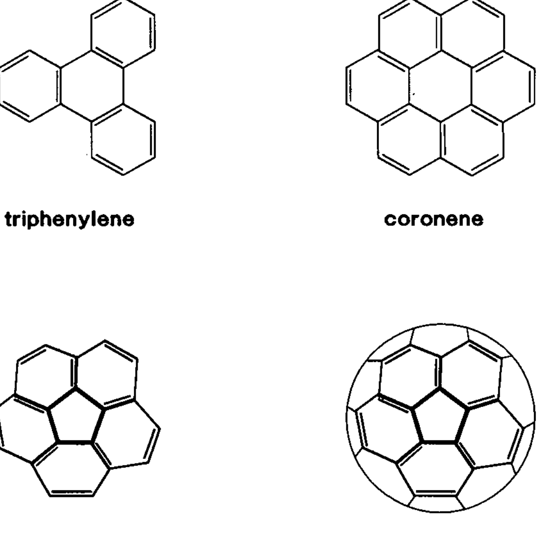

Fig. 3. The structures of the PAHs triphenylene, coronene and corannulene as well as an approximate structure for buckminsterfullerene (C_{60}).

148]. Larger fullerenes are not soluble in these solvents and alternative solvents, including quinoline and pyridine, have been used to extract very large fullerenes as large as C_{300} [149], and giant fullerenes as large as C_{500} [150].

Currently, column liquid chromatography using alumina columns is the method of choice for the separation C_{60} and C_{70}. Unfortunately, this classical technique is tedious, time consuming, and requires relatively large volumes of high-purity solvents, particularly when isolating C_{70}. Additionally, the reported yields of C_{60} by this method are currently limited to *ca.* 250 mg per column per day, or *ca.* 600 mg per column per day using improved procedures such as pre-adsorption onto alumina [151], and continuous column chromatography employing Kauffman columns [152]. The pace at which fullerene research can proceed will rely, in part, on the development of improved chromatographic methods for more rapid and efficient separation of fullerenes, as well as HPLC methods for testing the purity of fullerene extracts. HPLC techniques will also play an integral role in the separation and isolation of the higher fullerenes (*i.e.* C_{70}, C_{76}, C_{78}, C_{82}, C_{84}, C_{86}, C_{90}, C_{94}, etc.).

Due to the similarities between PAHs and fullerenes, many HPLC methods developed for PAH separation can be successfully adapted to the purification and analysis of fullerene mixtures. Good separation of C_{60} and C_{70} has been achieved on a dinitroanilinopropyl (DNAP) silica column with gradient elution from *n*-hexane–dichloromethane (50:50) over 35 min. The chromatographic characteristics of lower fullerenes on DNAP closely resembled PAHs with the retention of C_{60} nearly identical to that of triphenylene [153]. This early elution of C_{60} can be considered an extreme example of the effect of non-planarity on retention time in reversed-phase HPLC which is well documented with smaller molecules [154]. Although they have vastly different molecular masses, triphenylene (molecular mass 228.29) and buckminsterfullerene (molecular mass 720.67) have comparable diameters, as seen in Fig. 3. The relatively low solubility of the fullerenes in many of the HPLC solvent systems studied has limited column loadings and, therefore, the amount of material purified. The use of pure toluene, a solvent in which the fullerenes have good solubility, as a mobile phase has been used to isolate 23 mg of 93% pure C_{60} in less than 20 min [155]. The rapid isolation of C_{60} by HPLC methods should provide for higher overall daily yields (*i.e.* > 1 g/day) than that currently available by column liquid chromatography. Undoubtedly, techniques for the isolation of fullerenes higher than C_{60} will increasingly rely on HPLC.

A commercially available Pirkle-type phenylglycine-based semipreparative HPLC column was used to very selectively ($\alpha = 2.25$) separate C_{60} and C_{70} in less than 25 min using hexane as the mobile phase [156]. C_{60} and C_{70} were separated by reversed-phase HPLC with a C_{18} column using toluene–isopropanol (40:60) as eluent [157]. The separation of fullerenes has been studied on new multi-legged phenyl group bonded silica phases in capillary HPLC using *n*-hexane as the mobile phase. Two-legged biphenyl bonded silica phases provided the best separation of C_{60} and C_{70} [158]. Preparative-scale non-aqueous, reversed-phase separation of C_{60}, C_{70} and higher fullerenes has been accomplished using up to 68% dichloromethane in acetonitrile with C_{60} and C_{70} eluting in less than 10 min [159]. Fractions isolated by this method have identified by MS to include C_{76}, C_{78}, C_{82}, C_{84}, C_{86}.

Another interesting application of HPLC in fullerene research is in the isolation and identification of fullerene isomers [160]. HPLC with on-line MS has been used to separate and positively identify isomers of the C_{60} and C_{70} fullerenes. HPLC with UV detection was used to monitor the thermal conversion of these isomers to stable fullerenes. The conversion of the newly reported C_{60} isomer to stable C_{60} occurred with a half-life of *ca.* 1 h in de-aerated boiling toluene (111°C) in an argon atmosphere [160]. Reversed-phase HPLC has been used to isolate pure C_{76}, C_{2v}–C_{78}, D_3–C_{78} (C_{78} isomers), and a mixture of at least two C_{84} isomers [161]. Preliminary data on these higher fullerenes indicates that the chemistry of the higher fullerenes is diverse and distinctively different than that thus far reported for buckminsterfullerene and C_{70}. Advancement in the chemistry and physics of the higher fullerenes will rely, in part, on the optimization of the conditions for their formation as well as improved chromatographic methods for their isolation and characterization. Unfortunately, due to the current fervor of fullerene research, the present review of fullerene separations will likely be dated by the time it appears in print.

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Review

Environmental analysis of chlorinated aromatic thioethers, sulphoxides and sulphones

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ABSTRACT

Chlorinated aromatic thioethers discussed here are polychlorinated dibenzothiophenes, thianthrenes and diphenylsulphides. Relatively little is known about their occurrence, behaviour and fate in the environment. Polychlorinated dibenzothiophenes and diphenylsulphides have recently been found to be formed in waste combustion and analysed in pulp mill effluents. Chlorinated sulphoxides and sulphones are usually metabolites or oxidation products of different chlorinated aromatic compounds. Different gas chromatographic–mass spectrometric techniques are used in the analysis of the chlorinated thioethers. The sulphoxides and sulphones, because of their higher polarity, can be isolated from other compounds by liquid chromatography.

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1. INTRODUCTION

Polycyclic aromatic sulphur heterocycles (PASHs) and polycyclic aromatic hydrocarbons (PAHs) occur widely in the environment [1–5]. Dibenzothiophene (DBT) and some methyl-substituted dibenzothiophenes are persistent residues in the marine environment after oil accidents. Dibenzothiophene and its alkyl derivatives have been found to accumulate in eels, clams, short-necked clams, oysters and mussels [6–15]. These compounds in biota are supposed to originate mainly from crude oil pollution. Different crude oils contain 1–10% sulphur, of which about half is organically bound, mostly in aromatic structures [16]. The most important compound is dibenzothiophene [17].

The occurrence of polychlorinated polycyclic aromatic sulphur heterocycles (PCPASHs) in the envi-

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ronment has been reported recently. Polychlorinated dibenzothiophenes (PCDBTs) have been found in stack gases, bleached pulp mill effluents and aquatic organisms [18–21]. PCDBTs and polychlorinated thianthrenes (PCTAs) are environmentally and toxicologically interesting compounds because of their structural similarity to polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The structures of the compounds reviewed are described in Fig. 1. PCDBTs are sulphur analogues of PCDFs, and PCTAs are sulphur analogues of PCDDs. PCTAs and PCDBTs can in theory be formed analogously to PCDDs and PCDFs in different ways. Polychlorinated diphenylsulphides (PCDPSs) are sulphur analogues of the polychlorinated diphenylethers (PCDEs), which are common residues in the environment. There is no considerable knowledge about the potential toxicity of these kind of compounds. However, 2,3,7,8-tetrachlorothianthrene is suspected to possess dioxin-like activity [22], and recently preliminary toxicological studies have shown that the toxicity of PCDBTs is less than that of the most toxic co-planar PCBs [23].

Chlorinated aromatic methylsulphides, sulphoxides and sulphones are metabolites or oxidation products of different compounds [24–31]. Methylsulphonylation of xenobiotics leads to the formation of mercapto (–SH), methylthio ($-SCH_3$), methylsulphinyl ($-SOCH_3$) and methylsulphonyl ($-SO_2CH_3$) thioether derivative metabolites [32]. The formation of xenobiotic thioether derivatives is considered to be a pathway for the detoxification of reactive intermediates [32,33]. The metabolic pathway of halogenated aromatic hydrocarbons consists of three stages: oxygenation, glutathione thioether disposition and sulphoxidation [32–35]. The intestinal microflora is involved in the formation of these metabolites [36]. Many of these compounds possess some kind of biological activity [37–39].

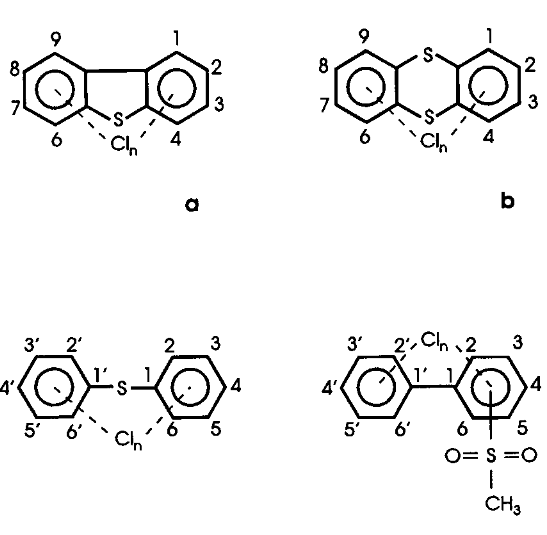

Fig. 1. Structures of the compounds reviewed: (a) polychlorinated dibenzothiophenes (PCDBTs); (b) polychlorinated thianthrenes (PCTAs); (c) polychlorinated diphenylsulphides (PCDPSs); (d) methylsulphones of PCBs.

2. EXTRACTION AND CLEAN-UP PROCEDURES

2.1. Chlorinated thioethers

PCDBTs have been analysed in stack gas, fly ash, bleached pulp mill effluent and aquatic biota samples [18–21,40].

The water samples were filtered twice, and the filters dried, weighed and Soxhlet-extracted with toluene for 48 h. The stack gas samples were extracted with toluene and washed with sulphuric acid. Column chromatography with basic aluminium oxide and activated carbon was used to separate the dioxin fraction from the sample extracts [41]. PCDBTs, PCDPSs and PCTAs behave during purification and fractionation with basic aluminium oxide and activated carbon in the same way as PCDFs and PCDDs [41,42].

2.2. Sulphoxides and sulphones

Fatty materials from lung, liver and adipose tissue were extracted with *n*-hexane after homogenizing with anhydrous sodium sulphate [43–45] and saponified with 2 *M* alcoholic sodium hydroxide or 1 *M* alcoholic potassium hydroxide solution [43,46] or extracted with 95% (v/v) dimethyl sulphoxide–water solution by shaking. Purification was done by silica gel column chromatography. The PCBs and other non-polar compounds were eluted with *n*-hexane, and sulphones, sulphoxides and other compounds with *n*-hexane–diethylether mixtures [43].

The sulphone-containing fraction could be extracted with concentrated sulphuric acid. The solu-

tion was then diluted with water and subsequently re-extracted with hexane, producing a pure sulphone fraction without any interfering compounds [47].

The methylsulphones and methylsulphoxides could also be extracted with dichloromethane and purified by gel permeation chromatography followed by alumina column chromatography [48].

2.3. TLC and HPLC of PCPASHs

In environmental samples, PCPASH compounds occur in a complex matrix of thousands of different organic compounds. The separation of the PCPASHs from interfering compounds in GC–MS and in GC proved to be difficult. Several different liquid chromatographic fractionations have to be applied to obtain a fraction with minimum amounts of interfering compounds [49,50]. Normally the first step is to separate the non-polar aromatic compound fraction, which contains a lot of halogenated and alkylated low-polarity compounds. This is usually done by silica gel or alumina column chromatography using hexane and hexane–dichloromethane as eluents. There does not exist any unambiguous method of separating PCPASHs from other compounds.

Reversed-phase (RP) TLC with RP-18 plates and acetonitrile–water eluents can separate PCDBTs from many interfering compounds but not completely from complex environmental samples [49]. In normal-phase NH_2-TLC with hexane the PCDBTs elute faster than the corresponding non-chlorinated compounds [49]. By oxidizing these compounds to the corresponding sulphones, they can be separated by RP-TLC or by RP-HPLC because of the difference in their polarity, but this makes the GC analysis more difficult [50,51].

The sulphones and sulphoxides, because of their different polarity, can be separated as a group from other non-polar compounds. In clean-up procedures used to prepare samples for the analysis of non-polar chlorinated compounds these are retained in the columns [52]. They also have longer retention times in GC columns usually used for residue determination [53]. From alumina or silica columns the sulphoxide–sulphone fraction can be eluted with more polar eluents, *e.g.*, ethyl acetate, after the PCBs and other non-polar compounds have been eluted with hexane or diethyl ether [44,46,54]. From Florisil columns these compounds can be eluted separately as a group using different diethyl ether–light petroleum mixtures [52].

In silica gel TLC when methylene chloride–hexane (50:50) is used as eluent, all compounds with a similar oxidation level (methylthio, methylsulphinyl and methylsulphonyl compounds) have similar R_F values [52].

Sometimes the methylthio and methylsulphinyl compounds are oxidized to sulphones to simplify the analysis [46,52]. These sulphones can further be desulphurized to facilitate normal GC analysis using non-polar columns [46].

The sulphones can be derivatized before analysis by preparing trimethylsilyl derivatives, methyl esters or different acetates [54,55].

Sulphoxides and sulphones, owing to their higher polarity, can easily be separated from other compounds by RP-HPLC. In RP-HPLC using a μBondapak C_{18} column and acetonitrile–water eluents the methylsulphone-PCBs elute before the methylthio-PCBs and PCBs [43].

3. GC AND GC–MS OF ENVIRONMENTAL SAMPLES

3.1. Chlorinated thioethers

Tetrachlorinated dibenzothiophenes (TeCDBTs) and tetrachlorinated dibenzo-*p*-dioxins (TeCDDs) have in low-resolution MS the same values of M^+, $[M+2]^+$ and $[M+4]^+$ ions. A resolution of about 20 000 is needed to separate TeCDBTs (MW=319.87880) from TCDDs (MW=319.89650). The retention times of the congeneric PCDBTs in GC with unpolar SE-30 and HP-5 columns are longer than those of the PCDDs; however, in many environmental samples there are several interfering peaks eluting in the same window as the PCDBTs if low resolution in GC–MS is used [42].

Several PCDBTs are available as model compounds in environmental analysis. The compounds are prepared by direct chlorination of the parent compound, DBT, when a mixture of different congeners is obtained, or by reacting PCBs with sulphur, when in some cases it is possible to obtain one isomer, although usually a mixture of a few isomers is obtained [19,20,41,56,57].

Buser and co-workers [19,20] have used different

GC–MS techniques to determine PCDBTs in fly ash samples and aquatic organisms. In electron impact (EI) mass spectra the PCDBTs show a strong molecular M^+ ion and the expected clustering due to chlorine and sulphur isotopes. The major difference in the EI mass spectra of the PCDDs and PCDBTs is the formation of a strong $M^+ - COCl$ in the former and a strong $M^+ - 2Cl$ in the latter compounds. QUAD MS–MS was found to be highly sensitive for detecting PCDDs and PCDFs via the reaction $M^+ \rightarrow [M - COCl]^+$, and mass-analysed ion kinetic energy (MIKE) MS–MS was found suitable for detecting the PCDBTs via the reaction $M^+ \rightarrow [M - 2Cl]^+$ [19].

Stack gas samples and bleached pulp mill effluent samples have been analysed for PCDBTs and PCDPSs with high-resolution GC–MS with a resolution of 20 000. Only particles in the effluent waters were studied, and it was assumed that PCDBTs are mostly particle-bound. Selected-ion monitoring was done with the values of M^+ and $[M + 2]^+$ ions of the TeCDBTs and pentachlorinated dibenzothiophenes (penta-CDBTs) (319.8788, 321.8758, 353.8398 and 355.8369). When the resolution was increased to 20 000, disappearance of the PCDDs was nearly complete [18,21].

3.2. Sulphones and sulphoxides

In MS fragmentation of methylsulphones the loss of CH_3 yields the $[M - 15]^+$ ion. The 1,2-phenyl migrates from sulphur to oxygen and the aryl methane sulphinate thus formed is fragmented to a phenolate ion, $[M - 63]^+$, by the loss of $SO–CH_3$. One of the major fragments, $[M - 114]^+$, is produced by the loss of the methylsulphone group and one chlorine atom [55,58,59].

Haraguchi *et al.* [48] have used selected-ion monitoring GC–MS with the values of M^+ and $[M + 2]^+$ ions for di- to tetrachlorophenylmethylsulphones, di- to hexachlorobiphenylmethylsulphones, tetra- to hexachloroterphenylmethylsulphones and dichloro-(diphenyl)dichloroethene (DDE)-methylsulphone. The values of the ions $[M - 16]^+$, $[M - 14]^+$ and $[M - 12]^+$ were used in the analysis of di- and trichlorophenylmethylsulphoxides and di- to pentachlorobiphenylmethylsulphoxides in human liver, lung and adipose tissues, human milk, human blood, blubber of blue whale, sardine, rainbow trout, Yaname, oyster and baby clam [48]. A 25 m × 0.25 mm I.D. fused-silica MPS-50 capillary column (Quadrex) in a JEOL JMS-DX300 mass spectrometer with a JMA-DA5000 data system was used [48].

In GC analysis electron-capture detection (ECD) and/or flame photometric detection (FPD) are normally used in the analysis of Cl–S compounds [43–46,52,59,60].

4. OCCURRENCE AND ENVIRONMENTAL CONCENTRATIONS OF PCPASHs

Stack gas samples contain several TeCDBT and penta-CDBT isomers that elute within the retention window of the prepared model compounds and have the typical $M^+/[M + 2]^+$ ion ratios for tetra- and pentachlorinated compounds. The concentrations of the TeCDBTs in stack gas samples are very high compared with the concentrations in bleached pulp mill effluents.

The isomer profiles observed in selected-ion monitoring chromatograms of TeCDBTs in stack gas samples and in pulp mill effluents are quite different [21]. The stack emissions contain faster-eluting (column HP-5) isomers [21]. This probably indicates a different route of formation of these compounds in waste combustion and in pulp bleaching.

Buser *et al.* [20] estimated the concentrations of PCDBTs in fly ash to be up to 55 ng/g, which is one order of magnitude below the concentrations of the PCDDs and PCDFs in these samples [20]. The PCDBTs detected were TeCDBTs and penta-CDBTs, including the 2,3,7,8-TeCDBT [20]. Aquatic organisms such as crabs, lobsters and worms from New York Bight/Newark Bay contain high levels of some PCDBTs. The main isomer found in aquatic organisms was the 2,4,6,8-TeCDBT. 2,3,7,8-TeCDBT was not found [19,40].

In pulp mill effluents analysed the concentrations of the TeCDBTs were $<1–60$ pg/l. In the pulp mill effluent samples the 2,3,7,8-TeCDBT isomer was the dominating TeCDBT isomer [21]. The ^{13}C-12-labelled 2,3,7,8-TeCDBT was used as a tentative internal standard in the quantitative determinations of the TeCDBTs in the pulp mill effluent samples, supposing that the MS response of the TeCDBTs is almost the same as that of the TeCDDs [21]. Penta-CDBTs and higher chlorinated PCDBTs were not found from the pulp mill effluent samples [21].

Hilker *et al.* [22] have reported on the finding of 2,3,7,8-tetrachlorinated thianthrene (TeCTA) in sediment from a sanitary sewer near a chemical plant that patented this compound. Recently, the determination of trichlorinated diphenylsulphides (tri-CDPSs) and tetrachlorinated diphenylsulphides (TeCDPSs) in stack gas and bleached pulp mill effluent samples has been reported [61]. 2,4,4′,5-TeCDPS (Tetrasul) has been used as pesticide and has been analysed by GC–ECD–FPD from food samples [62,63].

PCB methylsulphones are persistent and lipophilic compounds. Methylsulphones are accumulated in adipose tissue. Methylsulphone and methylsulphoxide metabolites of chlorinated benzenes (CPs), polychlorinated biphenyls (PCBs) and DDE have been detected in environmental samples and human tissues and milk [43–45,48,60,64–66].

These were identified as mainly tri-, tetra-, penta-, hexa- and heptachlorinated methylsulphones [44,67]. For example, in lung tissue obtained from a Yoshu victim about 60 different isomers of methylsulphone-PCBs were still present more than 15 years after intoxication [45]. The levels of methylsulphones were estimated at one twentieth of those of PCBs in the corresponding samples [60]. Seals from the Baltic have been found to contain at least 30 different methylsulphone-PCBs [53]. The major sulphone metabolites contain the methylsulphonyl group in the 4-position and the minor ones in the 3-position [33,47,60,68–70,71]. Tissue-specific, structure- and species-dependent high-affinity bindings and long retentions in lung, kidney and brain have been demonstrated [33,43,54,64,69–73].

From fish samples (striped bass, catfish, goldfish, carp, sucker) that contained high levels of chlorobenzenes and PCBs the metabolite sulphoxides and sulphones could not be found [52]. It is possible that these metabolites are not formed in fish or are not stored in the edible parts as are the parent compounds.

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Review

Different approaches for improving the precision in chromatographic analysis of environmental samples by optimum signal processing and correlation techniques

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ABSTRACT

An overview is given of the applicability of optimum signal processing and correlation techniques in chromatographic environmental analysis. Improving the precision and (sub)trace analysis is emphasized. The principles and problems of different approaches, particularly in the field of matched filtering and correlation (multiplex) chromatographic techniques, mainly based on previously published work, is discussed and illustrated with examples.

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1. INTRODUCTION

Protection of the environment is directly related to the capability of precise measurements of a variety of pollutants, in both air and water. A typical aspect of environmental analysis is the presence of a complex matrix. Usually, it is necessary to separate the component to be determined from the matrix; chromatography is an important tool in environmental analysis. Another property, often typical of the analysis of environmental samples, is the low concentrations of many species of interest; low detection limits are required to measure analytes with acceptable precision. In that respect, much successful work has been done in the field of optimization of separation conditions and development of sensitive low-noise detectors. Preconcentration of the sample is another approach; in general, large amounts of environmental samples are available. However, preconcentration is not always possible and the disadvantages are well known.

Generally, signal processing in chromatography is conventional, involving peak height or peak area determination. Surprisingly, optimum signal processing with respect to the minimization of the uncertainty in the results is rarely applied and it can be considered as an underdeveloped part of the chromatographic procedure. Nevertheless, it may reduce the detection limit, although not drastically; in practice, the reduction factor lies between about 2 and at most 5, compared with a proper integration procedure.

Reduction of the detection limit is not the only important reason to optimize signal processing. Chromatographic results are used for, *e.g.*, setting or monitoring regulations pertaining to environmental guidelines. If an important part of the chromatographic procedure, having a great influence on the final precision, is not well defined and when it is to a great extent dependent on unknown, uncontrollable and often unpredictable "integrator" software, then standardization and comparison of results from different laboratories are hardly possible. Therefore, one has to strive for well defined standardized optimum signal processing, including uncertainty calculations. The application of matched linear systems (matched filtering) in computerized chromatographic signal processing was recently investigated, with promising results [1,2]. It is an example of the application of chemometrics, *i.e.*, the use of advanced mathematical and statistical techniques in analytical chemistry.

Another way of using chemometrics is in the development of methods based on the mathematical and data-handling capacities of computers. *Correlation chromatography* (CC) is an example of this approach [3]. The most important advantage of CC over conventional chromatography is a rapid decrease in the detection limit at the cost of sample in a relatively short time. Possible changes to the sample composition are avoided, because no preconcentration is required. In addition, it can be used to monitor changes in concentration, being a kind of continuous chromatography.

Different modes of correlation chromatography have been developed. For instance, *differential CC* can be used to eliminate matrix peaks. In *simultaneous CC*, more than one sample can be "separated" in the same chromatographic column under identical conditions. *Single-sequence CC* is an intermediate between single-injection chromatography and correlation chromatography. Gradient elution liquid chromatography and temperature-programmed gas chromatography are still applicable, in contrast to other CC methods. Potentially, all these correlation technique have outstanding properties for application in environmental analysis.

In this paper, optimum signal processing, in particular matched filtering, and different modes of correlation chromatography are reviewed.

2. OPTIMUM ESTIMATION OF THE INTENSITY OF NOISY CHROMATOGRAPHIC PEAKS

Quantitative evaluation implies the determinations or, better, the estimation, of the "intensity" of relevant peaks. After calculation, this parameter gives a reliable measure of the amounts of components of interest. Normally, peak height or peak area is used as an intensity parameter. However, neither is optimum with respect to uncertainty in the results; an optimum estimation procedure uses all available and obtainable prior information to maximize the precision. This prior information consists of preknowledge of the (utility) signal and the noise. In both instances parameterized models are used. For chromatographic signals the model is a mathematical expression, describing the shape of the signal as a

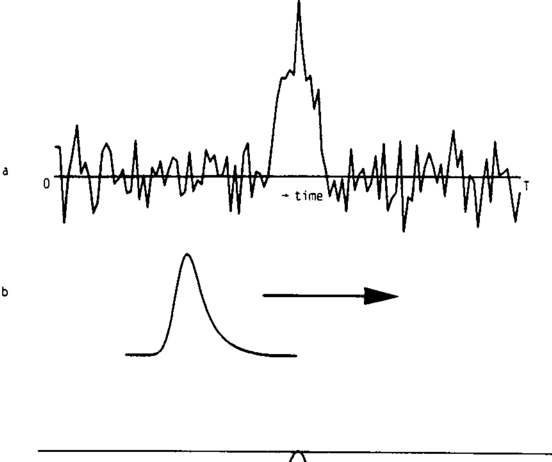
function of the time. Examples of signal models have been published [4]; the values of the parameters have to be determined such that the functions fit the real peak shape satisfactorily.

The usual goodness of fit criterion is χ^2, the sum of the squared deviations of the (discrete) signal amplitude values from the fitting function, weighted with the uncertainty in the datapoints:

$$\chi^2 = \sum_{i=1}^{m} \left\{ \frac{1}{\sigma_i^2} [y_i - y(t_i)]^2 \right\} \tag{1}$$

where m = number of data, y_i = data points, $y(t_i)$ = fitting function and σ_i^2 = uncertainty (variance) in the data points.

Noise can be either stationary, *i.e.*, the statistical properties are independent of the time, or non-stationary. Stationary noise, common in chromatography, can be modelled with the probability density function (PDF), the autocorrelation function (ACF) and, directly derived from the ACF by Fourier transformation, the power spectral density (PSD).



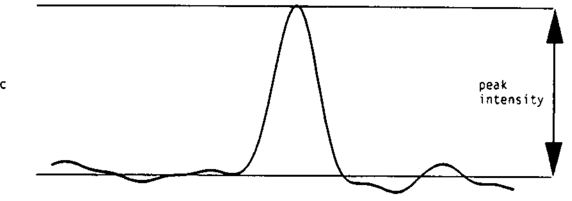


Fig. 1. (a) Signal $s(t)$ = peak $f(t)$ + noise $n(t)$; (b) model function $f_1(t-\tau)$, where τ = ime shift; (c) correlation detector output = integral of the product $s(t).\ f_1(t-\tau)$ as a function of τ.

3. CORRELATION DETECTION

Correlation detection is a relatively simple optimum signal-processing method if the noise is assumed to be "white", *i.e.*, the power of the noise is equally distributed along the frequency axis in the frequency range of interest. In other words, the PSD is flat. Correlation detection implies shifting a model peak shape along the chromatographic retention time axis and calculating the integral (area) of the product of the model shape and real signal for each time shift. Fig. 1 shows the result of the procedure applied to a noisy peak. The amplitude ("intensity") of the real signal is the only parameter of the peak not known in advance. In principle, each point of the peak resulting from the correlation detection procedure is directly related to the desired intensity of the real peak. However, the maximum (top) is optimum with respect to the minimum uncertainty. Note that the original peak shape is not maintained. The noise, $n(t)$, is also multiplied by the model function $f_1(t-\tau)$ and integrated over the interval T; τ is the time shift:

$$I_n = \int_{-T/2}^{T/2} f_1(t-\tau)n(t)\mathrm{d}t \tag{2}$$

Fig. 2 shows the result of multiplication of the noise with the (known) model of a skewed utility peak shape, a kind of non-stationary noise. The integral gives for each τ one point of the baseline of the correlation detector output. The standard deviation of I_n compared with the maximum of the correlation function determines the uncertainty in

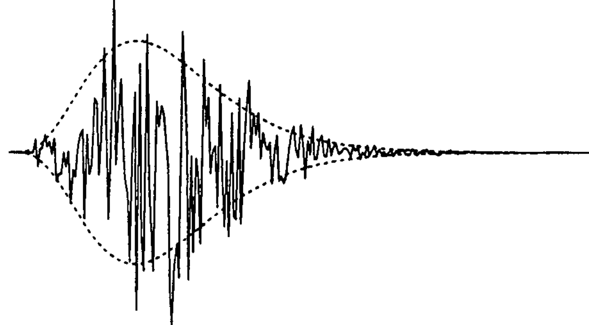


Fig. 2. Noise multiplied by a known signal model. The dashed lines denote the envelope of the product, determined by the model function. The standard deviation of the integral is decisive for the final precision (see text).

the determination of the intensity and eventually of the concentration of the component of interest. The signal-to-noise ratio is increased by a factor of about 1.5–2, depending on the peak shape and compared with integration, assuming optimum integration limits. The latter assumption is certainly not always realistic; determination of the optimum integration limits is more difficult than the determination of a peak top of correlation detection.

4. MATCHED LINEAR SYSTEMS (MATCHED FILTERING)

A signal-processing method, directly related to correlation detection, is matched filtering, *i.e.*, the application of matched linear systems (MLS). If white noise is assumed, both methods are essentially the same. With non-white noise, however, the matched filter is superior using pre-knowledge on the PSD of the noise. The matched filter acts by selectively enhancing or suppressing certain frequencies in the signal. If in certain parts of the PSD the noise dominates, these frequencies are suppressed, whereas the other frequencies are enhanced. Actually, all frequencies in the signal are weighted according to the ratio of signal power to noise power per frequency.

The (complex) frequency response of an MLS is

$$H(j\omega)_m = \frac{S^*(j\omega)}{N(\omega)} \cdot e^{-j\omega\mu} \qquad (3)$$

where $S^*(j\omega)$ = complex conjugate of the signal model in the frequency domain, $N(\omega)$ = power spectral density of the noise and μ = time shift introduced by the MLS.

Fig. 3 shows a realistic peak model and the (noise-free) output of a matched filter adapted for "white" noise and for so called $1/f$ noise or flicker noise, where the low frequencies are dominate. In this example, no noise is present in the input signal to show the influence of matched filtering on the peak shape. Flicker ($1/f$) noise frequently occurs in chromatography. The final output is similar to the result of a correlation detection procedure. Again, the desired information, the chromatographic peak intensity, is obtained from the output peak top.

Fig. 4 shows a noisy peak (b) with known shape (a). An example of area determination by integration is given in Fig. 4c, where the confidence interval is depicted by the bars. This confidence interval can be calculated [5] or measured. The output of the matched filter is given in Fig. 4d, including the significantly better confidence interval.

The possibilities of a matched filter are demonstrated in Fig. 5, showing two (simulated) noisy peaks. Hardly any difference can be observed. Both peaks have a peak height/σ_n ratio of 5, where σ_n is the standard deviation of the noise. However, the noise in the peak in Fig. 5a is "white", whereas in Fig. 5b a small rectangular part of the frequency spectrum (PSD) is about zero; in other words, in that part of the spectrum the noise power is very low. This means

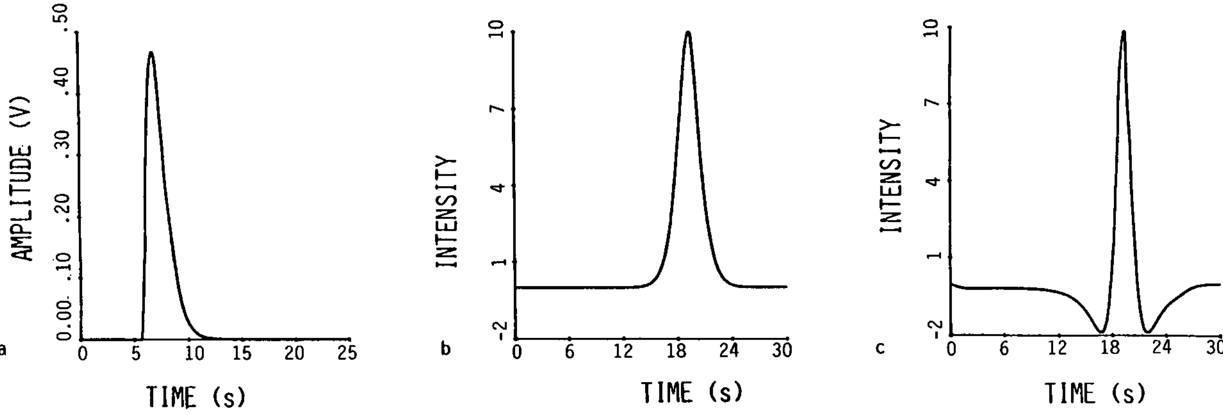
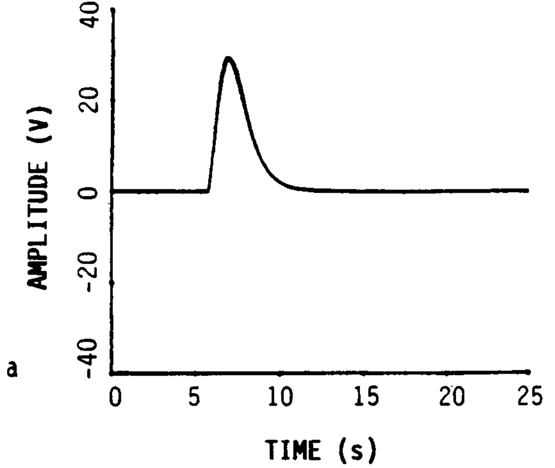
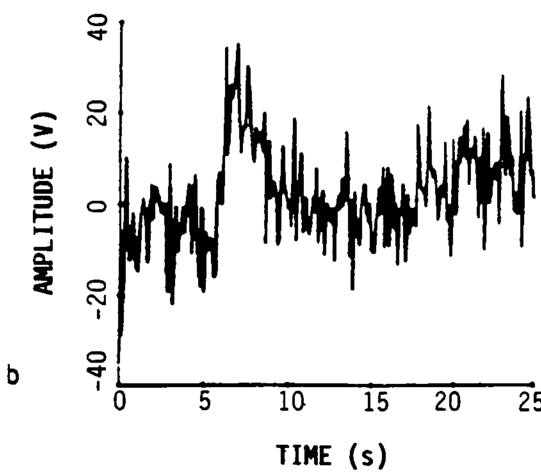
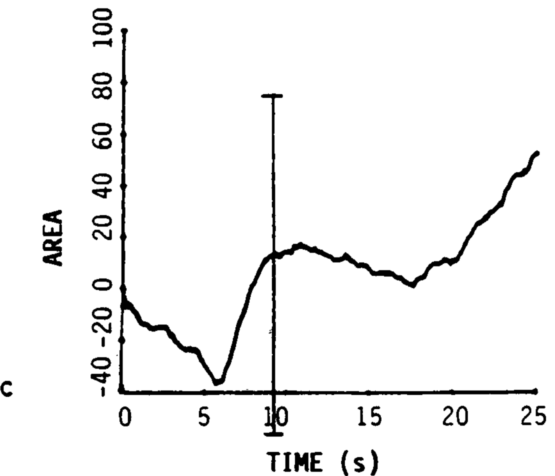


Fig. 3. (a) Peak model; (b) output of the matched filter, assuming noise with a flat (white) spectrum; (c) output of the matched filter, assuming flicker ($1/f$) noise, *i.e.*, the power of the noise, is inversely proportional to the frequency. The input peaks in (b) and (c) are taken to be almost noise-free in this instance to demonstrate the effect of matched filtering on the output peak shape.







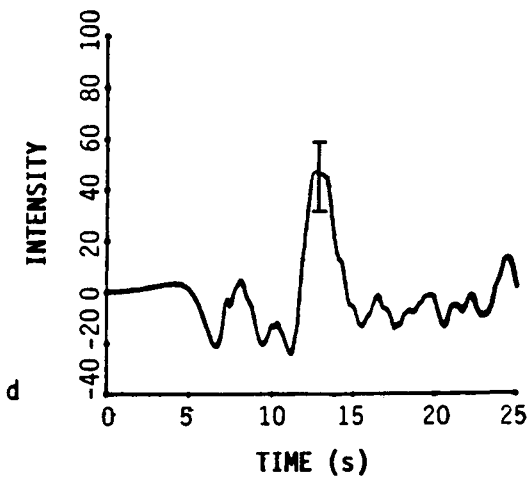


Fig. 4. (a) Signal; (b) signal + noise; (c) integral of noisy signal. The bars indicate the confidence interval. (d) Matched filter output.

that the frequency components of the signal can very precisely be determined, because of the high S/N ratio in that particular frequency range. In principle, the intensity of a peak can be estimated from each point of the frequency spectrum; a functional relationship exists between the spectral components given by the (known) signal model in the frequency domain.

Applying matched filtering to the peaks in Fig. 5 results in a higher precision of the intensity estimation of the peak in Fig. 5b compared with that in Fig. 5a; the precision gain factors are 29.6 and 2.75,

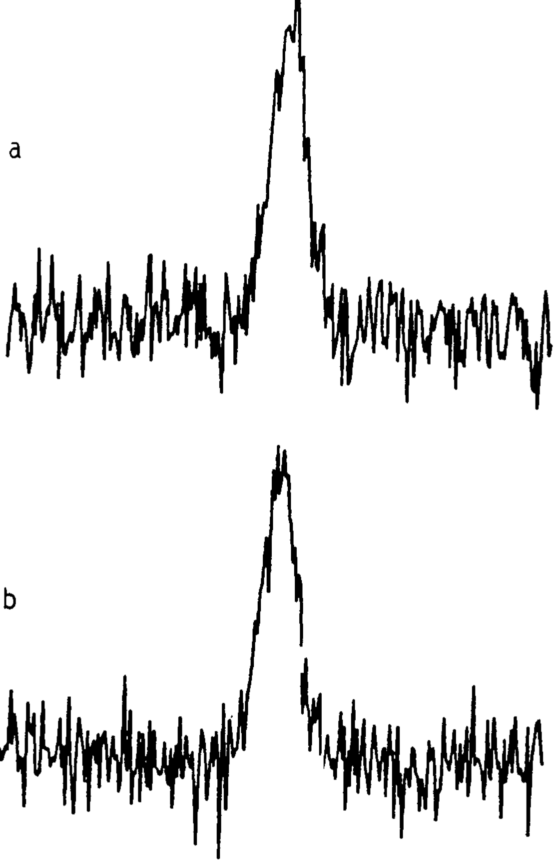


Fig. 5. (a) Peak with "white" noise; (b) the same peak with band-rejected filtered noise.

respectively. With integration hardly any difference between the two peaks can be observed.

5. CORRELATION CHROMATOGRAPHY

Correlation chromatography (CC) is a typical example of an on-line chemometric technique, with promising results in (ultra)trace analysis. In contrast to conventional chromatography, where essentially the response on a single injection impulse is determined. CC involves multiple injection chromatography. A schematic set-up with mechanical valves controlling the injection is shown in Fig. 6a. The response is a massive group of fused peaks, looking like noise with a greatly raised baseline. To the naked eye it is impossible to visualize separated peaks. However, the computer, using the known input function and the resulting output, can produce a "correlogram" very similar to a normal chromatogram, but with a drastic decrease in the noise. The longer the system is run, the higher the signal-to-noise ratio will be.

In trace analysis, trace compounds, otherwise not attainable by single injection techniques, can be detected at the cost of a larger amount of sample needed and a longer analysis time. Generally, in environmental analysis sufficient sample is present and an increase of a factor of 2 in the analysis time decreases the detection limit by one order of magnitude.

The principles of CC can be described by mathematical derivations. These derivations are not very meaningful for understanding what happens, so only a simple graphical explanation will be given here.

The most suitable input pattern in CC is a so called pseudo-random binary sequence (PRBS), a simple example of which is depicted in Fig. 7a. A PRBS has two levels, 1 and 0, corresponding to injection of sample and only eluent as an input, respectively. The PRBS is controlled by a clock, determining the minimum time Δt of the "1" or "0" state. A PRBS is a logical function, combining the properties of a true (binary) random pattern and a reproducible deterministic pattern. After a certain time interval, a sequence, the same pattern is repeated. In Fig. 7a two sequences are shown.

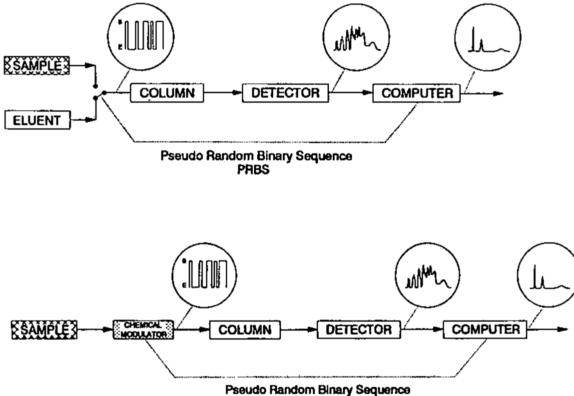


Fig. 6. (a) Set-up correlation chromatograph, mechanical modulation system; (b) chemical modulation system.

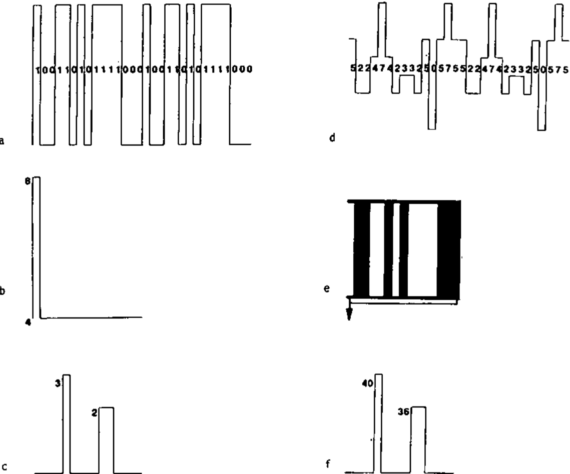


Fig. 7. (a) PRBS, two sequences, fifteen clock periods per sequence. (b) Autocorrelogram of the PRBS in (a), calculated by correlating (a) with the mask in (e). (c) Hypothetical normal chromatogram, response on a single pulse-shaped injection of a mixture of two components. (d) Detector signal resulting from a PRBS injection according to (a) of the same mixture as in (c) on the same column. (e) PRBS mask, similar to one sequence of the PRBS in (a), used for cross-correlation with the detector signal by shifting in time and adding. (f) Resulting correlogram.

Assume that a sample with two components is injected into a column, resulting in a chromatogram with two (not very realistic) block-shaped peaks appearing at $5\Delta t$ and $10\Delta t$ with amplitudes 3 and 2, respectively. The areas are 3 and 4 (Fig. 7c). Injection according to the PRBS shown in Fig. 7a will result in a series of overlapping chromatograms (Fig. 7d), which can easily be constructed.

Cross-correlating two functions means shifting one function along the other and calculating the average product for each time shift. This procedure is shown in Fig. 7d–f. A "mask" PRBS similar to the input PRBS (Fig. 7e) is cross-correlated with the output function. The cross-correlation procedure can easily be demonstrated and understood by reproducing Fig. 7e on a transparency and shifting it along the output pattern, multiplying the values (by 1 or 0) for each time shift and finally summing the product over one sequence. The values obtained as a function of the time shift give a correlogram (Fig. 7f) similar to the chromatogram, but with a non-zero baseline and higher amplitudes and area. In addition, cross-correlation of the PRBS with the detector baseline noise results in a much lower noise level in the correlogram, and continuing the correlation procedures reduces the noise level more and more. Cross-correlation of the mask PRBS with the similar input PRBS over one or a number of sequence lengths demonstrate why it is such a suitable input function in CC. Only for a time shift of 0 is the value of the correlation function 8; all other time shifts result in a constant value of 4 (Fig. 7b). This property actually makes CC possible.

As already mentioned, CC is potentially ideal in environmental trace analysis. A typical example, published some time ago [6], is given in Fig. 8, showing an HPLC trace and the corresponding correlogram of a mixture of polynuclear aromatic hydrocarbons (PAHs). The improvement with CC is considerable. A special injection system, which is the most important modification of an HPLC system to perform CC, is commercially available.

Much work in the field of CC has been done by the group of Kaljurand and Küllik [7]. They mainly directed their attention to the determination by CC of the degradation products of high-molecular-mass compounds. Koel (see ref. 7, p. 192), working in the same group, applied correlation steam–solid chromatography for the determination of alcohols and phenol in water samples. The detection limits were 10^{-4} g/l for phenol and 10^{-6} g/l for alcohols.

6. CHEMICAL MODULATION

Introducing multiple samples into the column by switching valves can be considered as a mechanical modulation (Fig. 6a), where all components are

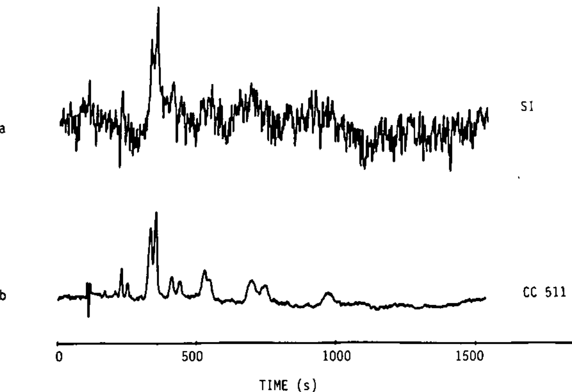


Fig. 8. Single-injection (SI) chromatogram and correlogram of polynuclear aromatic hydrocarbons (PAHs).

equally modulated. However, the concentration of the sample components introduced at the head of the column can also be changed chemically, which in certain instances offers advantages: extra selectivity is added to the system because the modulation can be specific for one or more components, and no moving parts are necessary (Fig. 6b). In addition, there is no separate carrier gas or eluent: the analyte (ambient air, water), possibly modified for optimum separation, is used as the mobile phase. This can be an advantage, but it may reduce the flexibility.

Much pioneering work in the field of chemical modulation in CC has been done by the group of Phillips *et al.* [8], who developed several destructive and non-destructive modulators. CC was called multiplex chromatography. The non-destructive thermal desorption modulator can be considered as a combination of preconcentration and multiple input chromatography. This modulator is a short segment of a fused-silica capillary at the head of a column. Varying the temperature of the modulator changes the adsorption and therefore the concentration of the components introduced into the column. A problem for deconvolution in CC can be the resulting injection waveform, resembling a derivation of a chromatographic injection.

Thermal decomposition modulators and the related thermal catalytic modulators [9,10] are of the destructive type. The principle is simple: heating a capillary tube or a hot wire directly in the carrier gas (air) causes reaction (oxidation) of sample molecules in the gas stream.

The first report on thermal decomposition modulation, by Lovelock [10], concerned modulating the output stream of a column before detection. A typical vacancy correlogram resulting from a hot wire thermal decomposition modulator in CC is shown in Fig. 9; more details can be found in ref. 11. An on–off switched platinum wire was used as a modulator. Only the first 0.33 s of a 1-s injection clock period was used to heat the wire (duty cycle injection), allowing the injection device to return to its initial condition within the clock period. This is essential to prevent memory effects, resulting in so-called ghost peaks.

An example of such a modulator in environmental analysis is demonstrated in ref. 9: methane in ambient air was monitored by CC (multiplex CC) with a thermal–catalytic modulator, selectively catalysing the decomposition of the methane.

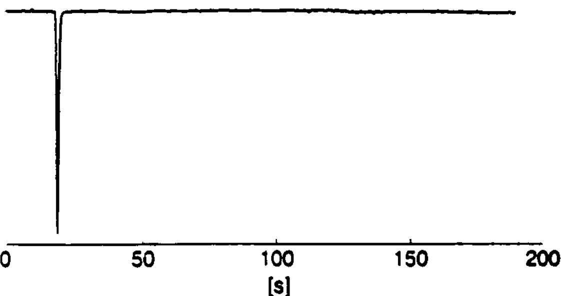


Fig. 9. Vacancy correlogram of 10 ppm of ethene in air, hot-wire modulator, 63-bit PRBS-based injection. Duty cycle injection (see text).

Another modification was reported in ref. 12, where the preliminary outlines of a spark modulator were presented. An important advantage of using a modulated spark train to initiate a reaction of the sample components is the absence of a time delay between the change in the control signal and the action of the spark. Therefore, no ghost peaks appear in the correlogram. However, the composition of the product peaks remains to be investigated in each special case.

Corney and Phillips [13] also reported on an electrochemical concentration modulator (ECM) for liquid chromatography, while an extensive study on the properties of an ECM in correlation chromatography was made by Engelsma *et al.* [14,15], who determined the phenol concentration in canal water.

Although the results are promising for application in environmental analysis, much work need to be done to optimize the ECM for practical application. In general, the relatively slow dynamic behaviour of a chemical modulator can be a source of ghost peaks and correlation noise, as shown by Engelsma [15].

7. DIFFERENT MODES OF CORRELATION CHROMATOGRAPHY

7.1. *Simultaneous correlation chromatography*

A modification of conventional CC is simultaneous correlation chromatography (SCC). The principle of SCC is to inject a number of different samples, if required with the same components, each according to a pseudo-random pattern, mutually

completely uncorrelated, and to calculate the different correlograms by cross-correlating the very complex output with the corresponding input pattern [16]. Because two completely uncorrelated PRBSs do not exist, a long PRBS with a length equal to the sum of the duration of the *n* different corresponding chromatograms has to be used and the analysis time is *n* times the duration of one chromatogram. SCC does not reduce the analysis time. All different samples are injected according to this PRBS, each with a different time shift equal to an integral number of chromatogram durations. So far only preliminary results have been published. A possible application is high-precision chromatography, if suitable reproducible injection systems can be developed.

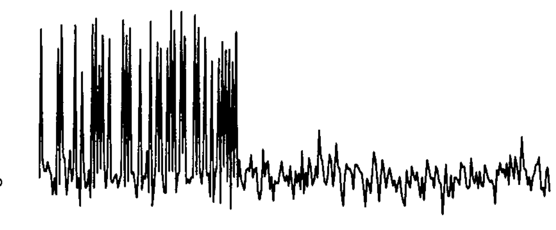
As has been demonstrated experimentally, calibration and measurements can be effected simultaneously in the same column under identical conditions, while the noise reduction property of CC is maintained. A very accurate calibration and determination can be achieved.

7.2. Single-sequence correlation chromatography (SSCC)

One of the disadvantages of CC is the demand for stationarity of the system, in other words, no varying conditions influencing the retention time, the peak shape, etc., are allowed. Modern chromatographs fulfil this condition more than satisfactorily, but temperature-programmed GC and gradient elution HPLC are out of the question. Recently a paper on a novel technique that overcomes this difficulty was published [17], in which the basic idea of injecting a large volume sample into the column was described. The width of this rectangular-shaped injection is too large for a satisfactory resolution of the narrow peaks, but is acceptable with respect to peak broadening of the broader peaks with longer retention times. The trick is to modulate the sample injection with a fine structure according to a PRBS and calculate the first part of the chromatogram by a deconvolution procedure without loss of resolution. The remainder of the chromatogram can be processed in the conventional way, if necessary after increasing the temperature or changing the eluent composition. For both kinds of peaks, broad and narrow, the singal-to-noise ratio will increase compared with normal pulse-like injection.

Fig. 10 shows the principle of a single-sequence injection, and the results obtained using different concentrations of a mixture of *m*-dihydroxybenzene, *o*-dihydroxybenzene, *p*-cresol, *o*-cresol, 2,3-dimethylphenol, 2,4-dihydroxyphenol and toluene with conventional single injection and with SSCC in HPLC are given in Fig. 11. The eluent was methanol–water (50:50, v/v). More details can be found in ref. 17.

SSCC has potential possibilities in environmental analysis, although the *S/N* ratio is not decreased as much as in conventional CC. However, it requires careful consideration of the chromatographic system including the components to be determined. In addition, the deconvolution is not as straightforward as in normal CC. The procedure has to be checked for interferences which must be avoided by a proper choice of the PRBS, or must be corrected for.



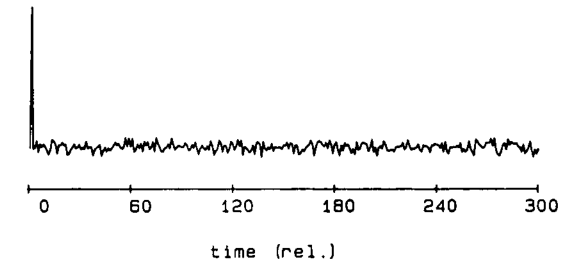


Fig. 10. (a) Single-sequence injection pattern. (b) Corresponding noisy detector signal (without column). (c) Calculated correlogram.

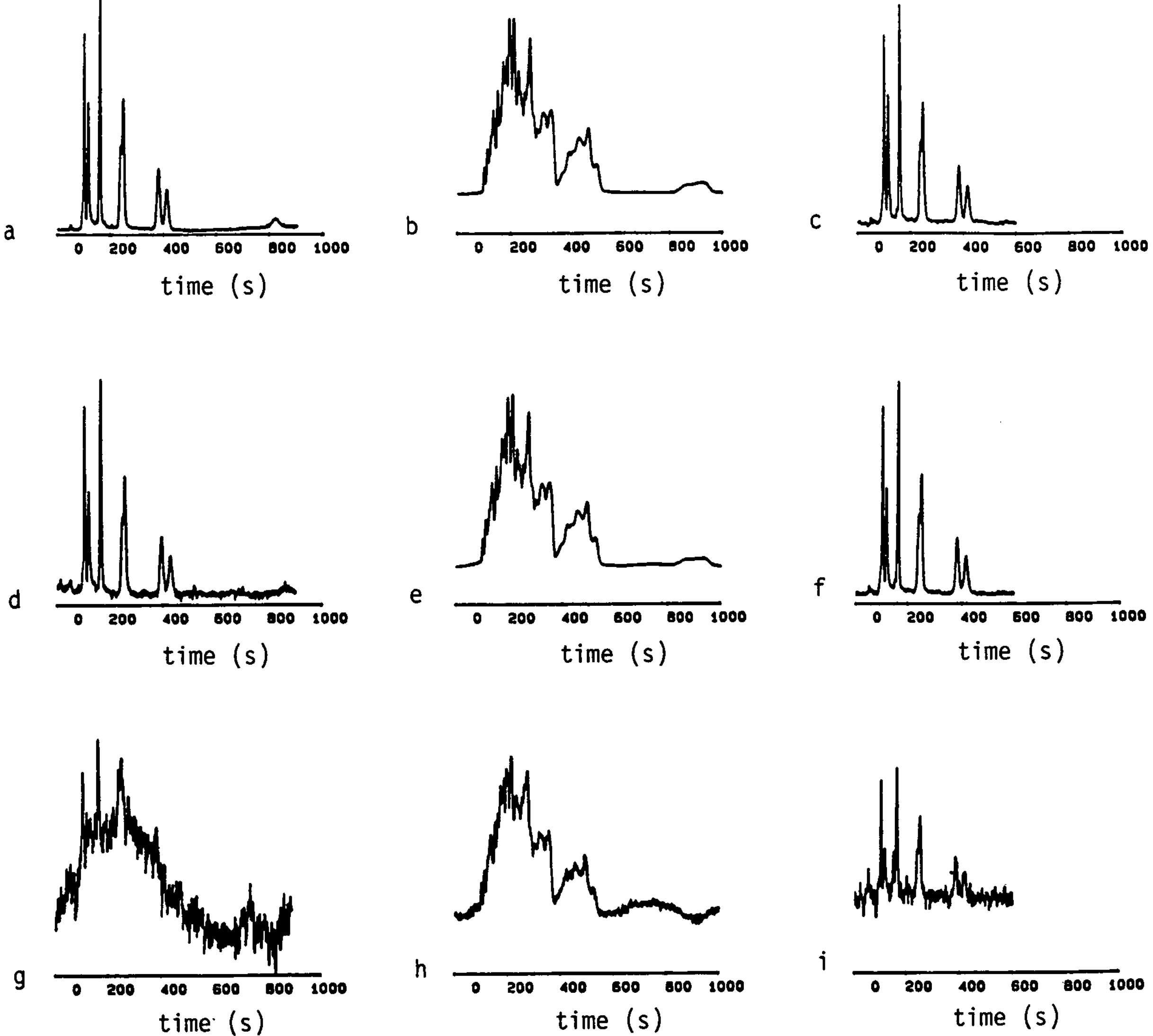


Fig. 11. Chromatograms (a, d, g), SSCC detector signals (b, e, h) and SS correlograms (c, f, i) of a mixture of *m*-dihydroxybenzene, *o*-dihydroxybenzene, phenol, *p*-cresol, *o*-cresol, 2,3-dimethylphenol, 2,4-dihydroxyphenol and toluene at different concentrations (from top to bottom: 3–5 μg ml^{-1}, 0.3–0.5 μg ml^{-1} and 30–50 ng ml^{-1} for each component).

7.3. *Differential correlation chromatography (DCC)*

As mentioned before, deviations from ideal behaviour of the correlation chromatographic system, particularly irreproducibility of the injections and non-linearity of the column and detector, cause ghost peaks and correlation noise. These effects depend strongly on the concentration differences between sample and eluent.

The presence of an unimportant main component can interfere in the determination of trace components by causing considerable correlation noise. A possible solution to this problem is to apply CC in the differential mode [18], apart from the develop-

ment of high-quality injection devices or utilizing the linear range of the distribution isotherm.

Concerning the known main components, the eluent is made as equivalent as possible to the sample. Essentially, CC is a differential technique and only differences between sample and adapted eluent or possibly another sample are measured. Differential correlation chromatography can be particularly useful in environmental analysis and in trace analysis of samples with a relatively complex matrix. It should be noted that negative peaks may occur, indicating a decrease in the concentration of certain components compared with the other sample. An example of a possible application of DCC is the monitoring of potential sources of pollution by determining at the sub-trace level the difference in concentration before and after the source of pollution. Variations in concentrations with time can also be observed, of course after taking some precautions concerning conservation of the samples.

8. CORRELATION CAPILLARY ZONE ELECTROPHORESIS (CCZE)

Much attention is being devoted to the development of capillary zone electrophoresis (CZE). The small amount of sample and the small volumes of the detectors required generally may cause high detection limits in capillary separation methods. This is particularly the case in CZE, because of rapid overloading of the column. The application of correlation techniques is obvious and recently some preliminary experiments were presented [19]. As usual, a special "injection" system has to be developed. The injection is not based on mechanical valves or chemical modulation, but a voltage is switched by a relay between two reservoirs, each connected to the column via a capillary. The set-up is shown in Fig. 12. The first experiments showed that correlation CZE is possible. Typical problems to be overcome are the influence of the high-voltage switching in the sensitive detector and the required stationarity of the system. Work on optimization of the system is in progress.

9. DISCUSSION

The possibilities of the application of correlation techniques in environmental analysis, both in optimum signal processing and in correlation chromatography, have been demonstrated. Particularly a drastic decrease in the uncertainty in the determination, or in other words enhancing the precision and the quality of measurement, may make these techniques extremely useful. In addition, the possibility of monitoring at sub-trace levels is very attractive. Nevertheless, the applications in practice have so far been restricted, for three reasons: these techniques are not easy to understand or easy to implement using laboratory-made software or simple modifications of existing apparatus; software for optimum signal processing or for CC is not commercially available; and injection systems for correlation CC are not available. Only recently has a suitable commercial injection system for correlation HPLC been described [6].

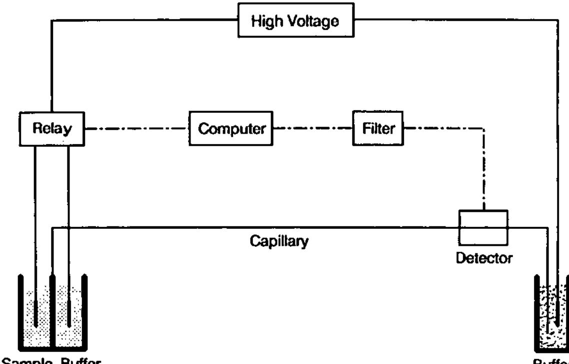


Fig. 12. Set-up of a correlation CZE system.

Much work still has to be done in the field of chemical modulation. In addition, chemical modulation is less general than conventional mechanical modulation CC and each modulator has to be optimized for a specific problem. In spite of all these problems, one may conclude that with a well designed system CC is not difficult for the experienced chromatographer, because a profound knowledge of the theoretical basis is not required.

The same arguments are valid when using the more complicated techniques (simultaneous CC, single sequence CC), but the demands on the system and on the knowledge of the operator required for optimum performance and interpretation of the results are higher.

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Review

Methods for the analysis of hazardous wastes

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ABSTRACT

This review covers the analytical methods for water and contaminated soils at hazardous waste sites. The different methods needed for rapid screening, target compound analysis and in-depth investigations, are presented. Field techniques, which are becoming commonplace, are also discussed.

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1. INTRODUCTION

The need to characterize the content of hazardous waste landfills has arisen from the public concern over their potential impact in the surrounding air and water. Certain chemicals deposited in landfills have been known to be potentially harmful if they volatilize in the air or leach in the groundwater.

Until recent years, a comprehensive characterization of chemicals in a landfill was an extremely onerous task. At best, gas chromatographs with an electron capture detector could reveal the presence of some industrial chemicals that were suspected to be present. Concentrations of metals were determined, one by one, by atomic absorption spectrophotometry.

The advent of gas chromatography–mass spectrometry (GC–MS) and inductively coupled plasma (ICP) greatly increased our capabilities, rekindling the interest in characterizing industrial effluents [1]. It now became possible to analyse replicate samples and address the question of analytical quality control. The fact that it was now possible to analyse samples in triplicate at a more reasonable cost, led to an awareness of the reliability and the limitations of our analytical capabilities. It also uncovered the problems of the representativeness of the samples.

This article discusses the methods that are used for different degrees of investigations: from the

broad screening methods used to characterize the degree of hazard of the waste, and target compound analyses where individual known toxic compounds are sought and quantitated, to methods that are used to for in-depth investigations and potential remediation. Soil, water and non-aqueous phase liquids are considered, but the primary emphasis is placed on leachate from the landfill and contaminated groundwater, because leaching tests are probably the most frequently used methods of characterizing solid wastes and because groundwater is the resource most threatened by hazardous waste sites. Some attention is given to field methods as well as laboratory methods, because the importance of rapid response for the assessment of remediation scenarios is increasingly being recognized.

2. SCREENING METHODS USED FOR WASTE CLASSIFICATIONS

Prior to doing any chemical analysis, any information that can be gathered regarding the origin of the waste should be used. Different countries have defined various criteria as to what constitutes hazardous wastes, but a consensus on a certain number of chemicals as hazardous has been reached by the OECD and UNEP [2]. The presence of any component listed in Table 1 is sufficient to classify the waste as hazardous. Some countries utilise concentrations to indicate whether or not waste containing one or more of the substances listed in Table 1 is hazardous, but doing so implies a high degree of monitoring and control which is beyond the means of many countries and hence is not the subject of an international concensus.

In any hazardous waste investigation, the primary need is to establish whether the waste poses an immediate threat to the investigator. In the first analysis, determining whether the waste is flammable, whether it may be mixed with water or whether it is corrosive or radioactive is often all that is necessary to proceed. This type of approach is used by emergency response teams [3] who need to know immediately how to respond to a problem. Their need for precision is not high, but they have to be rapid and correct in their assessment. Methods used include pH and starch iodine indicator papers, simple flame tests, portable gas analysers (OVA™) and Geiger counters. Many of the methods have been borrowed from the occupational health sector in industry.

The laboratory at the receiving dock of a hazardous waste landfill or incinerator uses much of the same technology in its decision to accept or reject a shipment of waste [4]. In addition, these laboratories have equipment such as GC, GC–MS and ICP that allow for more in-depth investigation or analyses for specific chemicals of interest such as polychlorinated biphenyls (PCBs).

Many of the screening methods, which were developed initially for the characterization of sewage effluents, are compiled in a handbook [5]. It covers parameters such as total dissolved solids, total organic carbon (TOC), biological oxygen demand (BOD), oil and grease, total phenols, cyanide, sulfate and nitrate. This handbook also contains specific methods for heavy metals such as lead, mercury and zinc. Many of the parameters are bulk or indicator parameters. They do not pertain to a single chemical but rather are used to give a comparative measure of the degree of contamination of a sample and its possible impact on the environment, and to indicate how it should be treated.

These tests are relatively inexpensive and may be performed very rapidly. Some of them can be easily done at the field site (pH, oil and grease, methane gas, total PCBs colorimetric test) and allow for a good primary response to describe the nature of the waste. Care must be exerted however in the interpretation of the results of some screening methods. Possibly the worst case is the analysis for total phenols using the colorimetric technique based on condensation with 4-aminoantipyrine. Although it is stated that only non-*p*-substituted phenols will produce the colour, attempts to correlate this with other techniques, such as HPLC, indicate that there are other discrepancies, mostly because phenol is used as the only reference standard [6]. In hazardous wastes, the presence of amines such as aniline interferes with the test [7]. TOC analysis should be used with caution in any samples where a large portion of the organic carbon is due to volatiles, because they will be largely lost when the sample is purged initially to remove the inorganic carbon as CO_2. Alternate methods similar to purge-and trap have been developed but are seldom used by commercial laboratories [8].

Waste generators must now assess the leaching

TABLE 1

INTERNATIONAL LIST OF HAZARDOUS SUBSTANCES

Adapted from ref. 2.

Code No.	Hazardous constituent
C 1	Beryllium and its compounds
C 2	Vanadium compounds
C 3	Chromium VI compounds
C 4	Cobalt compounds
C 5	Nickel compounds
C 6	Copper compounds
C 7	Zinc compounds
C 8	Arsenic and its compounds
C 9	Selenium and its compounds
C 10	Silver compounds
C 11	Cadmium and its compounds
C 12	Tin compounds
C 13	Antimony and its compounds
C 14	Tellurium and its compounds
C 15	Barium and its compounds; excluding barium sulfate
C 16	Mercury and its compounds
C 17	Thallium and its compounds
C 18	Lead and its compounds
C 19	Inorganic sulfides
C 20	Inorganic fluorine compounds; excluding calcium fluoride
C 21	Inorganic cyanides
C 22	Lithium, sodium, potassium, calcium, and magnesium as metals
C 23	Acidic solutions or acids in solid form
C 24	Basic solution or bases in solid form
C 25	Asbestos as dust or fibres
C 26	Phosphorus and its compounds; excluding mineral phosphates
C 27	Metals carbonyls
C 28	Peroxides
C 29	Chlorates
C 30	Perchlorates
C 31	Azides
C 32	Polychlorinated biphenyls and terphenyls
C 33	Pharmaceuticals or veterinary medicines
C 34	Biocides and phyto-pharmaceutical substances (*e.g.*, pesticides)
C 35	Infectious substances (*e.g.*, viruses)
C 36	Creosotes
C 37	Isocyanates, thiocyanates
C 38	Organic cyanides (*e.g.*, nitriles)
C 39	Phenols
C 40	Halogenated solvents
C 41	Organic solvents, non-halogenated
C 42	Organohalogen compounds excluding inert polymers
C 43	Aromatic compounds; polycyclic and heterocyclic
C 44	Aliphatic and other nitrogen organic compounds
C 45	Aromatic amines
C 46	Ethers
C 47	Explosives
C 48	Organic sulfur compounds
C 49	Polychlorinated dibenzofurans
C 50	Polychlorinated dibenzo-*p*-dioxins
C 51	Hydrocarbons and their oxygen, nitrogen or sulfur compounds

potential of their wastes and therefore their potential for groundwater contamination. Different governments have developed standardized protocols that must be followed by all industries within their territories. In the USA, the Environmental Protection Agency (EPA) has established standards for the treatment of restricted wastes and leaching procedures to evaluate the leaching potential of hazardous wastes. The Toxic Characteristic Leaching Procedure (TCLP) is designed to determine the leachability of metals, pesticides, semi-volatile and volatile organic compounds [9]. The leaching solution is dilute acetic acid. The leachate is then analysed using the SW846 methods [10]. In Canada, the province of Ontario has well defined pieces of legislation regarding the classification and the leaching of hazardous wastes, known as Regulation 309 [11]. It also advocates the use of a dilute acetic acid solution (0.5 *M*) to extract the wastes. The different types of leaching tests used world-wide have been described in a compendium report [12].

3. TARGET COMPOUND ANALYSIS

At the next level of response, samples are sent to the laboratory for characterization. The samples generally consist of water, soil or other solids, and oily waste. They are screened for the possible presence of chemicals that are known to be toxic and to be used in the environment. The reason for this approach is that it is easier and less expensive to determine, for example, if there are PCBs in a sample than to try to identify all the possible contaminants in the samples. Lists of priority substances, substances that are known to be toxic or to be generally deleterious to the environment and to human health, have been compiled. The best known and most comprehensive list is known as AppendixIX [13]. Appendix IX is a list of 222 compounds and is the shortened form of Appendix XVIII, which is a list of over 400 target chemicals contained in an appendix to the US EPA Hazardous Substance Act known as RCRA (Resource Conservation and Recovery Act). The list was shortened because almost half of the compounds on the original list could not be measured with any degree of accuracy using the existing methodology [14,15].

The analytical methods suggested by the US EPA for these compounds are contained in a manual generally referred to as the SW846 manual [10]. The SW846 manual was developed after the Effluent Guidelines Methods which were the first promulgated methods (1979, revised in 1984) for the analysis of water, and wastewater, including groundwater [16]. In the Effluent Guidelines Methods, the contaminants were divided into several analytical groups such as volatiles, base-neutrals and acids, pesticides (organo-chlorine) and PCBs, and metals. The methods are based on chromatography including GC, high-performance liquid chromatography (HPLC) or GC–MS for the organic compounds, and on spectroscopy, atomic adsorption (AA) or ICP for the metals. The volatiles are analysed by purge-and-trap–GC, whereas the semi-volatile analytes are extracted by dichloromethane and the extracts are analysed by gas chromatography with a selection of detectors.

The same framework is used for the SW846, except that it is recognized that many of the samples require some other form of preconcentration or cleanup, before they can be measured by the same methods as the effluents are. Much more autonomy is given to the individual analyst because of the diversity of matrices encountered. An excellent review of the SW846 methods and of their capability was written by Parr *et al.* [14]. Therefore, they will only be discussed briefly here.

Only the USA have proclaimed such a comprehensive list of methods. However, researchers and government authorities have designated target compounds and many methods, other than the US EPA methods, have been used world-wide. Those methods will be discussed here in parallel and grouped according to target groups of compounds, in water or liquids and soil or solids.

3.1. *Volatiles*

This is the first analytical group to be considered in dealing with liquid wastes. It covers chlorinated methanes and ethanes and the aromatic components of light petroleum distillates (benzene, toluene, xylenes, *etc.*). For water, purge-and-trap is the most used technology (ref. 10, method 5030) and was borrowed from the Effluent Guidelines Methods. Purge-and-trap, initially developed by Bellar and Lichtenberg [17], consists of bubbling gas through an aqueous sample to strip it of its most volatile components. The gas is passed through a

solid sorbent such as Tenax [16] or Carbotrap [18] onto which the analytes are adsorbed, then thermally desorbed on-line into GC or GC–MS. A combination of electron-capture and Hall detectors has been used for the simultaneous measurement of chlorinated and aromatic hydrocarbons [19].

Some investigators have used adsorption onto cartridges followed by thermal desorption [20,21]. Sorption from water directly onto cartridges allows for sampling groundwater down-hole [22]. Microcartridges [23] and uncoated capillary columns [24] have also been used instead of polymeric sorbents. Adsorption on cartridges is also used for air samples and volatile organic sampling trains (VOST) at incinerators where a given amount of air is pumped through the sorbent, which is then thermally desorbed [10 (method 3720), 25]. Purging directly onto a capillary column with whole column cryotrapping, *i.e.* cooling down the whole gas chromatograph to condense the analytes, was used successfully by Pankow and Rosen [26] for petroleum contaminated water. The use of a trap to dessicate the hot gas stream before it reaches the chromatograph is essential to prevent ice formation in the capillary column.

Purge-and-trap is the most often employed method in the USA, but in other jurisdictions and for samples where excessive foaming occurs, headspace analysis is also often used [10,27–31]. Headspace analysis is more conveniently conducted in the field. Groundwater samples are rapidly screened in the field using partially filled vials sealed with a septum. A volume of the headspace is injected into a portable gas chromatograph equipped with a photoionization detector such as the Photovac™. Detection limits down to 1 μg/l have been obtained for benzene [32]. Liquid–liquid extraction with pentane [33] or hexane [34] followed by injection into a GC or GC–MS has been used for the measurement of halogenated hydrocarbons and aromatic hydrocarbons in landfill leachate.

Solvents such as 1,4-dioxane or tetrahydrofuran, which are highly soluble in water, are not efficiently analysed by purge-and-trap or headspace analysis because they do not partition sufficiently into the gas phase. For such samples, the technique of dynamic thermal stripping (DTS) has proven effective [35]. DTS is similar to purge-and-trap, but the purging is done at a higher temperature onto a cartridge, which is thermally desorbed subsequently.

More unusual is the use of HPLC to measure benzene, toluene, ethyl benzene and xylenes [36]. The separation was done on a C_{18} column eluted with 75% methanol and 25% water. The detection limit was quoted as 5–10 μM.

Soil samples present a special challenge in volatile analysis. Any attempt at homogenizing or drying the sample will result in significant loss of analyte. The samples are therefore analysed wet and the dry weight is determined on a separate aliquot. For purge-and-trap analysis, the sample is first dispersed in polyethylene glycol or methanol. An aliquot of the sample extract is then added to water and analysed by purge-and-trap [10 (method 5030)]. Some investigators have simply added water to a soil sample in the purging vessel [37,38]. The sorbent desorption has most often been thermal, but extraction with CS_2, a method borrowed from the occupational health sector, has also been used [38].

Soil vapour analysis can also be conducted in the field. The headspace in a specially designed monitoring well is sampled and analysed using a gas chromatograph with a photo-ionization detector, which can measure most solvents, from aromatic hydrocarbons to chlorinated hydrocarbons. The limitations of the technique have been described by Hughes *et al* [39]. As in any headspace technique, the control of the sample temperature, is crucial to the accuracy of the results. There are obviously inherent problems to achieving temperature control in the field. Excellent replication (10% R.S.D.) can be attained if attention is paid to the potential leaks in the system and to the integrity of sampling syringes. Other investigators have used a mobile laboratory equipped with gas chromatographs where soil gas samples taken from stainless steel hollow probes could be analysed on site [40].

3.2. Semi-volatiles

Semi-volatiles is an operational term coined by the US EPA to designate compounds that are not sufficiently volatile to be analysed by purge-and-trap–GC, but could be volatilized in a hot injector and analysed by GC. The compounds range in volatility from dichlorobenzenes to benzo-[*ghi*]-perylene. The Effluents Guidelines Protocol [16] subdivides the semi-volatiles into three main groups: the base-neutrals, the acids, and the organo-chlorine pesticides and PCBs. Base-neutrals and acids are

TABLE 2

SAMPLE EXTRACTION TECHNIQUES FOR SEMI-VOLATILES, SW846 METHODS [10]

Method	Matrix	Description
3510	Aqueous	Separatory funnel liquid–liquid extraction with dichloromethane
3520	Aqueous	Continuous liquid–liquid extraction with dichloromethane
3540	Soils, sludges and wastes	Soxhlet with acetone–hexane (1:1) or toluene–methanol (10:1) or dichloromethane
3550	Soils, sludges	Sonication with same solvents as in 3540

separated by the extraction at pH 10 and pH 2, respectively, then analysed by GC–MS. The base-neutral fraction contains compounds such as polyaromatic hydrocarbons, phthalate esters, chlorinated ethers and nitrosamines. The acid fraction contains phenol, methyl phenols and chlorinated phenols. The pesticides and PCBs are extracted separately at neutral pH and the final analysis is conducted by GC with an EC detector. The SW846 equivalent to this method is method 8270, which describes the GC–MS determination [10]. The difference is mainly in the number of analytes for which the method is validated and in the alternate sample preparation techniques (Table 2).

As was touched upon in the screening test section, the analysis for phenols can be a very challenging task especially in complex matrices. When dichloromethane is used, emulsions are often generated which reduce the efficiency of extraction. Alternate solvents have been tried; benzene was found not to be efficient and butyl acetate entrained too many co-extractives. The best results were obtained with diethyl ether although many laboratories might be reluctant to use it because of its high volatility and instability [41].

Alternately, phenols have been analysed by HPLC using electrochemical [42–44], UV detection [45–47] or both [48–50]. The electrochemical detector has not received as wide acceptance as it could, probably because of the instability and maintenance requirements of the early models. The selectivity and sensitivity of that detector make it an invaluable tool in monitoring ionizable molecules in complex matrices. HPLC with UV detection at 202 nm was also proposed for rapid screening of 32 priority pollutants [51]. The method did not gain widespread acceptance, probably because of the difficulty of working at such a low wavelength.

Sorption on cartridges followed by solvent desorption has also been used as a sample preconcentration technique. In a study where naphthalene was the only analyte, Borden and Bedient [52] passed 100 ml of an aqueous sample through a C_{18} Sep-Pak (Waters/Millipore) and desorbed the analyte with dichloromethane before analysing it by GC. More often the adsorption on cartridges has been followed by thermal desorption directly into a GC–MS [53–55]. In such cases the sorbents have been either Tenax or Carbotrap (Supelco) which are thermally stable. Dynamic thermal stripping instead of direct adsorption has also been used [7,55]. Dynamic thermal stripping is best described as purge and trap at a higher temperature and is a direct extension of the method used for volatiles. The technique cannot be used for very high boiling compounds, but has been successfully used up to the boiling range of pyrene.

A modified thermal-desorption–GC–MS system, capable of analysing PCBs in soils and soil extracts, has been devised [56]. It consists of a thermal probe that is put in direct contact with the soil sample. A 3.5-m capillary column is housed in a piece of tubing with a coil heater instead of the conventional GC oven. For quantitative analysis, the soil sample must be extracted first with hexane, which is vaporized by the probe. The instrument is battery powered and therefore can operate in the field.

3.3. Inorganic compounds

The analysis for inorganic salts and metals at hazardous waste sites has received less attention, because the methods used vary less for complex matrices than those for organic compounds. Ion chromatography has revolutionized the way most anion analyses are conducted [5]. Cations may also be determined by ion chromatography, but are most of-

ten analysed as the metals by either AA or ICP [5,10]. For complex matrices, directly coupled plasma (DCP) is often preferred because it is less subject to interferences and will accept samples with higher organic content [57,58]. X-Ray fluorescence spectrometry was used to measure metals in leachate from wood fly ash [59].

The methods for metals in aqueous or solid matrices differ mostly by their acid digestion procedures. Depending on whether the sample will be introduced by in a flame or graphite furnace AA or an ICP detector, the initial digestion is done in nitric acid, followed by a second hydrochloric acid digestion for the flame instrument. Hydrochloric acid cannot be used in the graphite furnace. The methods are adequate for aqueous samples, most soils and sediments and sludges. Some waste materials, such as oil base paints, can cause difficulty and the analysts are always cautioned to verify the results by accompanying the samples with suitable spiked samples to prevent bias due to interferences. The digestion procedures are often adjusted by the analyst to cope with specific interferences, but these adjustments are not considered significant alterations and are seldom published as separate methods. An exception to this was the publication of an alternate preparation procedure for chromium in spent ores with the use of fusion with sodium carbonate and sodium peroxide instead of the digestion in nitric acid [60]. The concentrations reported were an order of magnitude higher with a method derived from that of the American Society for Testing of Materials (ASTM) than with the SW846 method. The most suitable methods varies depending on whether the hazardous waste resembles the original product or it has been mixed with a subtantial amount of soil and water.

Artifacts due to sampling equipment and filtration of samples in the field have also been identified. Sampling devices which cause a lot of sample turbidity led to less reproducible results [61]. While it is customary to filter all groundwater samples for metal analysis, discrepancies of up to an order of magnitude can be found in unfiltered compared to filtered samples [62]. If the data is to be used for exposure assessment, whether filtered or unfiltered samples should be used remains a topic of discussion. Retaining the redox environment from which the samples are taken is also important in obtaining representative results.

Field methods are very important for inorganic constituents that are subject to alterations in the presence of oxygen. Alkalinity, dissolved oxygen, hydrogen sulphide, ferrous iron must all be measured in the field to get an accurate reflection of the groundwater condition [63]. A field-portable X-ray fluorescence spectrometer is now available. Its use has now been investigated for the rapid screening of metals and offers great potential for on-site analysis [64]. It has been used in industries such as steel mills, foundries and even the petroleum sector. It is not as popular in the environmental field because it may not be as sensitive as other methods. However, since high concentrations of contaminants are usually present at hazardous waste sites, very sensitive detectors are not necessary [65].

4. IN-DEPTH INVESTIGATIONS

In spite of all the activity at hazardous waste sites, few detailed accounts of contaminants other than priority pollutants have appeared in the refereed literature. One of the first of such investigations described the organic contaminants near dump sites at Niagara Falls, New York. Compounds were identified using solvent extraction and vapour stripping followed by GC–MS [66]. Solvent extraction of contaminated groundwater and soils followed by GC–MS, accounts for almost all the analytical methods in these investigations [33, 67–71], but Reinhart *et al.* added derivatization of the acid fraction with diazomethane to improve the chromatography of phenols and carboxylic acids [33].

In spite of its inability to identify unequivocally many of the compounds, the GC–MS trace and spectra were still useful in helping to trace the source of a groundwater plume that was potentially emanating from two adjacent landfills [72]. In some investigations, dynamic thermal stripping or adsorption onto cartridges followed by thermal desorption was substituted for solvent extraction to prepare the samples, but GC–MS was still the main technique used to identify the compounds [7,44].

Alternate techniques were employed by scientists of the US Geological Servey to characterize fully their study sites. Pereira *et al.* [73] used probe distillation with high-resolution MS in addition to GC–MS to identify the organic bases derived from coal tar wastes. Goerlitz, a pioneer in the field of

groundwater contamination at hazardous waste site, was the first to advocate the use of HPLC at the site, to measure phenolic compounds at coal tar contaminated sites [74,75] and to look at compounds that were possible indicators of microbiological activity rather than only the contaminant themselves [76]. At the Cape Cod site, Barber [58] used a large variety of methods to characterize the extent of groundwater contamination: closed loop stripping, purge-and-trap analysis, liquid–liquid extraction and solid phase extraction onto *n*-octyl silica and XAD-8 resin were used as isolation methods. Derivatives were made to analyse for anionic surfactants by electron-capture negative ionization mass spectrometry. Linear alkyl benzenesulfonates were analysed by HPLC with a fluorescence detector. XAD isolates were also subjected to ^{13}C NMR and infra-red analysis as an attempt to identify the surfactants better.

Attempts were made to characterize the molecular weight distribution of groundwater contaminants at a Canadian site. Barker *et al.* [77] used dialysis to separate the DOC according to molecular weight groups, and then used IR to get an indication of the functional groups present within the different fractions and compared it to spectra of fulvic acids [77].

HPLC–MS was used by Ho *et al.* [78] to solve an analytical problem that had received fairly little attention. It is not possible to distinguish between diphenylamine and its carcinogenic N-nitroso derivative because of the thermal instability of the latter. They were able to prove the absence of N-nitrosodiphenylamine in a soil sample from a dumpsite although it contained a large amount of diphenylamine. Attempts have been made to characterize the non-extractable fraction of landfill leachate using anion-exchange chromatography–particle beam–MS and ICP–MS. Although good quality spectra were obtained, few compounds could be identified because they could not be matched with spectra contained in computerized libraries [79]. This is not surprising because these have been the result of GC–MS analyses. As the techniques of particle beam and thermospray–MS gain popularity, their usefulness should increase. Also, more complementary techniques such as FT–IR and ICP–MS will be needed to provide more structural information on the unknowns.

5. CONCLUSIONS AND FUTURE DIRECTION

Compared to the total worldwide effort in environmental analytical chemistry, there have been, in effect, relatively few attempts at fully characterizing hazardous wastes. Reports still routinely contain statements such as: "only 30% of the contaminants could be identified, the rest are suspected to be high-molecular-mass or polar material". This phrase summarizes what the agenda of the analytical chemists should be in the next decade. There is a need to complement GC–MS with techniques that will allow the measurement of polar and higher-molecular-mass compounds. Only a few references could be found mentioning the use of HPLC–MS for hazardous wastes, although the technique is more than 10 years old. GC–FT-IR has not gained wide acceptance as an environmental analytical tool either, mostly because of its lack of sensitivity. This is not a problem at hazardous waste sites where high concentrations of analyte usually prevail. As the need to remediate highly contaminated sites is increasingly recognized as the priority, more emphasis will be placed on finding suitable cost effective methods to analyse their chemical constituents.

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Review

Environmental chromatographic methods and regulations in the United States of America

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ABSTRACT

The five major monitoring programs and the pesticide program administered by the US Environmental Protection Agency (EPA) are reviewed. Gas chromatographic (GC) and high-performance liquid chromatography (HPLC) methods used by each EPA program for organic analysis are described. Quality control is the major difference amoung methods in different programs. Trends for the future of environmental analyses in the US are discussed.

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1. INTRODUCTION

The US Environmental Protection Agency (EPA) interprets legislation enacted by the US Congress and drafts regulations to implement and enforce the perceived intent. The EPA was created in 1969 by the US Congress to consolidate all environmental functions, previously performed by diverse agencies, under one regulatory body. Since the EPA's inception, over 25 major environmental acts, or amendments to existing acts, have been passed by the US Congress. While some environmental programs are administered directly by the EPA, others can be administered through individual states. Individual states may accept "primacy" for a particular EPA program, which means they have demonstrated analytical proficiency and accept responsibility for implementation and enforcement.

The EPA organizational structure has grown out of the needs of the agency to comply with Congressional mandates [1]. Fig. 1 shows a simplified schematic of the current structure of the EPA. The different EPA Offices are centered around types of sample media: water, solid waste, air, and pesticides. Each Office has set up its own research programs and developed its own analytical methodology. This has led to a proliferation of methods, many of which differ only slightly in the sample preparation or analytical methodology [2]. Table 1 lists the relevant legislative acts and the resulting series of analytical methods by each type of medium. Consider the determination of chlorobenzene for which 13 gas chromatographic (GC) methods are available. In the USA, where the bulk of the environmental analytical work is performed by private laboratories, this has led to confusion over the proper method to use with a specific sample.

This paper reviews the five major EPA monitoring programs for drinking water, wastewater, solid and hazardous materials, and air. This paper also reviews the EPA's pesticide registration program, which is heavily dependent on chromatographic techniques. The chromatographic methods for each program are listed and their commonalities and differences highlighted. Sampling techniques, column requirements, and detection systems are reviewed. The unique performance characteristics of GC and high-performance liquid chromatography (HPLC) are described. Quality assurance and control (QA/QC) procedures necessary for accurate environmental analyses are discussed.

2. ENVIRONMENTAL LEGISLATION AND THE FIVE MAJOR MONITORING PROGRAMS

2.1. Wastewater

The Clean Water Act of 1977 gave the EPA the responsibility to regulate industrial discharges into

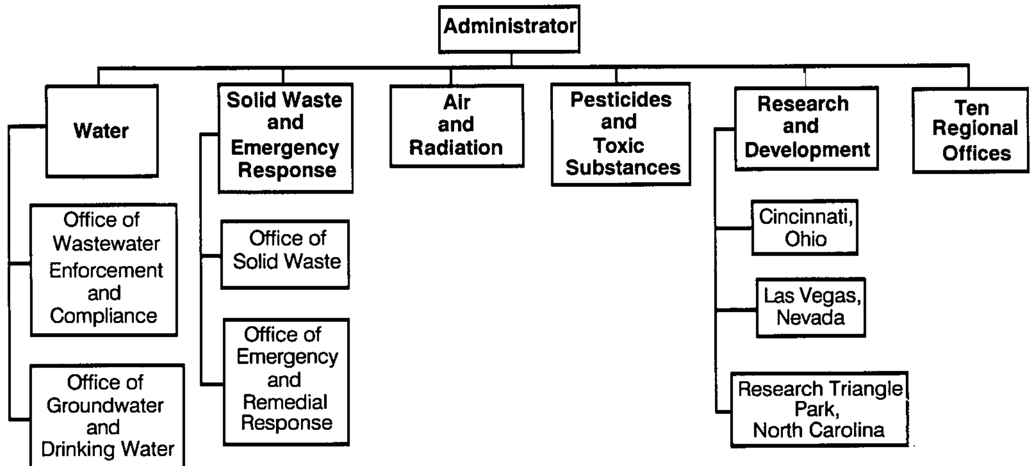

Fig. 1. Simplified structural diagram of the US EPA, showing the major monitoring program offices and regional research and development offices.

TABLE 1

KEY ENVIRONMENTAL LEGISLATION AND OFFICIAL ANALYTICAL METHODS FOR ORGANICS

Sample	Legislation and year of enactment	Methods
Wastewater	Federal Water Pollution Control Act (FWPCA) 1972, 1977 or Clean Water Act	EPA 600-Series
Drinking water	Safe Drinking Water Act (SDWA) 1974, 1977, 1986	EPA 500-Series
Solid waste	Resource Conservation and Recovery Act (RCRA) 1976, 1980, 1984 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) 1980 Superfund Amendments Reauthorization Act (SARA) 1986	SW-846 Methods (8000-Series) Statement of Work (SOW)
Air	Clean Air Act (CAA) 1970, 1990	Toxic Organic Series (TO)
Pesticides	Toxic Substances Control Act (TSCA) Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)	Company proprietary

surface waterways to maintain their quality. The EPA Office of Wastewater Enforcement and Compliance created the National Pollution Discharge Elimination System (NPDES) to permit the regulation of individual point sources. Guidelines have been established for the discharges of over 50 types of industries, and specific permits are negotiated between the wastewater generator and the regional EPA office or state, when state primacy applied. The 600-series of analytical methods [3] using GC, GC–MS, and HPLC have been developed by the Environmental Monitoring Support Laboratory in Cincinnati, OH, USA to aid in setting guidelines and enforcing permit requirements. Wastewater typically requires extraction, concentration, and cleanup with silica or florisil before introduction into the instrument.

2.2. Drinking water

Drinking water regulations [4] arise from the Safe Drinking Water Act passed by Congress in 1974. The EPA Office of Ground Water and Drinking Water protects public health through the National Primary Drinking Water Regulations. These regulatory limits are based on health criteria. As with all federal regulations, they are published in the Federal Register and set the maximum amount of organic and inorganic contaminants allowed in a drinking water supply. The National Secondary Drinking Water Regulations set contaminant levels which, if exceeded, will affect the aesthetic quality of a water supply. Secondary levels are EPA guidance, and as such, are not enforceable at the federal level. The EPA's Office of Drinking Water and Ground Water Protection adopted the 500-series of methods [5,6] for the determination of organic compounds in drinking water because the 600-series methods developed for wastewater analysis lack the sensitivity necessary to enforce the drinking water maximum contaminent levels (MCLs). The 200-series of methods [7] are used to determine inorganic components in both drinking water and wastewater.

Drinking water is a simple matrix, and filtration may be the only sample preparation step required. However, sample concentration is often needed, using either solid-phase extraction or methylene chloride extraction, to obtain the trace level (part-per-billion to part-per-trillion) required in the regulations. Direct injection is possible for some HPLC methods, such as the methods for the analysis of carbamate pesticides or the herbicide glyphosate, utilizing extremely sensitive and selective detection techniques (post-column reactions with fluorescence detection).

2.3. Solid and hazardous waste

There are two EPA programs with jurisdiction over solid and hazardous waste. The first program covers the more routine aspects of toxic transportation, storage and disposal (TSD) of hazardous ma-

terials. The second program establishes procedures to handle emergency releases of hazardous materials as well as dealing with hazardous waste sites that have been abandoned and require cleanup.

The Resource Conservation and Recovery Act (RCRA) of 1976, updated by the Hazardous and Solid Waste Amendments of 1984, directed the EPA to take "cradle to grave" responsibility for hazardous materials. This includes management of wastes through transportation, storage, and disposal. Most waste materials generated by the USA population are disposed of in municipal landfills or by incineration in waste disposal furnaces. However, hazardous waste must be treated separately (and more expensively). Determining whether a waste is hazardous has a great effect on the cost of disposal. Wastes are either listed as hazardous because of known hazardous compounds or are characterized to determine whether they are hazardous through four tests. Characterization tests determine reactivity, ignitability, corrosivity, and toxicity. The toxicity test is the most quantitative; it employs the Toxicity Characteristic Leaching Procedure (TCLP) to extract potentially toxic components into an acetic acid buffer solution. The solution is then analyzed for 31 organic components, using mostly GC–MS techniques.

The Office of Solid Waste has created four volumes of analytical methods covering a variety of analytes in soil, solid waste, oily matrices, and groundwater. The method compendium, entitled Test Methods for Evaluating Solid Waste: Physical/Chemical Methods, known colloquially as the SW-846 set of methods [8], contains the 8000-series methods for GC, GC–MS, HPLC, and HPLC–MS. Complex soil, sludge, and waste matrices are commonly analyzed by this program; some of the sample extraction and clean-up methods are listed in Table 2. Other programs often incorporate the sample preparation steps into the specific analytical method.

TABLE 2

EPA EXTRACTION AND SAMPLE CLEANUP METHODS USED IN TEST METHODS FOR EVALUATING SOLID WASTE (SW-846)

3510	Methylene chloride extraction
3520	Continuous liquid–liquid extraction
3540	Methylene chloride–acetone soxhlet extraction
3541	Automated soxhlet extraction
3550	Methylene chloride–acetone ultrasonic extraction
3580	Waste dilution
3610	Sample cleanup (alumina)
3620	Sample cleanup (florisil)
3630	Sample cleanup (silica gel)
3640	Sample cleanup (gel-permeation chromatography)
3650	Sample cleanup (acid–base partition)
3660	Sample cleanup (sulfur)
3665	Sample cleanup (sulfuric acid–permanganate)

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980, updated by the Superfund Amendments and Reauthorization Act (SARA) of 1986, prompted the EPA to create a unique program known as "Superfund" to clean up abandoned hazardous waste sites using funds derived from a special petrochemical tax. The EPA's Office of Emergency and Remedial Response administers the program. Analytical methods used for contamination assessment and cleanup monitoring are derived from other programs and modified to suit the special needs of Superfund. Generally, the quality assurance and control measures required by Superfund are more stringent, because data may be used in a court of law to help prove the source of pollution and recover cleanup costs for a contaminated site. Methods developed for this program are published in a contractual statement of work (SOW), which details the sample preparation, analysis, and QA/QC requirements to be used by analytical labs who work for the EPA under contract. Sample lots are awarded to various contract laboratories through a competitive bid process. Routine analyses are performed using GC–MS for 33 volatile organic compounds and 64 semivolatiles, and GC with an electron-capture detector for 28 target pesticides.

2.4. Air

The Clean Air Act of 1983, amended in 1990 (CAA), governs ambient air, stack sources, and moving sources of chemical contamination. To date, ambient air quality standards have been established for six components (NO_x, SO_2, ozone, CO, lead, and particulate matter smaller than 10 μm) using methods published in the US Code of Federal

Regulations. New Source-Performance Standard (NSPS) regulations for new sources suggest use of the toxic organic (TO) series of methods [9] for organics to verify compliance with individually negotiated permits, in much the same fashion as the wastewater NPDES program. The EPA Office of Air and Radiation is quickly moving to implement regulations to limit and additional 189 compounds in stack sources, moving sources, an ambient air as required by the recent CAA. New methods will need to be developed or adapted from other programs to determine the newly regulated contaminants.

2.5. Pesticides

From the EPA standpoint, pesticide analytical methods have been developed in support of registration petitions to the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA). The methods used can be company-proprietary and thus not widely available. This is in contrast to the published methods readily available for the other programs.

Pesticide determinations are made for samples such as drinking water, wastewater, and solid waste using EPA methods and in residual analysis of foodstuff (regulated by FDA) using American Society for Testing and Materials (ASTM), Association of Official Analytical Chemists (AOAC), and National Pesticides Survey (NPS) methods [10,11]. In addition, TSCA requires chemical manufacturers to notify the EPA using a Premanufacture Notice (PMN) describing structure, impurities, byproducts, environmental fate, and toxicology data. Company-proprietary methods (mostly HPLC) are used to generate assay, stability, residue, and metabolism data under the guidelines of Good Laboratory Practices (GLP).

3. EPA GAS CHROMATOGRAPHY METHODS

In the previous section, legislation was described as the driving force to develop different EPA programs, which in turn, developed different methods. In this section we review the instrumentation specified by the EPA methods. The sample introduction systems, sample concentration devices, and detectors generally used are described. The EPA methods are segmented by program and the detection technique specified in the method to show the diversity and commonality of program analyte requirements.

3.1. Sample introduction and concentration techniques

EPA GC methods utilize common sample introduction and concentration techniques for liquid and volatile samples. For liquid samples (*e.g.*, semivolatiles in extracts from water, air, and soil), the conventional flash vaporization injectors are used with packed columns. Both split and splitless injectors are also used with narrow and wide-bore fused-silica capillary columns specified in the newer EPA methods [12]. For compounds which might decompose under flash vaporization injection conditions, cold direct liquid injection or temperature-programmed vaporization techniques are preferred.

For volatiles and gaseous compounds, pneumatic sampling valves with cryogenic trapping are commonly used with several novel sample concentration devices developed specifically for water or air samples. Purge-and-trap devices are designed as online sample extraction and concentration systems for purgeable organics in water samples. First introduced in the late 1970s, they have become rugged and automated for routine determination of volatile compounds which are otherwise easily lost in the conventional liquid extraction process.

Headspace analysis is used as a quick turnaround method (CERCLA program) for evaluation of contaminated soil and groundwater at cleanup sites. Headspace sampling eliminates sample cleanup by using heat to desorb organics from a solid or liquid matrix into the headspace of an enclosed vial. The technique can be automated and is quantitative after appropriate calibration. Quick turnaround methods are used during cleanup of contaminated waste disposal sites where analytical data direct the cleanup efforts in progress. The same technique is particularly useful for screening purgeables in soil samples that often plague purge-and-trap systems due to cross-contamination problems [13].

For the determination of volatile organics in ambient air, adsorption systems using Tenax (Method TO-1) or carbon molecular sieves (Method TO-2),

TABLE 3

EPA GC METHODS

Method	Wastewater		Drinking water		Air	
GC–FID	603	Acrolein and acrylamide			TO-3	Volatile organic compounds (VOC)
	604	Phenols			TO-12	Non-methane organic compounds
	609	Nitroaromatics and isopherone			TO-13	Benzo[*a*]pyrene and PAHs
	610	PAHs				
GC–ECD	606	Phthalate esters	501.2	Trihalomethanes (liquid–liquid extraction)	TO-3	Volatile organic compounds
	608	Organochlorine pesticides and PCBs	504	EDB and DBCP	TO-4	Organochlorine pesticides and PCBs
	608.2	Organochlorine pesticides and PCBs	505	Organohalide pesticides and PCBs	TO-10	Organochlorine pesticides
	612	Chlorinated hydrocarbons	508	Chlorinated pesticides		
	627	Dinitroaniline pesticides	508A	Chlorinated pesticides and PCBs (derivatized)		
			515.1	Chlorinated acids		
			548	Endothall		
GC–PID	602	Purgeable aromatics	503.1	Volatile aromatics (purge and trap)		
GC–ELCD	601	Purgeable halocarbons	502.1	Volatile organic compounds (purge-and-trap)		
	611	Haloethers	502.2	Volatile organic compounds (purge-and-trap, ELCD and PID)		
			501.1	Trihalomethanes (purge-and-trap)		
GC–NPD	607	Nitrosamines	507	Nitrogen and phosphorus pesticides		
	645	Amine pesticides				
	633	Organonitrogen pesticides				
GC–FPD						
GC–MS	624	Purgeables	524.2	Purgeables (purge-and-trap, capillary column)	TO-1	Volatile organic compounds (capillary)
	625	Base/Neutrals (packed)	525	Organics (liquid–solid extraction, capillary column)	TO-2	Highly volatile organic compounds
	625.1	Base/Neutrals (capillary)	501.3	Trihalomethanes (SIM)[a]	TO-14	Volatile organic compounds (GC–MS and other detectors)
	680	Pesticides and PCBs			TO-13	Benzo[*a*]pyrene and PAHs by GC–MS and GC–FID
	613	Dioxin			TO-9	Dioxin (GC–HRMS)[a]
	1624	Volatile organic compounds (isotope dilution)			TO-7	N-Nitrosodimethylamine
	1625	Semi-volatile compounds (isotope dilution)				
GC–FT-IR						

[a] Abbreviations: SIM = single ion monitoring; HRMS = high-resolution mass spectrometry.

RCRA		CERCLA
8030	Acrolein, acrylamide, acetonitrile	
8040	Phenols	
8060	Phthalate esters	
8090	Nitroaromatics	
8015	Non-halogenated volatile organics (purge-and-trap)	
8060	Phthalate esters	Pesticides/Arochlors, Routine Analytical Services Statement of Work, OLMO1.8, 8/91
8061	Phthalate esters (capillary)	Pesticides, Special Analytical Services, Low Concentration Water SOW, 9/90
8080	Organochlorine pesticides	
8081	Organochlorine pesticides (capillary)	
8090	Nitroaromatics and cyclic ketones	
8120	Chlorinated hydrocarbons	
8121	Chlorinated hydrocarbons (capillary)	
8150	Chlorinated herbicides	
8151	Chlorinated herbicides (capillary)	
8100	PAHs	
8085	PCBs (derivatized)	
8032	Acrylamide	
8011	EDB and DBCP	
8045	Endothall	
8020	Volatile aromatics (purge-and-trap)	
8021	VOCs (purge-and-trap, ELCD–PID in series)	
8010	Halogenated VOCs (purge-and-trap)	
8021	VOCs (purge-and-trap, ELCD–PID in series)	
8080	Organochlorine pesticides and PCBs	
8150	Chlorinated herbicides	
8151	Chlorinated herbicides (capillary)	
8110	Haloethers	
8070	Nitrosamines	
8141	Organophosphorus compounds (capillary)	
8031	Acrylonitrile	
8145	Alkylphosphates	
8140	Organophosphorus pesticides	
8141	Organophosphorus compounds (capillary)	
8240	VOCs (purge-and-trap)	Volatile Organic Compounds, Routine Analytical Services Statement of Work, OLMO1.8, 8/91
8260	VOCs (purge-and-trap, capillary)	Semi-volatile Organic Compounds, Routine Analytical Services Statement of OLMO1.8, 8/91
8266	VOCs (isotope dilution)	Volatile Organic Compounds, Special Analytical Services Statement of Work for Low Concentrations in Water, 9/90
8250	Semi-volatile organics	Volatile Organic Aromatics, Special Analytical Services Statement of Work for Low Concentration in Water (based on 524.2), 9/90
8270	Semi-volatile organics (capillary)	
8276	Semi-volatile organics (isotope dilution)	
8280	Dioxin	
8275	TC–MS for semivolatile screening	
8290	PCDDs and PCDFs by HRGC–HRMS	
8410	Semivolatile organics (capillary)	
8415	Tris-2,3-dibromopropyl phosphate	
8430	Bis(2-chloroethyl)ether	

as well as cryogenic preconcentration trapping (Method TO-3) or SUMMA canister sampling techniques (Method TO-14) are used. These devices are used off-line or on-line with a GC or GC–MS system.

3.2. Detection techniques

Table 3 lists the EPA methods using gas chromatography with various detectors, and are categorized by the major EPA programs.

Gas chromatography is the traditional analytical technique for volatile organics. Because EPA methods often target trace levels of specific compound types in complex matrices, selective detection offers the most effective approach. Thermal conductivity detection (TCD) is not generally used in EPA methods because of its low sensitivity. Flame ionization detection (FID) is used, typically after extensive sample cleanup, and only if a more specific detector is not available.

Electron-capture detection (ECD) is used extensively for pesticides, chlorinated compounds, and phthalates in all five EPA programs. Nitrogen–phosphorous detectors are used for the analysis of nitrosamines, amines, acrylonitrile, and nitrogen- and phosphorous-containing pesticides. Flame photometric detection (FPD) is used for organophosphorous pesticides in method 8140 and draft method 8141. Electrolytic conductivity detection (ELCD) and photoionization detection (PID), rarely used elsewhere, are employed often in tandem for purgeable organics (Methods 502.2 and 8021). ELCD is very selective for halogenated compounds, while PID, using a low-energy lamp, is selective for aromatics.

Mass spectrometry (MS) with confirmatory capability is the preferred detector for survey methods and for screening samples as evidenced by its predominance in all five monitoring programs. When operating in the scanning mode, MS functions as a universal detector with only moderate sensitivity. Both sensitivity and selectivity can be increased significantly by using the single-ion monitoring (SIM) mode (*e.g.*, Method 501.3 for trihalomethanes in drinking water). Quite often, better detection limits can also be achieved by switching to a selective detector. For example, the detection limit for benzene using purge and trap and GC–MS can be lowered from 0.03 μg/l (in Method 8260) to 0.009 μg/l (Method 8021) by photoionization detection.

With improvements in interface technology and detection sensitivity, use of Fourier transform infrared spectroscopy (FT-IR) is increasing as an ancillary technique for GC. FT-IR often complements MS data by providing additional information for isomer and structure identifications. Three RCRA methods using GC–FT-IR (8410, 8415, and 8430) are currently being proposed.

4. EPA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHODS

The number of official EPA methods using HPLC has grown dramatically to more than 40 approved and draft methods [14–16]. In searching for more cost-effective methods, the EPA recognizes the applicability of HPLC for non-volatile, thermally labile, and polar materials. With the trend towards biodegradable pesticides, HPLC is becoming the preferred analytical method for most insecticides and their metabolites, herbicides, and plant growth regulators. Key target pesticides include carbamates, diquat and paraquat, triazine, phenylureas and glyphosate. In addition, HPLC is also amenable to acids, bases, surface active agents, dyes, and aromatic amines [17].

The advantages of HPLC include sensitive and selective detection for compounds with ultraviolet (UV) absorbance or natural fluorescence. HPLC also tolerates large-volume injections of aqueous samples, rendering it ideal for screening water samples. Reversed-phase chromatography is used almost exclusively in all EPA HPLC methods, with the exception of ion-exchange chromatographic methods for ion chromatography, glyphosate, and sulfonic acids. Gel-permeation chromatography (GPC) and liquid–solid chromatography are primarily used for sample cleanup.

4.1. Detection techniques

Table 4 lists the EPA HPLC methods segmented by detection system.

UV–Visible absorbance and fluorescence detectors are the primary detectors used. They are extremely sensitive for components with high molar absorptivity (ng levels) or fluorescence (pg levels).

TABLE 4

EPA HPLC METHODS

Method	Wastewater		Drinking water		Air		RCRA		CERCLA
HPLC–UV	610	PAHs	549	Diquat and paraquat	TO-5	Aldehydes and ketones	8310	PAHs	Pesticides/Aroclors, Routine Analytical Services Statement of Work, OLMO1.8 8/91, GPC cleanup
	631	Benomyl and carbendazim	550	PAHs	TO-6	Phosgene	8315A	Aldehydes and ketones (2,4-DNPH derivatized)	
	632	Carbamates and urea pesticides	550.1	PAHs	TO-8	Phenols and cresols	8316	Acrylamide, acrylonitrile and acrolein	
	604.1	Hexachlorophene and dichlorophene			TO-11	Formadehyde	8317	4,4′-Methylene bis(2-chloroaniline) (MOCA)	
	629	Cyanazine			TO-13	PAHs	8321	Azo dyes, amines, organophosphorus compounds by HPLC–TSP–MS[a]	
	635	Rotenone					8330	Nitroaromatics and nitramine explosives	
	636	Bensulide					8331	Tetrazene	
	637	2,2′-Dithiobis-(benzothiazole)					8332	Nitroglycerine	
	639	Bendiocarb					8333	Nitro compounds	
	640	Mercaptobenzo-thiazole					8350	Aromatic sulfonic acids by ion exchange chromatography	
	642	Biphenyl and orthophenyl phenol							
	643	Bentazon							
	644	Picloram							
HPLC–FL	610	PAHs	550	PAHs	TO-8	Phenols and cresols	8310	PAHs	
	641	Thiabendazole	550.1	PAHs	TO-13	PAHs	8318	N-Methylcarbamates (post-column reaction)	
			531.1	Carbamates (post-column reaction)					
			547	Glyphosate (post-column reaction)					
HPLC–EC[a]	605	Benzidines							
HPLC–MS							8321	Azo dyes, amines, organophosphorus compounds by HPLC–TSP-MS	
							8325	Benzidines and nitrogen-containing pesticides by HPLC–PB-MS[a]	
							8350	Aromatic sulfonic acids by ion-exchange chromatography	

[a] Abbreviations: EC = electrochemical detection; PB = particle beam; TSP = thermospray.

The advent of diode array detection (used in draft method 547) increases the utility of absorbance detectors to include spectral confirmation capability required to eliminate false positives [18]. Its potential for determining a wide variety of analytes at low levels has yet to be fully exploited. Electrochemical detectors are used for electroactive compounds such as benzidines and certain pesticides. Refractive index detection lacks sensitivity for environmental applications.

Mass spectrometry (LC–MS) is becoming a powerful tool for rapid evaluation of solid wastes and for screening wastewater samples [19]. Currently, three LC–MS methods are proposed for hazardous waste analysis (8321, 8325 and 8350) for RCRA, currently the most HPLC-progressive program. Method 8321 is close to approval and uses HPLC with UV and MS dectection for azo, anthraquinone, coumarin dyes, and organophosphorus pesticides. MS offers the possibility of a universal as well as selective and sensitive detector for HPLC, although its routine use is still hampered by the high system cost and operational difficulties. Rapid advances in LC–MS interface technology, in particular in electrospray and atmospheric pressure ionization (API), will improve the cost-effectiveness and applicability of this technique.

Carbamate and glyphosate analyses are currently performed routinely in many states. These EPA methods (531.1, 8318, and 547) utilize post-column reaction systems with fluorescence detection to provide sufficient sensitivity for direct injection of water samples at part-per-billion levels without concentration [20].

5. QUALITY ASSURANCE AND QUALITY CONTROL FOR EPA METHODS

Quality assurance (QA) and quality control (QC) is an important component for most methods developed by the EPA. Quality assurance programs cover all aspects of the laboratory process. The EPA defines QA as: *"The quality assurance process consists of management review and oversight at the planning, implementation, and completion stages of the environmental data collection activity, to ensure that data provided are of the quality required."*

Typical guidelines for managing QA in environmental laboratories are adopted from Good Laboratory Practices (GLP) and the ISO-9000 standards. The International Organization for Standardization (ISO) has developed a series of management standards (ISO 9000-9004) to assist manufacturing and service organizations in developing systems to ensure quality. The recently developed draft Good Automated Laboratory Practices (GALP) extend the GLP to data handling system [21]. Total quality management programs (*e.g.*, Juran Program), once only applied to manufacturing operations, are being applied to laboratory production [22]. Common components of a QA program include standard operating procedures (SOP), operator training programs, and performance evaluation samples from outside evaluators. To avoid conflict of interest, it is important to have a QA Officer, who reports independently from laboratory operations.

The EPA defines quality control as: *"The Quality Control process includes those activities required during data collection to produce the data quality desired and to document the quality of the collected data."* QC checks are performed at various points throughout the analysis to verify instrument and method performance. Many of the QC requirements are specified within the EPA method. One of the major differences between EPA methods that use the same technique for similar analyte lists in the QC requirements. Some programs have more intensive QC requirements because of the end use of the data. For example, the contract laboratory program (CLP) uses data to assign responsibility for cleanup costs at a contaminated site. Therefore, the data may have to stand alone in a court of law, long after the chemist who performed the analysis has left or the laboratory where the analysis was performed has ceased to exist. Table 5 compares the QC requirements of RCRA Method 8010 and drinking water Method 502.1 for halogenated volatile compounds. Although the analytes and technical aspects of the methods are very similar the QC checks and validation criteria are different, rendering it difficult to run both types of samples together in the same batch.

6. EPA CHROMATOGRAPHIC METHOD TRENDS

This section highlights a number of changes underway in regulations and technology that will im-

TABLE 5

COMPARISON OF METHOD PARAMETERS AND QC

Parameters	RCRA 8010 *Halogenated Volatile Organics*	Drinking Water 502.1 *Volatile Halogenated Organic Compounds in Water by Purge-and-Trap Gas Chromatography*
Analytes	39	50 (28 in common with 8010)
Sample introduction	Purge-and-trap	Purge-and-trap
Primary column	1% SP-1000 on Carbopack-B 60/80 mesh	Same
Detector	Halogen-specific	Same
Calibration	5 levels	3 levels (or more, depending on concentration range)
Quality control		
Initial response *vs.* subsequent standards	±15%	±20%
Spikes	5% or 1 per month	5%
Accuracy:		
Surrogate	$\pm 3\sigma$[a]	80–120%

[a] 3σ determined from historical laboratory performance.

pact EPA chromatographic methods over the next few years.

6.1. Method integration

The EPA structure has developed in the present fashion because of the requirements placed on the EPA by the US Congress. The EPA has designed most regulations by the type of medium involved: soil, air, water, and solid and hazardous waste. Many methods have been created by the various agency programs that are technically redundant, but have different QC requirements. The EPA has recognized that the proliferation of methods is confusing and counter-productive [23]. It has created the Environmental Monitoring Management Committee (EMMC) to consolidate methods across program lines [24,25]. Three methods are targeted for initial efforts, including GC analysis of volatile organic compounds. Agreement on a method among so many participants may be difficult, but the eventual savings in analysis time and expense will make the effort worthwhile.

6.2. Air methods

The EPA Air program is poised for growth and change. Implementation of the Clean Air Act amendments will require new methods and regulations [26]. Information on current contaminants and their pervasiveness will be required to assess the state of air quality. Routine monitoring will require the development of rugged methods suitable for a variety of skill levels.

Diffusive air sampling using prepacked adsorbents in sample tubes has been used extensively in industrial hygiene applications. It has been extended successfully to other environmental applications for air and water sampling in Europe. It is currently being evaluated in the USA for unattended ambient air sampling for toxic organics and for soil gas analysis. The advent of automated thermal desorption systems has rendered this technique extremely reproducible and cost-effective [27].

6.3. Trends towards automation, more efficient sample preparation techniques, and HPLC

The environmental laboratory of the future will emphasize automation and techniques that maximize sample throughput without sacrificing data quality. The EPA has recently drafted Good Automated Laboratory Practices to document guidelines in this regard. Trends to eliminate tedious and solvent intensive sample preparation techniques (such as soxhlet extraction, liquid–liquid extraction, etc.)

and to move towards more efficient techniques such as supercritical fluid extraction (SFE) [28], solid-phase extraction (SPE) using either cartridge or disk [29], and using HPLC (on-line through column switching or off-line using adsorption or GPC columns) are evident.

More HPLC methods are expected because of the advantages of the technique over GC for a large number of environmental pollutants. The EPA Office of Water must regulate an additional 25 compounds in drinking water every three years, and this may be an additional driving force to use HPLC for water testing. As requirements to monitor large numbers of compounds continue to grow, HPLC–MS will be preferred because of the broad-spectrum applicability and confirmatory nature of the technique.

7. DISCUSSION

Choosing an EPA method to match the analytical requirements can be a difficult task. Generally, the program areas require the use of their own methods or other well-established testing procedures such as ASTM methods or Standard Methods for the Examination of Water and Wastewater [30]. The RCRA program recently clarified in which cases the SW-846 methods must be used and the conditions under which more flexibility is allowed [31]. Generally the analyst must make a choice based on knowledge of the EPA program area involved and the concentration levels on which a decision might eventually be based. Until the EMMC is able to simplify the base of methods from which to choose, this will continue to be a task requiring careful consideration. This paper facilitates method selection by compiling all the methods for different programs under one cover, categorized by program and detection techniques.

In the past the EPA has been criticized for slow adoption of new technology. The EPA methods are well established and many have been in use for over 10 years, however the EPA moves slowly into new analytical technology because of the diversity of method users. Many private laboratories employ a variety of skill levels for sample preparation and instrument operation. Thus the methods, in addition to being accurate and precise, must be demonstrated to be rugged through time-consuming interlaboratory studies before promulgation. However, changes in this regard are evident in recent years. Newer technologies, such as HPLC–MS, solid-phase extraction disks, and thermal desorption-GC are being added to the EPA's repertoire of methods in quickening pace, thus increasing the number of tools available to the environmental analyst.

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Review

High-performance liquid chromatographic methods for the determination of N-methylcarbamate pesticides in water, soil, plants and air

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ABSTRACT

High-performance liquid chromatographic methods of analysis for 31 N-methylcarbamate pesticides and 46 of their metabolites in water, soil, plant and air samples are reviewed. Consideration is given to extraction, clean-up, chromatographic separation and detection techniques including UV absorbance, fluorescence, electrochemical and mass spectrometric detection.

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1. INTRODUCTION

N-methylcarbamate (NMC) pesticides comprise an important class of pesticides noted for their relatively short persistence in the environment. Since their introduction in the 1950s they have been used world-wide on a large number of crops. Of the 31 NMCs included in this review (Table 1), one, ethidimuron, is a herbicide and the rest are insecticides, acaracides, nematicides or molluscicides. There are no fungi-

TABLE 1

N-METHYLCARBAMATE PESTICIDES INCLUDED IN THIS REVIEW

A = Acaricide; H = herbicide; I = insecticide; M = molluscicide; N = nematicide.

No.	Common name(s)	Trade name(s)	Class
1	Aldicarb	Temik	I, N, A
2	Aminocarb	Matacil	I, A, M
3	Bendiocarb	Ficam	I
4	BPMC, fenobucarb	Bassa, Baycarb, Osbac	I
5	Bufencarb	Bux	I
6	Butacarb		I
7	Butocarboxim	Drawin 755	I, A
8	Butoxycarboxim	Plant Pin	I, A
9	Carbanolate	Banol	I
10	Carbaryl	Sevin	I
11	Carbofuran	Furadan, Curaterr	I, N, A
12	Cloethocarb	Lance	I, N
13	CPMC	Hopcide, Etrofol	I
14	Dioxacarb	Famid, Elocron	I
15	Ethidimuron	Ustilan	H
16	Ethiofencarb	Croneton	I
17	Formetanate	Carzol, Dicarzol	A, I
18	Isoprocarb, MIPC	Mipsin, Etrofolan	I
19	Methiocarb, mercaptodimethur	Mesurol, Draza	I, A, M
20	Methomyl	Lannate	I
21	Mexacarbate	Zectran	I, M, A
22	–	Mobam	I
23	MPMC	Meobal	I
24	MTMC	Tsumacide, Metacrate	I
25	Oxamyl	Vydate	I, N, A
26	Promecarb	Carbamult	I
27	Propoxur, arprocarb, PHC	Baygon, Blattanex, Unden	I
28	Thiofanox	Dacamox	I, A
29	–	Tranid	I, A
30	Trimethacarb	Landrin	I
31	XMC	Macbal	I

cides in the group. A structural feature they have in common is a hydrolyzable N-methyl group, a feature that has been exploited for residue analysis. Also included in the review are 46 metabolites of NMC pesticides (Table 2), some of which have lost the N-methylcarbamate moiety.

High-performance liquid chromatography (HPLC) is the favoured technique for determination of NMC pesticides as a class since many of them lack the thermal stability necessary for gas chromatographic determination. It has now been 20 years since the first reports of HPLC determination of NMCs were published [1–3]. Since then a large body of literature has been produced on this subject and it seemed appropriate to review this literature for the interest of those working in this field.

This review will attempt to deal with all aspects of determination of NMC residues in water (Table 3), soil (Table 4), plants (Table 5) and air by HPLC, including extraction, clean-up, chromatographic separation and detection. An attempt has been made at comprehensiveness, but no doubt omissions have occurred. A number of papers which include advances in analytical technology but in which no application to environmental analysis was made are also included because of their possible relevance to workers in the field (Table 6). In Tables 3–6 the units ppm, ppb and ppt are reported as used in the references.

TABLE 2

METABOLITES OF N-METHYLCARBAMATE PESTICIDES INCLUDED IN THIS REVIEW

No.	Metabolite	Parent compound
32	Aldicarb sulphoxide	Aldicarb
33	Aldicarb sulphone (aldoxycarb)	Aldicarb
34	Aldicarb oxime	Aldicarb
35	Aldicarb sulphoxide oxime	Aldicarb
36	Aldicarb sulphone oxime	Aldicarb
37	Aldicarb nitrile	Aldicarb
38	Aldicarb sulphoxide nitrile	Aldicarb
39	Aldicarb sulphone nitrile	Aldicarb
40	4-Formamido-3-methylphenol	Aminocarb
41	4-Methylamino-3-methylphenol	Aminocarb
42	4-Formamido-3-methylphenyl N-methylcarbamate	Aminocarb
43	4-Amino-3-methylphenyl N-methylcarbamate	Aminocarb
44	4-Dimethylamino-3-methylphenol	Aminocarb
45	4-Methylamino-3-methylphenyl N-methylcarbamate	Aminocarb
46	Butocarboxim sulphoxide	Butocarboxim
47	Butocarboxim sulphone (butoxycarboxim)	Butocarboxim
48	1-Naphthol	Carbaryl
49	7-Hydroxycarbaryl	Carbaryl
50	6-Hydroxycarbaryl	Carbaryl
51	4-Hydroxycarbaryl	Carbaryl
52	5-Hydroxycarbaryl	Carbaryl
53	Methylolcarbaryl	Carbaryl
54	1,6-Dihydroxynaphthalene	Carbaryl
55	1,5-Dihydroxynaphthalene	Carbaryl
56	1,7-Dihydroxynaphthalene	Carbaryl
57	3-Hydroxycarbofuran	Carbofuran
58	3-Ketocarbofuran	Carbofuran
59	2,3-Dihydro-2,2-dimethyl-7-benzofuranol	Carbofuran
60	2,3-Dihydro-2,2-dimethyl-3,7-benzofurandiol	Carbofuran
61	2,3-Dihydro-2,2-dimethyl-3-oxo-7-benzofuranol	Carbofuran
62	Ethiofencarb sulphoxide	Ethiofencarb
63	Ethiofencarb sulphone	Ethiofencarb
64	Ethiofencarb phenol	Ethiofencarb
65	Ethiofencarb phenol sulphoxide	Ethiofencarb
66	Ethiofencarb phenol sulphone	Ethiofencarb
67	Methiocarb sulphoxide	Methiocarb
68	Methiocarb sulphone	Methiocarb
69	Methomyl oxime	Methomyl
70	4-Dimethylamino-3,5-xylenol	Mexacarbate
71	4-Methylformamido-3,5-xylyl N-methylcarbamate	Mexacarbate
72	4-Methylamino-3,5-xylyl N-methylcarbamate	Mexacarbate
73	4-Formamido-3,5-xylyl N-methylcarbamate	Mexacarbate
74	4-Amino-3,5-xylyl N-methylcarbamate	Mexacarbate
75	Oxamyl oxime	Oxamyl
76	Thiofanox sulphoxide	Thiofanox
77	Thiofanox sulphone	Thiofanox

TABLE 3

HPLC METHODS USED TO DETERMINE N-METHYLCARBAMATE PESTICIDES IN WATER SAMPLES

CC = Column chromatography; EC = electrolytic conductivity; EL = electrochemical; FL = fluorescence; LLE = liquid–liquid extraction; LLP = liquid–liquid partitioning; MS = mass spectrometry; SPE = solid-phase extraction.

Chemical(s)	Extraction technique	Clean-up technique	Recovery (%)	Det.	Detection limit	Ref.
1, 5, 6, 9, 10, 14, 16, 17, 19, 20, 22, 26, 27, 30	LLE/dichloromethane	None	–	FL	–	4
10	LLE/dichloromethane	CC/Florisil	99.7	UV	–	5
1, 32–35	LLE/dichloromethane	Hexane wash	–	MS	0.3–0.6 ppb	6
3	LLE/dichloromethane	CC/Florisil	65–70	UV	1.8 μg/g	7
1, 4, 10, 11, 18, 20, 24, 27, 57	LLE/dichloromethane	None	62–109	UV	5–10 ng/g	8
4, 10, 11, 20, 25, 27	LLE/dichloromethane or SPE/C_{18}	None	98	MS	1 ppb	9
11	LLE/dichloromethane	None	94–100	UV	0.001 ppm	10
11	LLE/dichloromethane	None	82	UV	0.05 ppm	11
1, 11, 32, 33, 57, 58	LLE/dichloromethane	SPE/silica	>83	UV	1 ppb	12
25, 75	LLE/dichloromethane	None	92–98	UV	1 ppb	13
1, 2, 4, 10, 11, 19–21, 25, 27, 32, 33, 75	LLE/dichloromethane or SPE/C_{18}	None	19–93	MS	8–320 ng	14
10	LLE/chloroform	CC/silica	70–85	UV	1–2 ng	15
10	LLE/chloroform	None	–	MS	10–20 ppt	16
10	LLE/chloroform	Normal-phase HPLC	70–85	UV MS	1–2 ng	17
1, 20, 25, 32, 33	LLE/chloroform	None	77–95	FL UV	0.2 ng 10 ng	18
10	LLE/toluene or benzene	None	96	UV	5 ng	19
10, 20	LLE/hexane	None	–	EC	–	20
20, 69	LLE/ethyl acetate	LLP/dichloromethane	93.7	UV EL[a]	1–2 ng 0.1 ng	21
2, 40–45	SPE/XAD-4 resin	LLP/ethyl acetate	76–84	UV FL	0.5 ppb 0.05 ppb	22
1, 32, 33	SPE/XAD-2 resin	None	92–96	UV	12 ng	23
10, 11, 20, 48	SPE/C_{18} or direct injection	None	94–107	UV	0.9 ng	24
10, 48	SPE/C_{18} or direct injection	None	97–100	UV	–	25
1	SPE/C_{18}	None	97–115	UV	10 ppb	26
27	SPE/C_{18}	None	92–99	UV	20 ppb	27
11, 57–61	SPE/C_{18}	None	86–113	UV	0.4 μg/l	28
10, 48–56	SPE/C_{18}	None	99.8	UV	0.5 ng/ml	29
11	SPE/C_{18}	None	–	UV	0.04 ppb	30
11	SPE/C_{18}	None	94.5	UV	0.033 μg/ml	31
1, 32, 33	SPE/C_8	None	52–90	FL	2.5 ng	32
21, 70	Direct injection	None	–	UV	–	33
2, 4, 10	Direct injection	None	–	EL	0.14 ng	34
11, 57–61	Direct injection	None	–	UV	1–5 ppb	35
1, 2, 10,19, 20, 27	Direct injection	None	–	FL	0.1–0.85 ng	36
1, 32–39	Direct injection	None	–	UV	1–2 ng	37
1, 32, 33	Direct injection	None	–	UV	1–2 ng	38
1, 10, 11, 20, 25, 32, 33, 57	Direct injection	None	87–106	FL	0.5 ng	39
2	Direct injection	None	–	EL	53 pg	40
2, 10	Direct injection	None	–	EL	–	41
1, 10, 11, 19, 20, 25, 27, 32, 33, 57	Direct injction	None	94–98	FL	–	42
1, 11, 20, 25, 32, 33, 57	Direct injection	None	77–138	FL	0.3–0.6 ng	43

TABLE 3 *(continued)*

Chemical(s)	Extraction technique	Clean-up technique	Recovery (%)	Det.	Detection limit	Ref.
1, 10, 11, 19, 20, 25, 27, 32, 33	Direct injection	None	–	FL	0.7–26 ng	44
20, 25	Direct injection	None	–	FL	1 ng	45
11	Direct injection	None	99–105	UV	0.03 μg/ml	46
10	On-line trace enrichment/C_{18}	None	104–106	FL	0.4–2.0 ng	47
1, 32, 33	On-line trace enrichment/C_8	None	71–80	FL	70 ng/l	48
10, 11, 27	On-line trace enrichment/C_8 or C_{18}	None	–	UV	10–70 pg/ml	49

[a] Electrochemical detector was used for methomyl oxime only.

2. EXTRACTION

2.1. Water samples

Residues of NMCs are commonly extracted from water using liquid–liquid extraction or solid-phase extraction on C_{18} cartridges. The first report of HPLC analysis of NMCs in water was by Frei *et al.* [4] who used liquid–liquid extraction with dichloromethane followed by passage through anhydrous sodium sulphate and evaporation. This general approach has been followed using dichloromethane,

TABLE 4

HPLC METHODS USED TO DETERMINE N-METHYLCARBAMATE PESTICIDES IN SOIL SAMPLES

CC = Column chromatography; EL = electrochemical; FL = fluorescence; LLP = liquid–liquid partitioning; MS = mass spectrometry.

Chemical(s)	Extraction solvent	Clean-up technique	Recovery (%)	Det.	Detection limit	Ref.
25, 75	Methanol	None	98.6	UV	–	50
11, 57, 58	Methanol	Hexane wash	89–94	UV	30 ng	51
25	Methanol	None	95.5	UV	2 ng	52
10, 25	Methanol	CC/Florisil	–	MS	1–2 ng	53
11, 59	Methanol–water (4:1)	None	–	UV	–	54
11	Methanol–water (2:1)	None	93–97	UV	0.1 μg/g	46
7, 46, 47	Methanol–water (1:1)	CC/silica	81–96	UV	0.3 μg/g	55
11	Methanol–water (2:1)	LLP/dichloromethane	82.4	UV	0.05 ppm	11
1, 20, 25, 32, 33	Acetone or water	LLP/chloroform (acetone extracts only)	83–99	FL UV	0.2 ng 10 ng	18
1, 5, 6, 9–11, 14, 17, 19, 20, 22, 26, 27, 30	Acetone	CC/silica	–	FL	–	4
11, 57	Acetone	LLP/dichloromethane and CC/silica	90–96	UV	–	56
10, 11, 25, 27	Acetone–dichloromethane	CC/Florisil	–	MS	1 ng	57
10, 11, 25, 27, 48	Acetone–dichloromethane	CC/Florisil or SPE/Florisil or SPE/aminopropyl silica	98–110	UV	5 ng	58
10	Acetone–water	LLP/dichloromethane and CC/Florisil	106	UV	–	5
11	Acetonitrile	LLP/dichloromethane and CC/silica	90–100	UV	0.02 ppm	10
25, 75	Dichloromethane	SPE/silica	92	UV	1 ppb	13
1, 4, 10, 11, 18, 20, 24, 27, 57	Acidic ammonium acetate	LLP/dichloromethane and CC/Florisil	59–100	UV	0.05–0.1 μg/g	8
4, 10, 11, 27	Water	LLP/dichloromethane	–	MS	0.1 ppb	9

TABLE 5

HPLC METHODS USED TO DETERMINE N-METHYLCARBAMATE PESTICIDES IN PLANT SAMPLES

CC = Column chromatography; EC = electrolytic conductivity; EL = electrochemical; FL = fluorescence; GPC = gel permeation chromatography; LLP = liquid–liquid partitioning; MS = mass spectrometry; SPE = solid-phase extraction; TLC = thin-layer chromatography.

Chemical(s)	Extraction solvent	Clean-up technique	Recovery (%)	Det.	Detection limit	Ref.
2, 9–11, 19, 21, 27, 30	Acetone	LLP/dichloromethane–hexane and CC/Florisil	>70	UV	0.004–0.1 ppm	59
11, 57, 58	Acetone	LLP/dichloromethane–hexane and CC/Florisil	68–110	UV	0.02–0.05 ppm	60
11, 57, 58	Acetone	LLP/dichloromethane–petroleum ether and CC/Florisil	–	UV	–	61
10	Acetone	LLP/dichloromethane–hexane and CC/Florisil	90–91	UV	30–50 ppb	62
			78–81	FL	10 ppb	63
11, 57, 58	Acetone	LLP/dichloromethane–hexane and CC/Florisil	50–65	FL	0.01 ppm	64
1, 7, 32, 33, 46, 47	Acetone	Petroleum ether wash, LLP/chloroform and CC/silica or TLC	75–91	FL	0.02–0.1 μg/g	65
20, 25	Acetone	LLP/dichloromethane–petroleum ether and CC/Florisil	–	MS	50 ng	66
10	Acetone	LLP/dichloromethane–petroleum ether and CC/Florisil	93	FL	–	67
11	Acetone	LLP/dichloromethane–hexane and SPE/silica	91	UV	0.011 μg/g	68
1, 5, 19, 20, 25, 27, 32	Acetone	LLP/dichloromethane–petroleum ether	69–110	MS	0.025–1 ppm	69
10, 11, 16, 19, 26, 27	Acetone	LLP/dichloromethane and CC/Florisil	94–106	EL	0.5–2 ng	70
11, 57, 59–61	Acetone	LLP/chloroform, evaporation, dissolution in precipitating solution, LLP/cabon tetrachloride	60–100	UV	–	71
10	Acetone	LLP/chloroform, evaporation, dissolution in precipitating solution, LLP/carbon tetrachloride	72–81	UV FL	–	72
1–3, 5, 7, 9–11, 14–16, 18–21, 25–30, 32, 33, 46–48, 57, 67, 68, 76, 77	Acetone–dichloromethane–petroleum ether	SPE/aminopropyl-bonded silica	47–102	FL	0.025–0.5 ng	73
1–3, 5, 7, 9–12, 14–16, 18–21, 25–30, 32, 33, 46, 47, 57, 58, 67, 68, 76, 77	Acetone–dichloromethane–petroleum ether	SPE/aminopropyl-bonded silica	–	FL	1–10 ppb	74
10	Acetone–methanol	LLP/dichloromethane	78–104	UV	0.05 μg/g	75
10	Acetone–methanol	Add acetonitrile to separate aqueous layer, LLP/chloroform, CC/Florisil	–	–	5 ng	19
1, 3, 4, 7, 8, 10, 11, 14, 16, 18–20, 24–28, 30, 32, 33, 46, 57, 62, 63, 67, 68	Acetonitrile or dichloromethane/water	LLP/hexane and CC/Florisil or SPE (unspecified phase)	59–108	FL	40–80 ng/g	76

TABLE 5 *(continued)*

Chemical(s)	Extraction solvent	Clean-up technique	Recovery (%)	Det.	Detection limit	Ref.
10	Acetonitrile	LLP/dichloromethane	80–85	UV	0.02 μg/g	77
1, 10, 11, 19, 20, 25	Acetonitrile	Add NaCl, centrifuge, take acetonitrile layer for SPE/C_{18} or CC/Florisil	72–107	FL	–	78
32	Acetonitrile	Add NaCl, centrifuge, take acetotrile layer	74–76	FL	0.2 ppm	79
17	Acetonitrile (acidified)	Wash with dichloromethane (acidic conditions), LLP/dichloromethane (basic conditions), LLP/0.1 *M* sulphuric acid, LLP/dichloromethane (basic conditions)	>80	UV	0.02–0.05 μg/g	80
10	Benzene or petroleum ether	None	77–115 61–85	UV	0.005 ppm	81
10	Chloroform	LLP/acetonitrile, CC/Florisil	75–100	UV	–	5
10	Chloroform	CC/silica, SPE/C_{18}	95–104	UV	3 ng	82
11, 59–61	Chloroform or 0.25 *M* HCl	None	–	UV	2–4 ng	83
2, 10	Dichloromethane	CC/alumina-silver nitrate	80–90	UV	0.02 ppm	84
16, 62–66	Dichloromethane	None	35–103	UV	–	85
1, 32, 33	Dichloromethane	SPE/silica	86–96	UV	20–38 ng	86
4, 10, 11, 16, 18, 19, 27	Dichloromethane	None	–	FL	0.1 ng	87
2, 5, 10, 27	Dichloromethane	On-line clean-up (column switching)	55–100	UV	0.025–0.25 ppm	88 89
5, 10, 27	Dichloromethane	On-line clean-up (column switching)	67–100	UV	0.01–0.1 μg/g	90
10, 11, 27	Dichloromethane	LLP/acetonitrile, on-line clean-up (column switching)	71–89	FL	1.6 ng	91
1, 2, 10, 11, 21, 27, 57	Dichloromethane	None	–	FL	0.2–50 ng	92
5, 27	Dichloromethane	None	–	UV MS	–	93
10, 11, 48, 57	Dichloromethane (acidified)	CC/silica	–	UV FL	–	94
20, 69	Ethyl acetate	LLP/dichloromethane	101.4	UV EL[a]	1–2 ng 0.1 ng	21
1, 2, 5, 10, 11, 14, 17, 19, 20, 26, 27	Ethyl acetate	Transfer to water–methanol by evaporation, wash with *n*-pentane, LLP/chloroform, CC/Florisil	70–90	UV	20–1000 ng	95
1, 10, 11, 19, 20, 25, 27, 32, 33	Ethyl acetate	CC/alumina	–	FL	0.7–26 ng	44
20, 25	Ethyl acetate	Transfer to water by evaporation, wash with hexane, LLP/chloroform	61–81	UV	–	96
10, 20	Hexane–isopropanol	None	–	EC	–	20
10	Methanol	CC/Florisil	97	UV	0.05 μg/g	97
1, 10, 11, 19, 20, 25, 27, 32, 33, 57	Methanol	LLP/dichloromethane, GPC, SPE/Nuchar-Celite	52–97	FL	5–10 ppb	98
25, 75	Methanol	SPE/C_{18}; or hexane wash and CC/XAD-2 resin; or HPLC clean-up on C_{18}	90–99	UV	0.05 ppm	50

(Continued on p. 96)

TABLE 5 *(continued)*

Chemical(s)	Extraction solvent	Clean-up technique	Recovery (%)	Det.	Detection limit	Ref.
25	Methanol	None	87–103	UV	–	99
1	Methanol	Oxidation to aldicarb sulphone, LLP/dichloromethane, SPE/silica	–	UV	–	100
10, 48	Methanol	On-line clean-up (column switching)	86–97	UV	0.1 μg/g	101
1, 5, 10, 11, 19, 20, 25, 32, 33, 57, 67	Methanol	LLP/acetonitrile, wash with 20% NaCl and petroleum ether, LLP/dichloromethane, CC/Celite-Nuchar	55–103	FL	–	102
1, 5, 10, 11, 19, 20, 25, 33, 57	Methanol	LLP/acetonitrile, wash with 20% NaCl and petroleum ether, LLP/dichloromethane, CC/Celite-Nuchar	79–103	FL	–	103
1, 3, 5, 10, 11, 14, 18–20, 25, 27, 32, 33, 57, 58, 67	Methanol	LLP/acetonitrile, wash with 20% NaCl and petroleum ether, LLP/dichloromethane, CC/Celite-Nuchar	99	FL	–	104
10, 11	Methanol	LLP/acetonitrile, wash with 20% NaCl and petroleum ether, LLP/dichloromethane, CC/Celite-Nuchar	102–108	FL	–	105
5, 10, 11, 18, 19, 57	Methanol	LLP/acetonitrile, wash with 20% NaCl and petroleum ether, LLP/dichloromethane, CC/Celite-Nuchar	99	EL	0.4–0.7 ng	106
25	Methanol	LLP/dichloromethane, SPE/Florisil	86–94	UV	2 ng	107
25	Methanol	SPE/Nuchar-Attaclay	90–94	FL	0.02 μg/g	108
20	Methanol	None	80–90	UV	1 μg	109
10, 11, 19	Water	Hydroxylapatite removal of proteins, SPE/C_{18}	78–93	FL	0.01 ppm	110
20	Water + surfactant	LLP/dichloromethane	–	FL	–	111
17	Water–acetonitrile (acidified)	None	94	UV	–	112
11, 57	Water–dichloromethane with acid digestion	CC/silica-carbon-attaclay and CC/silica	77–85	UV	–	113
7, 46, 47	Water–methanol	CC/silica	73–105	UV	0.3 μg/g	55
20, 25	Matrix solid-phase dispersion isolation	None	72–129	FL	20 ppb	45
1, 32, 33	Centrifugation	None	–	FL	–	114
14	–	–	–	UV	4 ng	115
10	–	None	–	UV	0.002 ppm	116

[a] Electrochemical detector was used for methomyl oxime only.

chloroform, toluene, benzene, hexane, or ethyl acetate [5–21] (Table 3). In two studies [8,11] the water sample was acidified to pH 3 before extraction. In three other studies [12–14] NaCl or Na_2SO_4 was added to the water sample before extraction. Recoveries were generally good; however, since solubilities of the NMCs can vary quite dramatically, recoveries of individual NMCs may vary for any one method. Aldicarb sulphoxide in particular is extremely water soluble and recovery using liquid–liquid or solid-

TABLE 6

METHODS FOR DETERMINATION OF N-METHYLCARBAMATE PESTICIDES WHICH WERE NOT APPLIED TO ANALYSIS OF ENVIRONMENTAL SAMPLES

AA = Autoanalyzer; EL = electrochemical; FL = fluorescence; MS = mass spectrometry.

Chemical(s)	Detector	Detection limit	Ref.
1, 2, 5, 9–11, 19–22, 27, 30	UV	–	1
13, 18, 23, 24, 27, 31	UV	–	2
6, 11, 14, 17	FL	1–10 ng	3
1, 5, 9–11, 19–21, 27, 30, 32, 33, 48, 57	UV	1.1–61.3 ng	118
1, 5, 9–11, 19, 20, 23, 25–27, 30	IR	–	119
10	FL	–	120
1, 2, 10, 11, 19, 20, 27	FL	0.1 ng (methomyl)	121
1, 2, 10, 17, 19–21, 25, 48	UV	–	122
1, 32–35	UV	<1 μg/l	123
11, 59	UV	–	124
10	UV	*ca.* 0.04 μg	125
11, 57–59	UV	–	126
10, 27	UV	–	127
10, 11	UV	–	128
11, 57–61	UV	–	129
21, 70–74	UV	–	130
10, 48	UV	<3.5 ng	131
10, 11, 19, 20, 27, 48, 57	FL	–	133
1, 3, 5, 10, 11, 19, 20, 25, 27, 30, 32, 33, 48, 57, 67, 68	FL	10 ppb	134
3, 10, 11, 48, 57	FL	–	135
1, 19, 20, 27	FL	0.4–1 ng	136
1, 2, 10, 11, 13, 17–21, 25–27, 32, 33	FL	0.9–1.3 ng	137
1, 5, 10, 11, 19, 20, 25, 27, 32, 33, 57	FL	0.1 ng	138
10, 19, 27, 30	EL	1–1000 ng	139
	UV	1.2–13.6 ng	
1–3, 10, 19, 20, 32, 33	EL	5–100 pg	140
10	EL	0.03 ng	141
1, 2, 11, 20, 21, 23, 26, 27	MS	2.5–27.2 ng	142
	UV	1.1–18.2 ng	
1, 4, 10, 11, 27	MS	2–6 ng	143
4, 10, 11, 27	MS	40–50 ng	144
1, 4, 10, 11, 20, 25, 27	MS	0.02–2 μg	145
20	MS	–	146
1, 10, 11, 20, 25, 32, 33, 57	MS	–	147
10	MS	–	148
14	AA	200 ng	149

phase extraction may be poor. In the last-mentioned study [14] recoveries ranged from 19% for aldicarb sulphoxide to 93% for methiocarb using liquid–liquid extraction with dichloromethane, and from 10% for aldicarb sulphoxide to 98% for methiocarb using solid-phase extraction on C_{18}.

The first report of solid-phase extraction of an NMC from water for HPLC analysis was by Brun and McDonald [22] who used XAD-4 resin to extract aminocarb from lake water and rain water, achieving recoveries of 76–84%. Narang and Eadon [23] used XAD-2 resin to extract aldicarb and its oxidation products from drinking water and achieved recoveries of 92–96%. Other studies employing solid-phase extraction of water samples have used C_{18} [24–31] or C_8 [32] cartridges. In most cases recovery was over 85%.

Several HPLC studies have employed direct injec-

tion of unextracted, unconcentrated water samples [24,25,33–46]. This circumvents the extraction and clean-up steps with their inherent possibility of loss of analyte, but may not provide the detection limits required for environmental samples.

On-line trace enrichment of NMC residues on a precolumn prior to determination on an analytical column has been utilized by several workers to improve detection limits. She *et al.* [47] concentrated carbaryl residues on a C_{18} precolumn. Chaput [48] concentrated 10-ml water samples containing residues of aldicarb and its oxidation products on a C_8 precolumn. Marvin *et al.* [49] concentrated carbaryl, carbofuran and propoxur from 100-ml water samples on C_8 or C_{18} precolumns in a completely automated HPLC system.

2.2. Soil samples

Extraction of NMCs from soil is accomplished using solvent extraction [4,5,8–11,13,18,46,50–58] (Table 4). In most cases, recovery was better than 80%. Some of the earlier methods involved the use of chloroform or benzene, a practice which of course is no longer recommended for health reasons. De Bertrand *et al.* [58] compared four extraction solvents: methanol, and 1:1 mixtures of acetone–dichloromethane, acetone–methanol and acetone–ethyl acetate. The best recovery of carbaryl, carbofuran, oxamyl and propoxur was obtained with acetone–dichloromethane (1:1) (102%). Methanol extraction produced a co-extractive which prevented determination of oxamyl. Dekker and Houx [18] extracted subsoils with water and topsoils with acetone and found acetone to be better for methomyl and oxamyl and water to be better for aldicarb sulphoxide, another reflection of aldicarb sulphoxide's water solubility.

2.3. Plant samples

The solvent chosen to extract residues of NMC pesticides from plant tissue depends not only on the solubility of the chemical, but also on the nature of the information required. For example, the determination of dislodgeable residues for the purpose of establishing safe re-entry times for workers after crops have been sprayed requires a surface extraction of residues. On the other hand, determination of residues in fruits or vegetables to ensure the safety of food for consumers normally requires homogenization of the whole sample to extract the total residue.

A number of solvents have been used for the extraction of NMC residues from plant tissue ranging in polarity from water to petroleum ether [5,19,20, 44,45,50,55,59–116] (Table 5). The most effective strategy is to choose the solvent, whenever possible, such that the recovery of analytes is maximized and extraction of co-extractives is minimized. For highly water-soluble compounds such as formetanate and butocarboxim the water content of the extraction solvent may be increased [55,112], thus minimizing the amount of potentially interfering co-extracted material. Similarly, relatively non-polar solvents such as dichloromethane may efficiently extract less polar compounds without removing an excess of plant pigments. Unfortunately, compounds of intermediate polarity such as oxamyl are most effectively extracted in a solvent such as methanol which also removes an abundance of co-extractives. In such a situation an efficient clean-up step is normally required before determination.

The most inclusive study of extraction of NMCs from plant tissue was that of De Kok *et al.* [73], who determined recoveries of 21 NMC pesticides and 10 of their metabolites in a wide range of crops. They used an extraction solvent consisting of acetone–dichloromethane–petroleum ether (1:1:1) for fruits and vegetables. Recovery of NMCs was almost invariably better than 70%, but recovery of the most polar metabolites, butocarboxim sulphoxide and aldicarb sulphoxide, was always less than 60%.

A novel technique that was used on very small samples (0.5 g) was that of matrix solid-phase dispersion isolation [45]. Instead of using a solvent to extract the analytes, this technique involved blending a small amount of homogenized sample with C_{18} sorbent (40-μm particle size), transfering the mixture to a glass chromatography column and eluting the analytes with dichloromethane. This essentially combined extraction and column chromatographic clean-up, but would appear to be useful only for very small samples.

2.4. Air samples

Only one method for HPLC determination of airborne NMC pesticide residues was found [117].

This method employed a glass absorption tube packed with 10% Carbowax 400 on 80/100 mesh Supelcoport to absorb airborne propoxur. Air was sampled at a rate of 5 l/min for a number of hours and absorbed propoxur was eluted from the absorption tube with methanol. Recoveries were better than 85%.

3. CLEAN-UP

Most environmental sample extracts will require a preliminary clean-up procedure before determination by HPLC. The extent of clean-up required is dependent on the type of sample being analyzed, the detection limit required and the detection technique employed. As might be expected, about 80% of the methods reported for analysis of water samples summarized in Table 3 did not require clean-up of samples or extracts. On the other hand, almost all methods for soil and plant samples summarized in Tables 4 and 5 required at least some clean-up. Selective detection techniques such as fluorescence or mass spectrometry may minimize the need for clean-up by ignoring co-eluting co-extractives. The need for clean-up is indicated when it is not possible to separate an analyte from an interfering co-extractive on the HPLC column at the required level of sensitivity. It may also be desirable to clean-up samples in order to prolong the life of HPLC columns or prevent contamination of detectors.

The goal of clean-up is to remove as much interfering co-extracted material and as little of the analyte(s) as possible. Development of a clean-up method is often difficult since co-extractive compounds most similar to the analyte are those which are most likely to interfere with analysis, and also the most difficult to remove without also removing the analyte. Also, as was the case for the extraction step, the range of polarities of NMC pesticides makes it difficult to develop one clean-up procedure which is equally effective for all.

The clean-up techniques most commonly employed for extracts containing residues of NMC pesticides are liquid–liquid partitioning or a chromatographic clean-up such as column chromatography or solid-phase extraction (SPE). Many methods require a combination of both. Surprisingly, only one report of the use of gel permeation chromatography as a clean-up method for NMC determination was found [98]. A useful strategy, in the case of chromatographic clean-up techniques, is to employ a different stationary phase in the clean-up step than will be used in the determination step. For example, a Florisil SPE clean-up may be effective prior to determination on a C_{18} column.

The most inclusive clean-up method for HPLC determination of NMC pesticides was that reported by De Kok *et al.* [73]. This simple method employed an aminopropyl-bonded silica SPE column for clean-up of a dichloromethane extract of 21 NMC pesticides and 10 metabolites. Recovery from the clean-up step was quantitative, but losses occurred in the extraction step as previously mentioned.

Several methods [88–91,101] employed an on-line clean-up of plant extracts using a precolumn and column switching to direct fractions of interest from the precolumn to the analytical column. This useful approach essentially permits automation of column chromatographic or SPE clean-up steps.

Two methods [71,72] utilized an ammonium chloride–orthophosphoric acid solution to precipitate methyl anthranilate and other interfering plant materials. Another [110] used a column packed with hydroxylapatite, a form of calcium phosphate, to remove protein from vegetable extracts.

4. CHROMATOGRAPHIC SEPARATION

Most HPLC methods for NMC pesticides have employed reversed-phase chromatography with C_{18} or C_8 columns and aqueous mobile phases. Some methods employed normal-phase LC on silica columns [3,4,10,16,19,59,60–64,66,75,84,88–90,93,95, 101,118,119]. Nondek *et al.* [120] used an alumina column with an *n*-heptane–2-propanol mobile phase. Almost all normal-phase methods were reported before 1984. Diol [89,90] and nitrile [6,89,90] stationary phases have also been used in the normal-phase mode and cyclohexyl [28,79] and phenyl [82,121] stationary phases in the reversed-phase mode.

Sparacino and Hines [118] studied retention and resolution of 14 NMC pesticides and metabolites in normal- and reversed-phase modes on a variety of columns. Silica, cyanopropyl and propylamine columns were studied in normal-phase mode with two mobile phase systems (isopropanol–heptane and dichloromethane–heptane). C_{18} and ether phase

columns were studied in reversed-phase mode with three mobile phase systems (water–methanol, water–tetrahydrofuran and water–acetonitrile). Although normal-phase mode was for the most part satisfactory, reversed-phase mode gave generally superior results. The C_{18} column and water–acetonitrile mobile phase gave overall best performance in terms of resolution of the pesticides and UV transparency of the mobile phase. Aten and Bourke [122] reported retention volumes for eight NMCs on a C_{18} column with six different mobile phases.

Two methods [107,114] employed unmodified silica HPLC columns with aqueous (*i.e.*, reversed-phase) mobile phases with good success. This was especially useful for the isocratic separation of aldicarb and its oxidation products [114]. Another method [123] separated aldicarb and its oxidation and hydrolysis products isocratically on a cyanopropyl bonded stationary phase with a water–acetonitrile mobile phase.

Kikta *et al.* [124] explored the influence of column temperature on retention using a C_{18} column and found that at 27°C carbofuran could not be completely resolved from 2,3-dihydro-2,2-dimethyl-7-benzofuranol, a metabolite of carbofuran. At 70°C with a modified water–methanol ratio in the mobile phase the elution order was reversed and complete resolution was achieved.

5. DETECTION

5.1. UV absorbance

UV absorbance has been the most commonly used detection method in HPLC determination of NMC pesticides [1,2,118,122–131, and relevant references in Tables 3–5] probably because of its wide applicability and consequent presence in most HPLC systems. However, UV is subject to interference from sample co-extractives and also lacks sensitivity for some compounds, two factors which limit its usefulness for analysis of environmental samples. Sparacino and Hines [118] studied absorption maxima and extinction coefficients for 14 NMC pesticides and metabolites and found that with the exception of carbaryl (222 nm), methomyl (233 nm) and Mobam (223 nm) absorption maxima occurred at 202 nm or less. This is a region where plant co-extractives also commonly absorb strongly.

In order to overcome these limitations some workers have employed derivatization of NMCs either before HPLC determination or on-line following chromatographic separation. Nelsen and Cook [11] improved the resolution of carbofuran from soil co-extractives by performing base and acid washes of soil extracts which converted carbofuran to its phenolic moiety. The phenol was well resolved from co-extractives. Any phenol initially present as a metabolite was removed in the initial base wash.

Li *et al.* [55] hydrolyzed butocarboxim and its oxidation products. This released methylamine which they derivatized with 1-fluoro-2,4-dinitrobenzene (FDNB) to form N-methyl-2,4-dinitroaniline which was determined by HPLC. Lauren and Agnew [83] reacted the phenolic metabolites of carbofuran with FDNB to form 2,4-dinitrophenyl ether derivatives and achieved detection limits of 2–4 ng. Pietrogrande *et al.* [82] hydrolyzed carbaryl and derivatized the resulting 1-naphthol with 4-aminoantipyrine. The derivative was determined by HPLC–UV at 460 nm, a wavelength at which co-extractive compounds are not likely to absorb. The detection limit was 3 ng.

A recent method reported by Tena *et al.* [131] employed a post-column derivatization reaction for UV detection of carbaryl and 1-naphthol. The post-column reaction required the delivery of three reagents to accomplish the hydrolysis of carbaryl with NaOH, diazotization of sulphanilic acid with $NaNO_2$ and coupling of 1-naphthol with diazotized sulphanilic acid. Derivatization provided stonger absorption at 280 nm and also allowed monitoring of the chromatogram at 506 nm, thus minimizing the possibility of interference from co-extractives. An interesting aspect of the method was that the flow cell of the UV detector was packed with C_{18} bonded silica (60–100 μm) which served to retain and concentrate the derivative in the flow cell and thereby allow determination at low concentration levels; the detection limit was <3.5 ng. In order to maximize sensitivity, a post-column pump was used to deliver water to the flow stream down stream from the reactor to dilute the aqueous acetonitrile mobile phase and favour retention of the derivative on the C_{18} solid-phase in the flow cell. A fifth reagent (acidified ethanol) was delivered when required, through a switching valve located just prior to the flow cell, to elute the derivative from the C_{18}

solid-phase after each peak had been completely integrated.

5.2. Fluorescence

Fluorescence detection is not nearly as widely applicable as UV detection, since most NMCs do not possess native fluorescence. However, for those which fluoresce, or can be made to fluoresce by derivatization, fluorescence detection offers a degree of selectivity and sensitivity often an order of magnitude or more over that offered by UV. Frei and co-workers [3,4] were the first to report the use of fluorescence detection for the determination of NMC pesticides. Fourteen NMCs were rendered fluorescent by derivatization with dansyl chloride prior to injection. Detection limits were between 1 and 10 ng. This approach was used to determine carbaryl [62,63] and carbofuran [64] residues in vegetables.

Moye and Wade [92] introduced a fluorometric enzyme inhibition detector for NMCs. In this system, the effluent from a reversed-phase LC column was incubated with cholinesterase, which was introduced via a post-column reagent-delivery pump, and the resulting partially inhibited cholinesterase was reacted with N-methyl indoxyl acetate to produce a fluorophore. The presence of a cholinesterase inhibitor was indicated by a reduction in the baseline fluorescence. Detection limits ranged from 0.2 ng for carbofuran to 50 ng for aldicarb.

A significant development occurred in 1977 when Moye *et al.* [121] introduced a post-column derivatization reaction for NMCs. Sodium hydroxide introduced by a post-column reagent delivery pump was used to hydrolyze the NMC at 90°C and release methylamine. This methylamine was subsequently reacted with a mixture of *o*-phthalaldehyde (OPA) and 2-mercaptoethanol, introduced by a second post-column pump, to form a highly fluorescent derivative identified as (1-hydroxyethylthio)-2-methylisoindole [132]. The detection limit for methomyl was as low as 0.1 ng.

In a series of studies, Krause refined the chromatographic and derivatization parameters [133, 134], introduced a complex extraction and clean-up procedure for crop samples [102] and validated the method through collaborative studies [103,104]. The method was rapidly adopted by a large number of workers for determination of various NMCs in a variety of substrates including water [18,32,39,42, 43,48], soil [18,76] and plant tissue [65,73,76,78,79, 87,91,98,110,111,114].

During this time there were also reports of the determination of naturally fluorescent aryl NMCs (aminocarb, carbaryl, carbofuran, 3-hydroxycarbofuran and Mobam) without derivatization [22,67,72, 94,105,135]. Detection of 0.5 ng carbaryl was reported [135].

In 1983 Nondek *et al.* [36,120] reported the use of a catalytic solid-phase reactor consisting of a column packed with an anion-exchanger resin maintained at 100–120°C for hydrolysis of NMCs. This reactor eliminated the need for the NaOH post-column reagent delivery pump and allowed detection of as little as 0.1 ng aldicarb and 0.85 ng methiocarb. She *et al.* [47] successfully used this technique with a less expensive anion exchanger to determine carbaryl in polluted water samples. Jansen *et al.* [136] miniaturized the solid-phase reactor to render it compatible with narrow-bore LC on 1 mm I.D. columns and observed detection limits of 0.4 ng for methomyl and 1.0 ng for propoxur. In a study which extended the application of the solid-phase reactor to determination of 22 NMC pesticides and 10 metabolites on crop samples De Kok *et al.* [74] used magnesium oxide in place of the anion exchanger and reduced reaction band broadening to zero.

Another approach to hydrolysis of NMCs prior to derivatization with OPA–2-mercaptoethanol was proposed by Miles and Moye [44,137] who employed a photolytic reactor consisting of a UV lamp inserted in the centre of a woven coil of PTFE tubing. This also eliminated the need for one post-column pump. Detection limits were about 1.0 ng.

A useful simplification of the post-column reaction technique which eliminated the need for both an NaOH post-column pump and solid-phase or photolytic reactors was reported by McGarvey [138]. In this approach the hydrolysis and derivatization steps were combined by the use of a single reagent, OPA–2-mercaptoethanol in 0.01 *M* KOH, which was delivered by a single post-column pump. The detection limit for eleven NMCs by the single-stage derivatization system was about 0.1 ng. Reproducibility of retention times and peak heights was also good, coefficients of variation averaging 0.2% and 2.3%, respectively. This technique has been

used to determine oxamyl in potatoes [108], and oxamyl and methomyl in crops and water [45].

5.3. Electrochemical detection

Electrochemical detection is possible for analytes capable of being oxidized or reduced at moderate electrode potentials. It offers a degree of selectivity through adjustment of electrode potential, but has seen limited use for detection of NMC pesticides. Anderson and Chesney [34] studied oxidative electrochemical detection of aminocarb, BPMC and carbaryl at applied potentials of up to 1.2 V, but successfully detected only aminocarb which exhibited a detection limit of 140 pg. Alawi and Rüssel [21] determined methomyl oxime, but not methomyl, by electrochemical detection and observed a detection limit of 100 pg. Mayer and Greenberg [139] determined carbaryl, methiocarb, propoxur and trimethacarb at applied potentials of 1.31 to 1.37 V and observed detection limits ranging from 1–1000 ng. Only methiocarb and carbaryl exhibited potential for detection by this method at reasonable sensitivity.

Anderson *et al.* [40] determined aminocarb in water (detection limit 53 pg) using a microarray electrochemical detector. Olek *et al.* [70] determined six NMC pesticides in vegetables with detection limits of 0.5–2 ng. Thomas and Sturrock [140] could detect only aminocarb at an applied potential of 1.3 V but detected aldicarb, aminocarb, bendiocarb, carbaryl and methiocarb at 1.9 V. They were able to achieve a detection limit of 5 pg for aminocarb at 1.3 V and about 0.1 ng for the rest at 1.9 V. Von Nehring *et al.* [41] determined aminocarb and carbaryl at 1.25 and 1.3 V and Kawai *et al.* [141] determined carbaryl at 0.75 V (detection limit 0.03 ng).

Krause [106] used a post-column pump delivering NaOH solution to hydrolyze six NMC pesticides to their phenolic moieties and detected the phenols at 0.55 V. Detection limits in fruit and vegetable extracts ranged from 0.25 ng for carbaryl to 0.65 ng for bufencarb.

5.4. Mass spectrometry

Several workers have reported the use of mass spectrometry (MS) for detection of NMCs. The earliest reports [16,17] involved use of a fraction collector to collect carbaryl peaks as they were eluted from a UV detector and confirmation of their identity off-line using low- and high-resolution field desorption MS. Schmid *et al.* [93] used off-line electron impact MS to identify HPLC fractions containing bufencarb and propoxur.

Wright and co-workers [6,142] employed on-line HPLC–MS with a moving belt interface and a 2-propanol–hexane mobile phase and methane chemical ionization and obtained readily identifiable mass spectra. Detection limits ranged from 2.5 to 27.2 ng for nine NMCs. Cairns *et al.* [66] also used a moving belt interface with a hexane–isopropanol–dichloromethane mobile phase and methane chemical ionization mass spectrometry to determine methomyl and oxamyl. A protonated molecular ion was observed for methomyl (m/z 163) but not for oxamyl.

Voyksner and co-workers [9,143] applied on-line thermospray LC–MS to the determination of several NMCs using an aqueous methanol mobile phase and post-column addition of ammonium acetate for the ionization. Detection limits were in the range 1–8 ng. Voyksner and co-workers [144,145] also optimized conditions for direct liquid introduction LC–MS with an aqueous acetonitrile mobile phase and observed detection limits of 40–50 ng. Shalaby [146] employed on-line thermospray HPLC–MS for determination of methomyl with an aqueous acetonitrile or aqueous methanol mobile phase and post-column addition of ammonium acetate.

Bellar and Budde [14] determined 12 NMCs in water samples using on-line thermospray LC–MS with ammonium acetate in the aqueous acetonitrile mobile phase, and obtained detection limits ranging from 8 ng for methiocarb to 320 ng for carbaryl. Chiu *et al.* [147] used the same technique, except with methanol instead of acetonitrile in the mobile phase, and obtained detection limits in the low nanogram range. Bellar *et al.* [148] also used a particle beam interface for LC–MS determination of carbaryl and found that post-column addition of ammonium acetate solution resulted in enhanced positive ion abundance.

Liu *et al.* [69] used thermospray LC–MS with selected ion monitoring for determination of seven NMCs in fruits and vegetables, but obtained poor sensitivity for aldicarb, aldicarb sulphoxide, metho-

myl and oxamyl. Durand *et al.* [57] and Barceló *et al.* [53] studied the influence of different LC eluents in positive and negative ion mode thermospray LC–MS spectra of carbaryl, carbofuran, oxamyl and propoxur. They found that in positive ion (PI) mode the reversed-phase eluents produced a base peak corresponding to $[M + NH_4]^+$, but that relative intensities of other adduct ions varied with varying eluents. Thus the potential for positive identification by thermospray LC–MS may be extended by the use of different LC eluent mixtures. In negative ion (NI) mode carbaryl exhibited a base peak attributed to $[M - CONHCH_3 + CH_3COOH]^-$ and oxamyl a base peak attributed to $[M - CON(CH_3)_2 + HCOO]^-$. Sensitivity for carbaryl and oxamyl was 1.5 and 3 orders of magnitude better, respectively, in PI mode than in NI mode. Carbofuran and propoxur did not show any response in NI mode.

5.5. Other detection techniques

Ramsteiner and Hörmann [149] utilized a cholinesterase inhibition autoanalyzer for detection of dioxacarb and calculated a detection limit of 200 ng. The chief advantage of this method was that there was no interference from non-cholinesterase-inhibiting co-extractives.

In one study [20] chlorine selective electrolytic conductivity was evaluated for 37 pesticides including methomyl and carbaryl. The detector was found to be suitable for methomyl but unsuitable for carbaryl. It was speculated that adaptation of S- and N-selective electrolytic conductivity to HPLC would increase the potential applications of the system, but no subsequent related studies were found.

Another study of the behaviour of pesticides in normal-phase HPLC on a silica column [119] used IR detection at 5.75 μm to monitor 12 NMC insecticides. The study used 1-mg/ml pesticide standards and made no suggestion that IR would be suitable for trace analysis.

6. CONCLUSIONS

It is clear that a large and very diverse body of literature exists describing the determination of NMC pesticides by HPLC. This represents a well-developed field of study, but one in which, nonetheless, there is much room for further work. Of particular usefulness will be additional developments in sensitive and selective detection systems which minimize the need for clean-up of samples.

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Relationships of structures of nitro-polycyclic aromatic hydrocarbons with high-performance liquid chromatography retention order

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ABSTRACT

Forty six structurally related nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) and their corresponding parent PAHs were employed to study the relationships between structure and HPLC retention time. Using reversed-phase HPLC, larger molecules had longer retention times, while saturation of the aromatic rings shortened the retention time. Isomers with a perpendicular nitro group had shorter retention times than if the nitro substituent-was parallel to the ring system. The addition of a nitro group caused a substantial decrease in retention time when compared to its parent PAH. When using normal-phase HPLC, an additional benzo ring increased the retention time. The presence of one or two nitro groups on the molecule also increased the retention time, while saturation of a benzo ring decreased the retention time. These results suggest that the polarity of the PAH or nitro-PAH is the principal factor for determining its HPLC retention time.

INTRODUCTION

Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are a class of mutagenic and carcinogenic environmental pollutants [1–8]. Since nitro-PAHs may pose adverse effect to human health, it is necessary to identify the toxic nitro-PAHs present in the environment, to study their metabolic activation, and to determine their genotoxic effects. HPLC has been the major analytical tool in the biological stud-

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ies of nitro-PAHs, including separation of the metabolites and nucleoside adducts [5,7,8]. The metabolites separated include epoxides, *trans*-dihydrodiols, phenolic products, quinones, tetrahydrotetrols, nitroso-PAHs and amino-PAHs [7]. However, to date, HPLC has been used much less frequently for separating the parent nitro-PAHs present in environmental mixtures [2,9–11]. This may be due to the fact that geometric isomers of nitro-PAHs, such as 1-nitropyrene and 2-nitropyrene, are generally difficult to separate by conventional analytical methods, including HPLC [2,9–11]. Thus, little has been known concerning the relationships between structure and HPLC retention time of nitro-PAHs [2,11]. It has been reported that certain structural features of nitro-PAHs can affect the biological activities, such as mutagenicity and tumorigenicity, as well as the chemical properties of these compounds [5–7,12–16]. Among these, orientation of the nitro group has been found to be a decisive structural feature [6,7]. It is known that when a compound is eluted from an HPLC column, polarity and molecular size of the molecule are important factors in determining the HPLC retention time [2,11–14]. Polarity is largely related to the type, number, and location of the functional group(s) in the molecule. Nitro orientation would also alter polarity and therefore affect HPLC retention times of nitro-PAHs. In order to determine the relationships between structures and HPLC retention times, 46 nitro-PAHs were chosen for this study and several different HPLC columns (reversed-phase and normal-phase; monomeric, polymeric, and chiral stationary phase) were employed.

EXPERIMENTAL

Materials

The following PAHs and nitro-PAHs were purchased from Aldrich (Milwaukee, WI, USA): naphthalene (N), biphenyl (Bp), fluorene (F), anthracene (A), phenanthrene (Ph), 9,10-dihydrophenanthrene (H_2-Ph), pyrene (Py), 1,2,3,6,7,8-hexahydropyrene (H_6-Py), benz[*a*]anthracene (BA), chrysene (Ch), benzo[*a*]pyrene (BaP), benzo[*e*]pyrene (BeP), 1-nitronaphthalene (1-N-N), 2-nitronaphthalene (2-N-N), 4-nitrobiphenyl (4-N-Bp), 2-nitrofluorene (2-N-F), 9-nitrophenanthrene (9-N-Ph), 1-nitropyrene (1-N-Py), and 3,4-dihydrobenz[*a*]anthracen-1(2H)-one. 4,5-Dihydrobenzo[*a*]pyrene (H_2-BaP), 7,8,9,10-tetrahydrobenzo[*a*]pyrene (H_4-BaP), 1-nitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene (1-N-H_4-BaP), 3-N-H_4-benzo[*a*]pyrene, 6-N-H_4-benzo[*a*]pyrene, 1-nitrobenzo[*a*]pyrene (1-N-BaP), 3-nitrobenzo[*a*]pyrene (3-N-BaP), 6-nitrobenzo[*a*]pyrene (6-N-BaP), 9,10,11,12-tetrahydrobenzo[*e*]pyrene (H_4-Bep), 1-nitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene (1-N-H_4-BeP), 3-N-H_4-benzo[*e*]pyrene, 4-N-H_4-benzo[*e*]pyrene, 1,2,3,6,7,8,9,10,11,12-decahydrobenzo[*e*]pyrene (H_{10}-BeP), 4-nitro-1,2,3,6,7,8,9,10,11,12-decahydrobenzo[*e*]pyrene (4-N-H_{10}-BeP), 1-nitrobenzo[*e*]pyrene (1-N-BeP), 3-nitrobenzo[*e*]pyrene (3-N-Bep), 4-nitrobenzo[*e*]pyrene (4-N-Bep), 1,3-dinitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene (1,3-Di-N-H_4-BeP), 1,6-dinitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene (1,6-Di-N-H_4-BeP), 1,8-dinitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene (1,8-Di-N-H_4-BeP), 1,3-dinitrobenzo[*e*]pyrene (1,3-Di-N-BeP), 1,6-dinitrobenzo[*e*]pyrene (1,6-Di-N-BeP) and 1,8-dinitrobenzo[*e*]pyrene (1,8-Di-N-BeP) were prepared as previously described [12–15,17]. 4-Nitro-1,2,3,6,7,8-hexahydropyrene (4-N-H_6-Py) was synthesized by nitration of 1,2,3,6,7,8-hexahydropyrene (H_6-Py) with sodium nitrate in trifluoroacetic acid, a modified method of Spitzer and Stewart [18] and 4-nitropyrene (4-N-Py) was prepared by dehydrogenation of 4-N-H_6-Py with 2,3-dichloro-5,6-dicyano-1,6-benzoquinone [19,20]. 4,5,9,10-Tetrahydropyrene (H_4-Py) was synthesized by hydrogenation of pyrene (Py) catalyzed by palladium on charcoal [21]. 4,5,7,8,9,10,11,12-Octahydrobenzo[a]pyrene (H_8-BaP) was similarly synthesized from H_4-BaP [21]. 9-Nitroanthracene (9-N-A) [14], 7-nitrobenz[*a*]anthracene (7-N-BA) [22], 6-nitrochrysene (6-N-Ch), 2-nitro-9,10-dihydrophenanthrene (2-N-H_2-Ph), 2-nitro-4,5,9,10-tetrahydropyrene (2-N-H_4-Py) [23], 2-nitro-4,5,7,8,9,10,11,12-octahydro-BaP (2-N-H_8-BaP), 7-nitrodibenz[*a,h*]anthracene (7-N-DiB-[*a,h*]A), and 9-nitrodibenz[*a,c*]anthracene (9-N-DiB[*a,c*]A) were similarly prepared by nitration of anthracene, benz[*a*]anthracene (BA), chrysene (Ch), 9,10-dihydrophenanthrene (H_2-Ph), 4,5,9,10-tetrahydropyrene (H_4-Py), 4,5,7,8,9,10,11,12-octahydrobenzo[*a*]pyrene (H_8-BaP), dibenz[*a,h*]anthracene (DiB[*a,h*]A), and dibenz[*a,c*]anthracene (DiB-[*a,c*]A), respectively, with sodium nitrate in trifluoroacetic acid. Nitration of 4,5-dihydro-BaP gave 6-

nitro-4,5-dihydrobenzo[*a*]pyrene (6-N-H_2-BaP) as a major product, and 12-nitro-4,5-dihydrobenzo[*a*]pyrene (12-N-H_2-BaP) and 1,6-dinitro-4,5-dihydrobenzo[*a*]pyrene (1,6-Di-N-H_2-BaP) as minor products [24]. Nitration of 7,8,9,10-tetrahydrobenzo[*a*]pyrene with two equivalent molar ratio of sodium nitrate in trifluoroacetic acid yielded 1,3-dinitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene (1,3-Di-N-H_4-BaP), 1,6-dinitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene (1,6-Di-N-H_4-BaP) and 3,6-dinitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene (3,6-Di-N-H_4-BaP) as major products and 1,3,6-trinitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene (1,3,6-Tri-N-H_4-BaP) as a minor product [24]. 2-Nitroanthracene (2-N-A) was obtained as a gift from Dr. F. A. Beland. 1,3-Dinitropyrene (1,3-Di-N-Py), 1,6-dinitropyrene (1,6-Di-N-Py), and 1,8-dinitropyrene (1,8-Di-N-Py) were prepared by nitration of pyrene with two equivalent amounts of sodium nitrate in trifluoroacetic acid, and were separated by a normal-phase HPLC system employing a Zorbax SIL semi-preparative column (250 × 9.4 mm I.D.), eluted with hexane–methanol–acetonitrile (72:2:1, v/v/v) at a flow-rate of 5.6 ml/min. Under these conditions, 1,3-dinitropyrene, 1,6-dinitropyrene, and 1,8-dinitropyrene eluted at 10.6, 12.8, and 17.0 min, respectively. All the known compounds, either purchased or synthesized, were characterized by comparison of their UV–visible absorption and mass spectra with the published data. For the identification of the new compounds, high-resolution nuclear magnetic resonance spectral analysis was employed. The orientation of the nitro substituents of each compound were also characterized by spectral analysis [12–16,23,24].

Chromatography

The HPLC system was composed of two Beckman/Altex (Fullerton, CA, USA) Model 100 pumps, a Beckman/Altex 420 gradient controller, a Beckman/Altex Model 210 injector, a Waters Assoc. (Milford, MA, USA) Model 440 absorbance detector set at 254 nm, a Kipp & Zonen (Delft, Netherlands) Model BD41 dual-pen strip-chart recorder, and optionally, a Hewlett-Packard (Palo Alto, CA, USA) Model 3390A reporting integrator, or with a Hewlett-Packard 1040A detection system with the Data Processing Unit option. The columns used were: Zorbax ODS (250 × 4.6 mm I.D.) (Du-Pont Medical Products, Wilmington, DE, USA); Zorbax SIL (250 × 4.6 mm I.D.); Vydac 201TP54 ODS (250 × 4.6 mm I.D.) (The Separations Group, Hesperia, CA, USA) and a Pirkle-type chiral stationary phase column (250 × 4.6 mm I.D.) (Regis, Morton Grove, IL, USA) packed with (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine covalently bonded to spherical particles of γ-aminopropylsilanized silica. All columns have a particle size of 5 μm. All mobile phases, as described in Tables I and II, were HPLC-grade solvents and were degassed before use. Each injection was 20 μl or less in volume. The retention time was recorded by the 1040A detection system, and the ultra violet absorption spectrum of the material in each chromatographic peak was recorded, so that the identity of the chromatographic peak could be confirmed. After the retention time of each compound had been determined, a mixture of two or more compounds was chromatographed in order to confirm the relative elution order and reproducibility of retention times. To eliminate the possible UV photolytic decomposition of the compounds, the laboratory was equipped with UV-absorbing films placed above the light diffusion panels.

RESULTS AND DISCUSSION

In this paper, we employ a series of structurally-related nitro-PAHs to study the relationships between structure and HPLC retention. For determining the effect of the nitro substituent on HPLC retention time, the HPLC retention times of the corresponding parent PAHs were also determined. The structures and abbreviations of the parent PAHs and nitro-PAHs used in this study are shown in Fig. 1.

It has been previously reported that, depending on the geometric location of the nitro substituent, one of two orientations will predominate [6,7,12]. Orientation of the nitro group can be well determined by spectral analysis of the compounds, including uv-visible absorption, mass and proton nuclear magnetic resonance (NMR) spectra [6,7,25–27]. Compounds of the first type have their nitro substituents perpendicular or nearly perpendicular to the aromatic moiety so that steric hindrance with the adjacent protons can be minimized. Nitro-PAHs of the second type have their nitro substituents parallel (co-planar) or nearly parallel to the

TABLE I

HPLC RETENTION TIMES OF POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR PARTIALLY SATURATED DERIVATIVES ON DIFFERENT REVERSED- AND NORMAL-PHASE COLUMNS

The eluents for the HPLC analyses are: methanol–water (90:10, v/v) at a flow-rate of 1.5 ml/min for the Zorbax ODS column; methanol–water (90:10, v/v) at a flow-rate of 1.0 ml/min for the Vydac ODS column; tetrahydrofuran–hexane (3:97) at a flow-rate of 3.0 ml/min for the Zorbax SIL column; and ethanol–acetonitrile–hexane (2:1:27, v/v/v) at a flow-rate of 1.0 ml/min for the Pirkle column.

Compound	Retention time (min)			
	Zorbax ODS	Vydac ODS	Zorbax SIL	Pirkle Ph-Gl
Naphthalene (N)	3.8	3.4	4.0	4.5
Biphenyl (Bp)	4.6	3.8	3.9	4.6
Fluorene (F)	5.7	4.4	4.3	4.9
Anthracene (A)	6.2	5.0	4.3	5.6
Phenanthrene (Ph)	5.8	4.4	4.1	5.8
9,10-Dihydrophenanthrene (H_2-Ph)	6.3	4.9	5.0	5.0
Pyrene (Py)	8.2	6.3	6.5	6.8
4,5,9,10-Tetrahydropyrene (H_4-Py)	9.0	5.8	3.97	4.3
1,2,3,6,7,8-Hexahydropyrene (H_6-Py)	14.0	6.7	3.99	4.1
Chrysene (Ch)	10.6	11.1	5.0	7.1
Benz[*a*]anthracene (BA)	10.5	11.2	4.9	6.9
Benzo[*a*]pyrene (BaP)	16.3	12.3	3.9	7.9
4,5-Dihydrobenzo[*a*]pyrene (H_2-BaP)	17.7	16.2	2.9	6.2
7,8,9,10-Tetrahydro-BaP (H_4-BaP)	27.4	22.7	3.98	6.4
4,5,7,8,9,10,11,12-Octahydro-BaP (H_8-BaP)	28.7	23.7	3.96	4.0
Benzo[*e*]pyrene (BeP)	14.7	13.9	6.4	8.3
9,10,11,12-Tetrahydro-BeP (H_4-BeP)	27.0	18.5	3.79	7.9
1,2,3,6,7,8,9,10,11,12-Decahydro-BeP (H_{10}-BeP)	43.6	18.8	3.78	7.7
Dibenz[*a,c*]anthracene (DiB[*a,c*]A)	18.5	14.2	5.2	9.2
Dibena[*a,h*]anthracene (DiB[*a,h*]A)	19.0	14.3	5.0	9.3

TABLE II

HPLC RETENTION TIMES OF NITRO-POLYCYCLIC AROMATIC HYDROCARBON DIHYDRODIOLS AND THEIR PARTIALLY SATURATED DERIVATIVES ON DIFFERENT REVERSED AND NORMAL PHASES

The eluents for the HPLC analyses are: methanol–water (90:10, v/v) at a flow-rate of 1.5 ml/min for the Zorbax ODS column; methanol–water (90:10, v/v) at a flow-rate of 1.0 ml/min for the Vydac ODS column; tetrahydrofuran–hexane (3:97, v/v) at a flow-rate of 3.0 ml/min for the Zorbax SIL column; and ethanol–acetonitrile–hexane (2:1:27, v/v/v) at a flow-rate of 1.0 ml/min for the Pirkle column.

Nitro-PAH	Nitro orientation	Retention time (min)			
		Zorbax ODS	Vydac ODS	Zorbax SIL	Pirkle Ph-Gly
1-N-N	Parallel	3.2	3.1	4.3	8.0
2-N-N	Parallel	3.4	3.5	4.2	7.7
4-N-H_6-Py	Parallel	11.2	5.3	6.43	4.9
4-N-H_{10}-BeP	Parallel	26.2	13.5	6.44	10.1
4-N-Bp	Parallel	3.8	3.6	6.15	7.6
2-N-F	Parallel	4.5	4.0	4.9	8.6
2-N-H_2-Ph	Parallel	5.3	4.2	3.24	5.0
2-N-H_4-Py	Parallel	6.6	5.3	11.2	6.8
2-N-H_8-BaP	Parallel	17.1	13.6	12.3	6.4

TABLE II (*continued*)

Nitro-PAH	Nitro orientation	Retention time (min)			
		Zorbax ODS	Vydac ODS	Zorbax SIL	Pirkle Ph-Gly
2-N-A	Parallel	5.3	4.7	4.45	8.8
9-N-A	Perpendicular	2.0	2.6	4.55	6.3
9-N-Ph	Parallel	5.8	4.0	5.9	10.2
6-N-Ch	Parallel	8.7	7.6	5.6	3.4
7-N-BA	Parallel	7.5	6.9	5.7	4.5
6-N-H_2-BaP	Perpendicular	8.5	9.2	3.86	7.8
12-N-H_2-BaP	Parallel	12.2	12.4	10.2	15.6
9-N-DiB[*a,c*]A	Perpendicular	10.5	9.3	9.3	12.8
7-N-DiB[*a,h*]A	Perpendicular	12.5	9.4	8.0	11.6
4-N-Py	Parallel	7.0	5.9	6.7	9.6
1-N-Py	Parallel	6.8	5.5	7.8	8.0
1-N-H_4-BaP	Parallel	20.1	20.5	5.0	10.5
3-N-H_4-BaP	Parallel	20.0	22.8	5.2	10.7
6-N-H_4-BaP	Perpendicular	16.6	15.0	5.54	10.8
1-N-H_4-BeP	Perpendicular	15.2	9.6	5.4	8.4
3-N-H_4-BeP	Parallel	23.5	16.2	4.63	10.8
4-N-H_4-BeP	Parallel	24.6	16.6	4.3	12.4
1-N-BaP	Parallel	14.0	9.4	8.02	14.0
3-N-BaP	Parallel	14.2	9.7	8.03	10.9
6-N-BaP	Perpendicular	11.9	8.4	6.99	14.5
1-N-BeP	Perpendicular	8.3	7.1	8.35	12.1
3-N-BeP	Parallel	13.0	12.6	6.98	13.0
4-N-BeP	Parallel	12.6	11.9	6.57	12.4
1,3-Di-N-Py	Parallel	6.6	5.4	10.6	4.6
1,6-Di-N-Py	Parallel	5.3	4.7	10.9	3.4
1,8-Di-N-Py	Parallel	5.5	4.9	13.3	3.2
1,3-Di-N-H_4-BaP	Parallel	14.0	10.5	4.0	12.5
1,6-Di-N-H_4-BaP	Mixed[a]	15.3	14.0	2.8	12.2
3,6-Di-N-H_4-BaP	Mixed	13.1	15.0	4.3	12.0
1,3-Di-N-H_4-BeP	Mixed	16.6	11.6	7.65	12.9
1,6-Di-N-H_4-BeP	Mixed	12.4	9.9	8.76	15.3
1,8-Di-N-H_4-BeP	Mixed	8.8	7.6	9.3	12.2
1,3,6-Tri-N-H_4-BaP	Mixed	16.6	15.1	3.76	9.2
1,6-Di-N-H_2-BaP	Mixed	8.7	9.9	10.1	15.5
1,3-Di-N-BeP	Mixed	6.7	10.1	8.06	11.8
1,6-Di-N-BeP	Mixed	7.4	6.6	5.0	12.3
1,8-Di-N-Bep	Perpendicular	5.5	4.4	5.2	12.0

[a] "Mixed" indicates that the nitro-PAH compound contains at least one nitro substituent with a parallel orientation and one nitro substituent with a perpendicular orientation.

aromatic moiety. Based on the analysis of the spectral data, the orientations of the nitro-PAHs are shown in Table II. In order to find out how nitro orientation can affect HPLC retention time, 46 nitro-PAHs of both types were chosen for this study. The compounds were selected on a structure comparison basis. For example, there are four sets of geometric mononitro-PAH isomers: (1) 1-N-BaP, 3-N-BaP, and 6-N-BaP; (2) 1-N-BeP, 3-N-BeP, and 4-N-BeP; (3) 1-N-H_4-BaP, 3-N-H_4-BaP, and 6-N-H_4-BaP; and (4) 1-N-H_4-BeP, 3-N-H_4-BeP, and 4-N-H_4-BeP (see Fig. 1). Each set contains the iso-

mers with different nitro orientations, and therefore, is relevant for determining the effect of nitro orientation on HPLC retention. To examine the effect of the nitro group(s) on HPLC retention, some representative parent PAHs were included for study.

To compare the separation capability of each type of column, a monomeric Zorbax ODS column, a polymeric Vydac ODS column, a normal-phase Zorbax SIL column, and a Pirkle-type chiral stationary-phase column were employed. The retention times of the parent PAHs and the nitro-PAHs

Parent PAHs	NO_2-PAHs	Parent PAHs	NO_2-PAHs
Biphenyl (Bp)	4-N-Bp	Pyrene (Py)	1-N-Py 4-N-Py 1,3-Di-N-Py 1,6-Di-N-Py 1,8-Di-N-Py
Naphthalene (N)	1-N-N 2-N-N	4,5,9,10-tetrahydropyrene (H_4-Py)	2-N-H_4-Py
Fluorene (F)	2-N-F	1,2,3,6,7,8-Hexahydropyrene (H_6-Py)	4-N-H_6-Py
Anthracene (A)	2-N-A 9-N-A	Benz[a]anthracene (BA)	7-N-BA
Phenanthrene (Ph)	9-N-Ph	Chrysene (Ch)	6-N-Ch
9,10-Dihydrophenanthrene (H_2-Ph)	9-N-H_2-Ph		

Fig. 1.

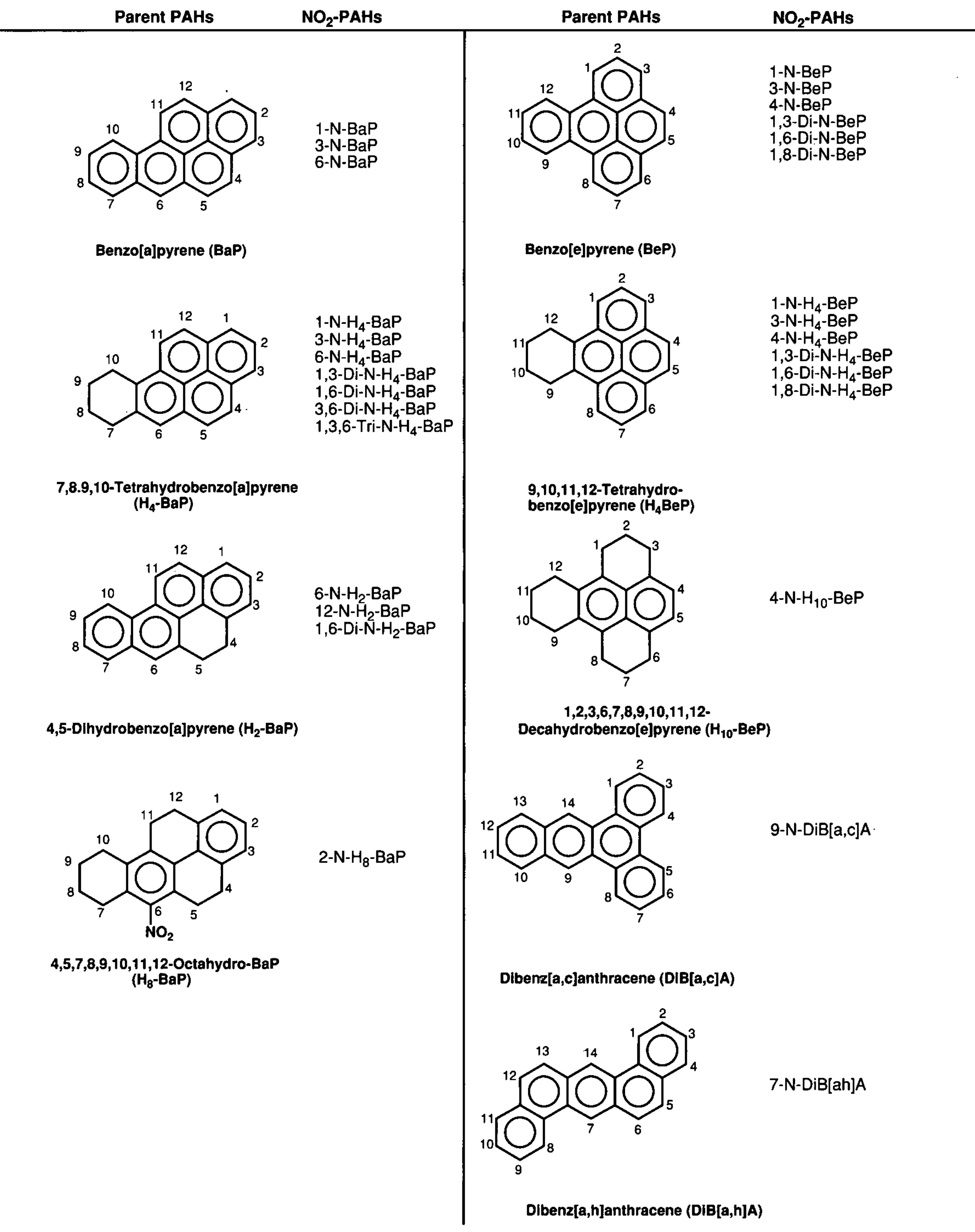

Fig. 1. Structures and abbreviations of the polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs used in this study.

were determined accordingly (Tables I and II). In order to obtain suitable retention times for nitro-PAHs, a number of different solvent systems and solvent flow-rates have been tested. The final conditions chosen are shown in Tables I and II.

Separation of the parent PAHs by reversed-phase and normal-phase HPLC

To determine the effect of the nitro substituent on HPLC retention, the HPLC chromatographic conditions chosen for eluting the parent PAHs were identical to those used for nitro-PAHs. Thus, optimum conditions were not chosen for separation of these compounds. As expected, due to the low polarity of the PAH molecules, the monomeric Zorbax ODS column and the polymeric Vydac ODS column provided much better separation than the normal-phase Zorbax SIL column (Table I). Both the Zorbax SIL and the Pirkle-type CSP columns separated the parent PAHs poorly (Table I). Between the two ODS columns, the monomeric Zorbax ODS column provided better separation (retention times ranging from 3.8 to 43.6 min) than the Vydac column (retention times ranging from 3.3 to 22.7 min). These results contrast to the previous finding that the polymeric ODS Vydac column had better separation for the phenolic derivatives of BA, BaP and chrysene than the monomeric Zorbax ODS column [28]. In general, in these two reversed-phase HPLC systems, PAHs with a larger molecular size had a longer retention time. For example, the increased retention times of naphthalene, anthracene, pyrene, chrysene, and BaP (Table I) is in accord with the increased molecular size of the compounds. For the Zorbax ODS column, another general phenomenon is that retention time increases when one or more aromatic rings are saturated (Table I). An example is the large increase of the retention time from BeP (14.7 min) to H_4-BeP (27.0 min) and H_{10}-BeP (43.6 min). However, this phenomenon is not prominent with the polymeric Vydac ODS column.

Separation of nitro-PAHs by the reversed-phase and normal-phase HPLC

The order of the nitro-PAHs listed in Table II is based on the identity of the aromatic moiety of the molecules. For instance, 1-N-N, 2-N-N, 4-N-H_6-Py, and 4-N-H_{10}-BeP all have a naphthalene aromatic ring system, and thus, are grouped together. Such an arrangement facilitates the determination of the relationship between structural features and HPLC retention order.

Reversed-phase HPLC. Both the monomeric Zorbax and the polymeric Vydac ODS column show similar efficiency on the separation of nitro-PAHs. In general, retention time increased when molecular size increased (Table II). For example, 4-N-H_6-Py has a longer retention time than 1- and 2-N-N, but has a shorter retention time than 4-N-H_{10}-BeP (Table II). Because of higher polarity, nitro-PAHs have shorter retention times than their corresponding parent PAHs. In addition, nitro-PAHs with a perpendicular orientation have a shorter retention time than the geometric isomers which have a parallel orientation. For example, 6-N-BaP and 6-N-H_4-BaP have shorter retention times than 1- and 3-N-BaP, and 1- and 3-N-H_4-BaP, respectively (Table II). Similarly, 1-N-BeP and 1-N-H_4-BeP have shorter retention times than their geometric isomers, 3- and 4-N-BeP and 3- and 4-N-H_4-BeP. Since a nitro-PAH with a perpendicular orientation is more polar than the geometric isomer which has a parallel (co-planar) orientation [29], our results suggest that polarity of the nitro-PAHs is an important factor in determining the HPLC retention time.

With an additional nitro substituent, dinitro-PAHs have shorter retention time than the mononitro analogue (Table II). When a dinitro-PAH has both nitro groups adopting a perpendicular or nearly perpendicular orientation (*e.g.*, 1,8-Di-N-BeP and 1,8-Di-N-H_4-BeP), it has a shorter retention time than the dinitro-PAH isomer(s) which have only one or no nitro group with a perpendicular orientation (*e.g.*, 1,3- and 1,6-Di-N-BeP, and 1,3- and 1,6-Di-N-H_4-BeP). These results further indicate that polarity of a molecule is a decisive factor in determining the HPLC retention time (order).

Normal-phase HPLC. Separation of nitro-PAHs by the normal-phase Zorbax SIL column is much less efficient than by the reversed-phase HPLC column. Retention time increases when molecular size increases (Table II). When a benzo ring is saturated, retention time is also decreased. Contrary to the reversed-phase HPLC, due to polar character of the nitro substituent, mononitro-PAHs have longer retention times than the parent PAHs. Similarly, a

TABLE III

RELATION OF STRUCTURAL FEATURES AND HPLC RETENTION OF NITRO-POLYCYCLIC AROMATIC HYDROCARBONS

The symbols "+" and "−" denote the HPLC retention time increased and decreased, respectively.

Structural feature	Effect on retention time			
	Reversed-phase		Normal-phase	Pirkle-type
	Zorbax ODS	Vydac ODS		
Increase in molecular size	+ + +	+ +	+	+
Addition of a benzo ring	+ +	+ +	+	+ +
Addition of a tetrahydrobenzo ring	+ +	+ +	±	+ +
Saturation of a benzo ring	− −	−	−	±
Addition of a nitro group				
(parallel orientation)	− −	− −	+	±
(perpendicular orientation)	− − −	− − −	+ +	±
Addition of two nitro groups				
(parallel orientation)	− − −	− − −	+ +	±
(perpendicular orientation)	− − − −	− − − −	+ + +	±

dinitro-PAH has a much longer retention time than its mononitro analogues (Table II). As expected, a mononitro-PAH with a perpendicular nitro group has a longer retention time than the isomer with a parallel nitro group. The same phenomenon is observed for dinitro-PAHs (Table II).

Chiral Pirkle HPLC. The Pirkle-type chiral stationary phase column employed has been shown to efficiently resolve enantiomers of a large number of compounds, including the ring-oxidized derivatives of PAHs [30–32]. However, although it was reported that several nitro-PAHs were better separated by chiral Pirkle HPLC than by the other columns [33], our study indicates that the large number of nitro-PAHs used in this study were not well separated. In most cases, but not all, retention time increased when the molecular size increased. Nitro orientation does not significantly affect the retention order. Thus, the chiral character of the stationary phase does not facilitate the separation of nitro-PAHs.

Relationships between structures and HPLC retention times of nitro-PAHs

Greibrokk *et al.* [11] reported the separation of nitro-PAHs and found that, eluted from a normal-phase silica HPLC column with dichloromethane–hexane, the retention order was dependent on (i) the location of the nitro group, (ii) the number of protons *peri* to the nitro substituent, and (iii) the length/breadth ratio of the molecule. We previously reported a study on the separation of ring-oxidized derivatives of nitro-PAHs and found that polarity is the major factor for determining HPLC retention time [29]. We have now examined the relationships between structure and HPLC retention time employing the parent nitro-PAHs. The relationships are summarized in Table III. For both reversed-phase HPLC systems, a larger molecule results in a longer retention time. In contrast, saturation of an aromatic ring shortens the retention time. Among the isomeric nitro compounds, the isomer with a perpendicular nitro group has a shorter retention time than the isomer with a parallel nitro substituent. Comparison of the retention times of the nitro-PAHs and their parent PAHs indicates that a nitro group decreases the retention time substantially.

For the normal-phase chromatography, an additional benzo ring results in an increase of retention time. Saturation of a benzo ring also leads to a shorter retention time (*e.g.*, H_4-BeP *vs.* BeP). The presence of one or two nitro groups to the molecules causes a longer retention time on a normal-phase HPLC system. The opposite effect of a nitro

group on the retention time to the reversed- and normal phase HPLC systems clearly indicate that polarity of the molecules is an important factor in determining the HPLC retention time.

As described previously, certain structural features, particularly the nitro orientation, are important factors in determining the biological activities of nitro-PAHs. In this paper, we have found the relationships between several of the structural features and the HPLC retention order of nitro-PAHs. Thus, our results provide useful information concerning both the HPLC chromatography and the biological significance of the environmental nitro-PAHs.

NOTE

The opinion or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Food and Drug Administration.

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Trace determination of volatile organic compounds in water by enrichment in ultra-thick-film capillary traps and gas chromatography

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ABSTRACT

The technique by which volatile organic compounds are enriched in coated or uncoated capillaries, and desorbed for qualitative gas chromatographic determination, was adapted for quantitative analysis by using a 1-m length of ultra-thick-film trap (145-μm film thickness) to extract the volatile compounds flowing through the trap at 0°C. The trapped volatiles are thermally desorbed, cryotrapped on an analytical capillary column and determined by gas chromatography with flame ionization detection. It was found that analytes such as dichloromethane and chloroform did not break through when sampling 1 ml of water. With this sampling volume, relative standard deviations well below *ca.* 2% and *ca.* 2–4% were obtained for most of the analytes at levels of 1 ppm (v/v) and 1 ppb (v/v), respectively.

INTRODUCTION

The determination of low concentrations of volatile organic compounds in water and water-containing samples mostly requires a concentration step prior to gas chromatographic (GC) measurement. Concentration procedures include closed-loop stripping [1], open-loop or dynamic stripping, also known as purge-and-trap [2,3], and liquid–liquid extraction [4–6], which could conceivably contribute to environmental pollution owing to the relatively large volumes of halogenated solvents that are used in some applications. A simple, rapid and sensitive method that does not require a preconcentration step is the direct aqueous injection technique in conjunction with electron-capture detection (ECD) and/or flame ionization detection (FID) [7,8]. It has also been shown that trace components can be stripped from large aqueous samples passed through long, uncoated copper and polyethylene capillaries [9]. So far, however, this observation has not been implemented in a protocol for quantitative trace analysis.

Kaiser and Rieder [10] developed a qualitative method for the enrichment of high-boiling organic compounds in potable water by liquid–liquid enrichment in a polysiloxane-coated capillary column and Blomberg and Roeraade [11], having succeeded in coating glass capillaries with an ultra-thick polysiloxane stationary phase film, demonstrated the application of these thick-film capillaries to the qualitative analysis of organic compounds in water. Similar traps were produced in our laboratory by the insertion of a polysiloxane rubber tube into a fused-silica tube with 0.53 mm I.D. to produce traps with a film or lining thickness of 145 μm [12]. The finished product had an I.D. of 0.24 mm. It was found that these traps can be applied very successfully to the qualitative analysis of airborne organic volatiles [12–14] and apolar organic compounds in aqueous samples. Although it was demonstrated [15] that the full thickness of the polysiloxane rubber lining of these traps is utilized to retain volatile compounds, it was found that small and highly volatile compounds are not retained very effectively on

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traps short enough to be subjected to thermal desorption in the injector of the gas chromatograph. These observations also apply to the extraction of volatile organic compounds from aqueous samples. The use of longer traps and the trapping of volatiles at subambient temperatures therefore seemed to be unavoidable, even though traps longer than about 70 mm would require additional equipment for the desorption of the volatiles. As far as the analysis of airborne volatiles is concerned, reasonably accurate information on the breakthrough volumes of volatile compounds can be obtained by the extrapolation of the results achieved with shorter traps. However, no information is available on the influence of parameters such as temperature and flow-rate on the efficiency with which organic compounds are extracted from water by an ultra-thick-film capillary trap.

The main aim of our research on the application of capillary traps to the GC determination of volatile organic compounds is to make technology and procedures available that would enable any normally equipped analytical or quality control laboratory to make at least a preliminary or emergency assessment of problems arising from the presence of organic contaminants in, for example, air of gas samples, beverages, drinking water or process effluents, without having to invest in expensive instrumentation or time-consuming conversions of existing equipment. In this paper we report the results of experiments carried out to determine the scope and limitations of long ultra-thick-film traps for the trace determination of organic volatiles in aqueous samples.

EXPERIMENTAL

Long (1-m) fused-silica open-tubular traps were produced by inserting polysiloxane rubber tubing (0.65 mm O.D. × 0.30 mm I.D.) (Silastic medical grade tubing; Dow Corning, Midlands, MI, USA) into polyimide-coated fused-silica tubes (0.70 mm O.D. × 0.53 mm I.D.) according to the procedure described by Burger *et al.* [12] to give ultra-thick-film traps of 0.24 mm I.D. and with a film or lining thickness of 145 μm. Several traps were connected in series, installed in a GC oven and conditioned at 280°C for 80 h.

A spiked water sample containing dichloromethane, chloroform, bromoform, tetrachloroethylene, benzyl chloride, heptane, benzene, toluene, *p*-xylene, and *p*-cymene at the 1 ppm (v/v) level was prepared by injecting 10 μl of a mixture of these compounds through a PTFE-backed septum into the vortex of 1 l of magnetically stirred purified water (obtained with a Milli-Q system; Millipore, Bedford, MA, USA) in a 1-l round-bottomed flask, the neck of which was shortened to leave a relatively small headspace volume (sample A). Water obtained from the Milli-Q system contained a large number of organic compounds at a level of <10 ppb and had to be stripped of these interfering compounds by boiling away about 50% of the original volume. A water sample containing the above-mentioned analytes at a level of 1 ppb (v/v) (*p*-cymeme 2 ppb) was prepared by adding, according to the previously described procedure, 1 ml of a freshly prepared 1 ppm sample to 999 g of this purified water (sample B).

Two ultra-thick-film traps, installed in capillary column cages, were connected with shrinkable PTFE tubing. Using shrinkable PTFE tubing, 0.5- and 1.2-m lengths of fused-silica tubing (0.53 mm I.D.) were connected to the inlet end of the first trap and the oulet end of the second (guard) trap, respectively. The connected traps were immersed into an ice–water bath and purified water was sucked through the traps until all air bubbles had been purged from the trap. This normally took only a few minutes. The outlet end of the fused-silica extension was closed by inserting it into a silicone-rubber septum, whereafter the inlet end was inserted through a septum or an opened stopcock into the spiked water sample. Water was allowed to flow through the traps for 1 h, about 1 ml of water being collected in a weighed vial. The outlet end of the fused-silica extension was again closed and the inlet end removed from the water sample, then the septum was removed from the outlet end of the tubing and the contents of the traps and extensions were allowed to siphon to waste. The water collected in the vial during the first part of the experiment was carefully weighed and represented the total volume of water sampled in the experiment. To achieve the specified flow-rate the tip of the outlet extension had to be *ca.* 85 cm below the surface of the water in the inlet reservoir.

The guard trap was installed in the first oven of a

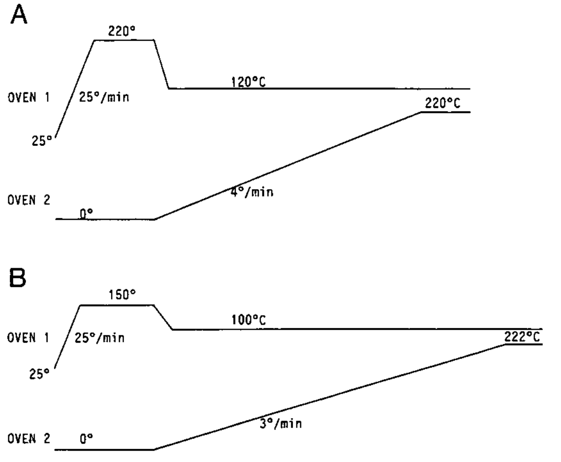

Fig. 1. Temperature programmes used for the desorption and analysis of volatile organic compounds in water. (A) Determination of analytes in sample A at a level of 1 ppm; (B) determination of analytes in sample B at a level of 1 ppb.

Siemens Sichromat 2 gas chromatograph. The volatile organic compounds were thermally desorbed and transported by the carrier gas (helium at 35 cm/s) to an analytical capillary column (40 m × 0.3 mm I.D., glass, coated with immobilized PS-255 with a film thickness of 5 μm) installed in the second oven. The two ovens of the gas chromatograph were temperature programmed according to the profiles depicted in Fig. 1. After it had been established that no breakthrough to the second (guard) trap had taken place, the first trap was subjected to a similar desorption and analysis procedure. Quantification of the FID response was done with a Hewlett-Packard Model 3385A integrator.

Direct on-column, aqueous injection of water containing the analytes at a level of *ca.* 1 ppm was carried out with a Carlo Erba Model 5300 gas chromatograph fitted with a cold on-column injector and a glass capillary column (40 m × 0.3 mm I.D.) coated with PS-255 with a film thickness of 5 μm and connected to a 10 m × 0.32 mm I.D. deactivated fused-silica retention gap.

RESULTS AND DISCUSSION

As far as solubility in water and extractability by the apolar trap lining are concerned, no internal standard can be expected to behave exactly like analytes such as the smaller halogenated compounds. It is therefore clear that the only way to achieve reproducible quantitative results is to employ conditions that ensure quantitative extraction of all the analytes. This requires a relatively low flow-rate of the water through the trap. A flow-rate of *ca.* 1 ml/h was chosen for this purpose. Although this may seem to be excessively time consuming, it must be kept in mind that several traps can be loaded simultaneously and then stored for analysis at a later stage, or that two traps can be used in a loading-and-analysis cycle in an automated system.

The extraction of analytes from water by the polysiloxane lining of a trap cannot be compared with the almost irreversible adsorption of organic molecules on an activated charcoal trap. With ultra-thick-film traps breakthrough is generally not due to overloading or saturation of the traps, but to the movement of the analyte molecules through the trap by normal chromatographic processes. Although the increased rate of mass transfer at higher temperatures would have allowed the use of higher flow-rates, the limiting factor in this method is the relatively low capacity of the trap for the more volatile analytes. Taking the results of studies on temperature-programmed liquid chromatography [16] into consideration, sampling was carried out at the lowest possible temperature (0°C) to increase the capacity of the trap. A series of analyses were carried out with water spiked with the analytes at concentrations of *ca.* 1 ppm. It was found that breakthrough of dichloromethane became detectable at a sampling volume of *ca.* 2 ml. This means that dichloromethane cannot be quantitatively extracted and retained in the trap when sample volumes larger than about 2 ml are used. As the main aim of this study was the development of a method for volatile water contaminants including dichloromethane, further determinations were carried out with sample volumes of about 1 ml.

The results obtained with water containing the ten analyte compounds at a level of 1 ppm are given in Table I and a typical gas chromatogram is shown in Fig. 2A. The relative standard deviations (R.S.D.s) of the absolute peak areas are fairly good, especially if it is taken into consideration that the integration system used does not allow any manipulation of the accumulated data, such as baseline adjustment. For comparison of these results with

TABLE I

AREA COUNT REPEATABILITY FOR THE ANALYSIS OF WATER CONTAINING ORGANIC COMPOUNDS AT A CONCENTRATION OF *ca.* 1 ppm (SAMPLE A) (n = 5)

Compound	Average uncorrected area counts[a]	Standard deviation	R.S.D. (%)
Dichloromethane	72 468	954	1.32
Chloroform	34 088	138	0.41
Benzene	253 328	3333	1.32
Heptane	39 652	9348	23.58
Toluene	233 146	2224	0.95
Tetrachloroethylene	91 448	1380	1.51
Benzyl chloride	231 860	1199	0.52
p-Xylene	230 420	10 334	4.49
Bromoform	5397	982	18.20
p-Cymene	184 619	1253	0.68

[a] Attenuation: 100 × 2^8.

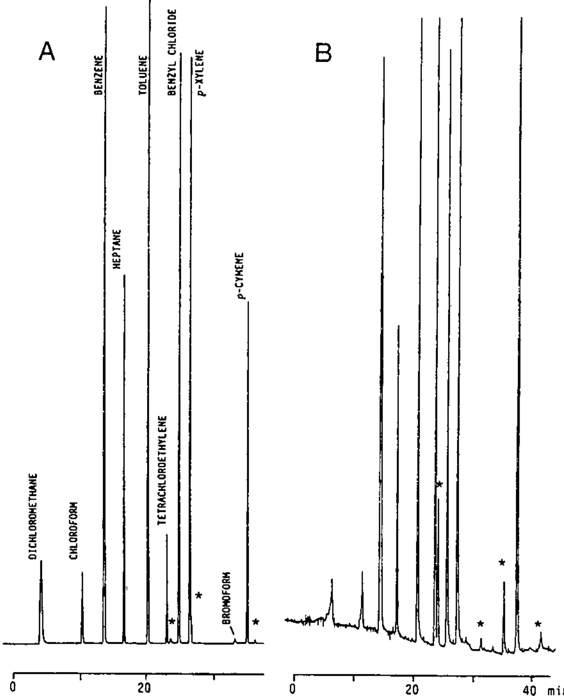

Fig. 2. Typical gas chromatograms of volatile organic compounds extracted from water samples in a 1-m ultra-thick-film trap. Water samples containing the analytes at levels of (A) 1 ppm and (B) 1 ppb. GC conditions as described under Experimental and in Fig. 1. Asterisks indicate artefacts and/or contaminants.

those obtainable with direct aqueous injection [7], 5 μl of a spiked water sample containing the analytes at a level of *ca.* 1 ppm were injected on-column into an apolar column fitted with a 10-m retention gap. With such relatively small injection volumes and FID, the determination of these analytes at a level of 1 ppb was found to be out of the reach of the direct injection method.

The exceptionally high R.S.D. for heptane in Table I is the result of steadily decreasing area counts over the five days during which the determinations at the 1 ppm level were made. Owing to its low solubility in water, this compound probably became concentrated in the headspace gas, resulting in a decrease in its concentration in the water. It is impossible to preserve the integrity of water samples spiked at these levels for longer than 1–2 days at the most. The results in Table I were nevertheless used here to illustrate the effect that storage of samples may have on the accuracy and reproducibility of results. This problem was found to be less pronounced at the 1 ppb level. The large R.S.D. for bromoform can be ascribed to the low FID response to this compound, resulting in a larger effect of integration errors on the area counts obtained for bromoform than for other analytes having normal FID responses. The relatively high R.S.D. obtained for *p*-xylene can be ascribed to the incomplete separation of this compound from an impurity, probably a cyclosiloxane produced by thermal decomposition of the polysiloxane lining of the trap. To avoid this problem in analyses at lower concentration levels, the desorption temperature was lowered from 220 to 150°C, which still gave complete desorption of all the compounds, and the programming rate was changed from 4 to 3°C/min.

At the 1 ppb level, the low molar response factors of the halogenated compounds resulted in poorer R.S.D.s. It was found to be impossible to determine bromoform with its very low response factor at a level of 1 ppb. The results of an area count reproducibility study are given in Table II. A typical gas chromatogram obtained at a level of 1 ppb is shown in Fig. 2B.

As dichloromethane and possibly also chloroform cannot be substantially enriched by using larger volumes of the water sample, it does not seem to be feasible to determine these compounds at concentrations much below 100 ppt with the present

TABLE II

AREA COUNT REPEATABILITY FOR THE ANALYSIS OF WATER CONTAINING ORGANIC COMPOUNDS AT A CONCENTRATION OF *ca.* 1 ppb (SAMPLE B) (n = 5)

Compound	Average uncorrected area counts[a]	Standard deviation	R.S.D. (%)
Dichloromethane	8817	802	9.10
Chloroform	5433	690	12.70
Benzene	35 488	1414	3.98
Heptane	32 721	1452	4.44
Toluene	71 624	525	0.73
Tetrachloroethylene	118 748	2340	1.97
Benzyl chloride	76 560	1834	2.40
p-Xylene	148 395	3150	2.12
p-Cymene	350 720	10 751	3.07

[a] Attenuation: 1×2^6.

method, in conjunction with FID. If ECD is employed, however, these compounds should be readily detectable at a level of 1 ppt. As far as the compounds with larger molar response factors are concerned, acceptable R.S.D.s were obtained without having to increase the volume of water siphoned through the trap, although the more favourable partition coefficients of the higher compounds allow larger volumes of water to be passed through a trap before breakthrough occurs. However, at such low levels the collection of reliable quantitative data was hampered by residual impurities in the water used for the preparation of spiked water samples and especially by the thermal decomposition products of the polysiloxane rubber lining of the trap. If appropriate precautions are not take, the accumulation of polisiloxane decomposition products can lead to the appearance of large interfering peaks, even in analyses at the 1 ppb level. It was found to be essential to cool the trap relatively slowly to at least 120°C before the analytical column is cooled for the next analysis. The trap is also left at the desorption temperature only long enough to effect quantitative desorption of the analytes in question. If these precautions are not observed, strongly tailing decomposition product peaks are produced which might overlap with, or obscure, the peaks of the analytes.

CONCLUSIONS

Although it cannot be claimed that ultra-thick-film traps are universally applicable to the determination of all volatile organic compounds in any type of water sample, the method described in this paper could bring the determination of certain water contaminants within reach of laboratories without ECD instrumentation, provided that instrumentation is available for the thermal desorption of analytes from the trap (double-oven gas chromatograph or ohmic heating) and cryofocusing of the desorbed volatile compounds. Depending on the R.S.D. that is considered acceptable, it is possible to determine the more volatile organic halides at levels of, for example, 1–10 ppb and the results reported here can serve as guidelines for the development of methods for the determination of similar compounds. Interference of artefact peaks produced by the decomposition of the polysiloxane trap lining can cause problems when less volatile compounds have to be determined at levels of 1 ppb or lower. However, the xylenes, *p*-cymene, etc., did not break through when 10 ml of water were sampled and the problem resulting from the increasing size of artefact peaks that are formed when desorption temperatures have to be increased to achieve relatively rapid and quantitative desorption of high-boiling compounds can therefore be offset by sampling larger volumes of water, allowing higher attenuation values to be used. General guidelines will have to be established for the determination of such higher boiling compounds as far as the flow-rate at which these compounds can be extracted from water and the temperature and time required for complete desorption from the trap are concerned.

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Use of adsorbents to collect selected halocarbons and hydrohalocarbons of environmental interest from large air volumes

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ABSTRACT

Hydrohalocarbons are the proposed replacement compounds for the chlorofluorocarbons. They will initially have very low concentrations, on the order of a ppt (v/v), in the global atmosphere. Neither gas chromatography–electron-capture detection, nor gas chromatography–mass spectrometry, have adequate sensitivity to measure such concentrations directly from air samples; concentration techniques are required to achieve this. We have examined a range of commercially-available adsorbents, including activated charcoals, carbon molecular sieves, porous polymers, and graphitized carbons, for their suitability as ambient temperature concentrating traps for a range of man-made halocarbons and hydrohalocarbons (CFC-12, CFC-11, CFC-13, HCFC-22, HCFC-123 and HFC-134a). From our measurements of specific retention and desorption volumes it was found that no one adsorbent could both collect all of the target compounds with high efficiency, and also allow efficient recovery by thermal desorption. A sequence of adsorbents is required. We designed a 30 cm long × 0.64 cm O.D. trap containing HayeSep D_B (a porous polymer), Carboxen 1000 and Carbosieve S-II (both carbon molecular sieves) to collect all of the target compounds from a 5 l air sample at 25°C and allow efficient recovery with 500 ml of nitrogen carrier gas at 200°C. Good comparability was demonstrated between the adsorbent trapping system and direct loop injection analysis for CFC-12 in ambient air. Precision for all of the compounds analyzed with the adsorbent trap was better than 4%, and improved to better than 1% when ratioed to CFC-12.

INTRODUCTION

The chlorofluorocarbons (CFCs) are man-made compounds used in refrigeration and cooling systems, foam blowing, aerosol propellants and industrial solvents. In the atmosphere they can migrate to the stratosphere where photolysis releases the chlorine atoms, which can in turn participate in reactions which remove ozone [1]. As a result of this threat to the ozone layer the CFCs, and other selected man-made halocarbons, have been targeted for elimination by the end of the year 2000 by countries ratifying the Montreal Protocol [2]. In many Western countries, including the USA and Canada, national regulations now require the elimination of CFC usage by the end of 1995 [3]. Industry is turning to alternative compounds to replace the CFCs, in particular the hydrohalocarbons (HHCs) [2]. These include the hydrochlorofluorocarbons (HCFCs), which are non-fully-halogenated analogues of the CFCs, and the hydrofluorocarbons (HFCs) which are both non-fully-halogenated and contain no chlorine. The presence of the hydrogen atom in these molecules makes them accessible to OH radical reaction in the troposphere, so reducing their atmospheric lifetime relative to the CFCs, and

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thus the amount reaching the stratosphere [4]. The HFCs, since they contain no chlorine, should have no impact on stratospheric ozone but are strong infrared adsorbers and may contribute to "greenhouse warming" [5].

We anticipate a rapid rise in HHC concentrations over the next decade or more as the CFCs are phased out and replaced. HCFC-22 is already in use and is present in the atmosphere at about 100 ppt (v/v) [6]. HFC-134a is the first HFC to be used commercially with its recent introduction in some automotive air conditioners [7]. This compound has not yet been detected in the ambient atmosphere. With the exception of HCFC-22, the HHCs will initially have minute atmospheric concentrations, on the order of a few ppt, compared with tens or hundreds of ppt for the CFCs. Direct gas chromatographic (GC) analysis of small loop injections of air will not be sufficiently sensitive for such concentrations. In addition, GC–electron-capture detection (ECD) sensitivity for HHCs is moderate to very poor compared with the CFCs.

Whether GC–ECD or GC–mass spectrometry (MS) is used, sample concentration is required. In our experience, large volume sample concentration of the condensable gases by cryogenic techniques (cryotrapping) results in frequent blockage of the traps by carbon dioxide and water vapor, and is often not suitable for field use. Adsorbents offer the possibility of ambient temperature collection and selective trapping to remove interferences such as water vapor. Adsorbent tubes are small, light, and cheap compared to metal flask samples, and might be used for portable multiple sampling units. In addition, some of the artifacts of flask sampling might be avoided.

We examined a variety of adsorbents for their suitability for trapping some representative CFCs and HHCs at ambient temperature, and subsequent recovery by thermal desorption. This information was used to design a trap for field use to concentrate the target compounds from several liters of air.

METHODS

The adsorbents tested are listed in Table I. Each adsorbent was used to make one or more short chromatographic columns in 0.64 cm or 0.32 cm

TABLE I

ADSORBENTS EXAMINED IN THIS STUDY

Adsorbent types: A = activated charcoal; CS = carbon molecular sieve; G = graphitized carbon; P = porous polymer; MS = molecular sieve. Suppliers: Alltech, Deerfield, IL, USA; Supelco, Bellefonte, PA, USA; Hayes Separations, Bandera, TX, USA.

Adsorbent	Supplier	Type	Mesh	Specific surface area (m^2/g)	Bulk packed density[a] (mg/ml)	Column dimensions, length × O.D. (cm)
SK4 Carbon	Alltech	A	80–100	n/a[b]	460	2.86 × 0.32
CT Carbon	Alltech	A	80–100	n/a[b]	470	2.54 × 0.32
Carbosieve S-II	Supelco	CS	80–100	1000	680	2.54 × 0.32
Carbosieve S-III	Supelco	CS	60–80	550	700	3.18 × 0.32
Carboxen 1000	Supelco	CS	60–80	1200	490	5.08 × 0.32
Carboxen 1001	Supelco	CS	60–80	500	580	3.18 × 0.32
Porapak Q	Supelco	P	100–120	510	340	22.9 × 0.32 22.9 × 0.64
HayeSep D_B	Hayes Separations	P	80–100	781	360	6.99 × 0.32 3.51 × 0.64 22.9 × 0.64
Carbopak B	Supelco	G	60–80	100	460	15.9 × 0.32
Graphpac GB	Alltech	G	Granular	100	450	15.2 × 0.64
Tenax TA	Alltech	P	20–35	35	220	15.2 × 0.64
Tenax GR	Alltech	G/P	80–100	24	320	15.2 × 0.64
Molecular sieve 5A	Supelco	MS	80–100	700-800	660	22.9 × 0.64

[a] Determined by weighing 7.62 × 0.64 cm O.D. tubes packed with adsorbent using a column vibrator.
[b] Information not available.

TABLE II

CFC, HCFC AND HFC COMPOUNDS STUDIED IN THIS WORK

Halocarbon	Formula	M_r (g/mol)	Boiling point (°C)	Standard concentrations (v/v, by mole fraction)
HFC-134a	$C_2H_2F_4$	102.02	−26.0	1021, 734, 156 ppm
HCFC-22	$CHClF_2$	86.47	−40.7	1058, 150, 56 ppm, 494 ppb
CFC-12	CCl_2F_2	120.91	−29.8	2.48 ppm, 438 ppb
CFC-11	CCl_3F	137.37	23.8	264, 253 ppb
HCFC-123	$C_2HCl_2F_3$	152.9	27.9	16 ppm, 581 ppb
CFC-113	$C_2Cl_3F_3$	187.38	47.5	357, 193 ppb

O.D. stainless-steel tubing (dimensions in Table I). The columns were connected to a gas chromatograph (Hewlett-Packard, Sunnyville, CA, USA; Model 5890A) equipped with an electron-capture detector operated at 350°C and a standing current of 1 nA. The compounds of interest (Table II) were introduced to the front of the column by making loop injections of gaseous gravimetric standards (in air) into the nitrogen carrier gas stream (Fig. 1) with a 6-port gas sampling valve (Valco Instruments, Houston, TX, USA). High-concentration standards (Table II) were used to account for the non-optimal chromatographic conditions and the poor ECD sensitivity of the hydrohalocarbons (particularly HFC-134a). Various loop volumes, from 0.05 to 5 ml, were used to obtain a satisfactory peak for individual compounds, concentrations, and experimental conditions. In later stages of the study, HCFC and HFC sensitivities were improved by adding a mixture of 2% oxygen in nitrogen to the carrier gas at the ECD inlet to give O_2 concentrations in the ECD of around 0.2%. This allowed smaller loops and/or lower concentration standards to be used (Table II).

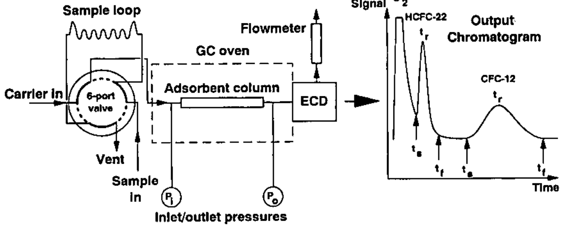

Fig. 1. Schematic of system used to test adsorbents for specific retention volumes and specific desorption volumes, together with an idealized chromatogram. t_s = Peak start time; t_r = compound retention time; t_f = peak finish time.

Peak retention, start, and end times were obtained by examining the ECD signal output to a recording integrator (Hewlett-Packard, Model 3396A). Times were corrected by reference to a non-retained species (oxygen from the standard air balance). Experiments were conducted at a range of column temperatures and carrier flow-rates. Since the primary interest in this study was collection of samples at ambient temperature, and subsequent thermal desorption of collected species at 200°C, attempts were made to include measurements at, or approaching, these temperatures. We also examined flow-rates in the range likely to be used under actual sampling conditions (order of 10–100 ml/min).

It is customary to evaluate adsorbent efficiency by calculating the specific retention volume (V_R) for each compound of interest [8]:

$$V_R = (t_r - t_0)\frac{T_c}{T}\cdot\frac{3}{2}\cdot\frac{(P_i/P_o)^2 - 1}{(P_i/P_o)^3 - 1}\cdot\frac{F}{V_s}\cdot\left(1 - \frac{P_w}{P_a}\right) \quad (1)$$

where t_r and t_0 are the retention times of the compound and non-retained species respectively (min), P_i and P_o are the column inlet and outlet pressures respectively, T_c and T are the trap operating and ambient temperatures (K) respectively, F is the carrier gas flow-rate at ambient temperature (ml/min), V_s is the volume of adsorbent in the column (ml), P_w is the vapor pressure of water at ambient tem-

perature, and P_a is ambient pressure. This equation corrects for gas compressibility in the adsorbent column and yields retention volumes that are appropriate for conditions of ambient pressure and temperature in the adsorbent column. The last term corrects the volumetric flow-rate measured by soap bubble flow meter, and is omitted where solid state flow sensors are used.

Note that V_R in this expression is normalized to adsorbent volume, rather than the more conventional use to adsorbent mass. This is more useful in the present study where the quantity of adsorbent to be used in sampling tubes of given or maximum dimensions is considered. Conversion to units of volume per adsorbent mass can readily be achieved using the adsorbent densities in Table I.

Eqn. 1 measures the approximate 50% breakthrough volume (BTV) of the compound. A measure of the maximum sample volume (V) can be derived from [9]:

$$V = V_R(1 - 2/\sqrt{N}) \quad (2)$$

where N is the number of theoretical plates at the experimental conditions at which V_R is measured. Rather than attempt to estimate N, but to still obtain a measure of maximum sample volume, the visually determined peak start time, t_s (see Fig. 1), was used in place of t_r in eqn. 1. The detection threshold varied with peak height and width, but we estimate that in no case did it exceed 1% of the peak area. We therefore refer to the specific retention volume so calculated as the 99% specific retention volume: $V_{R,0.99}$.

We can similarly measure the desorption time, t_f, for peaks returning to the baseline. Again we estimate that the detection threshold was >99% for all peaks, and calculate the 99% specific desorption volume, $V_{D,0.99}$, from eqn. 1 using t_f in place of t_r.

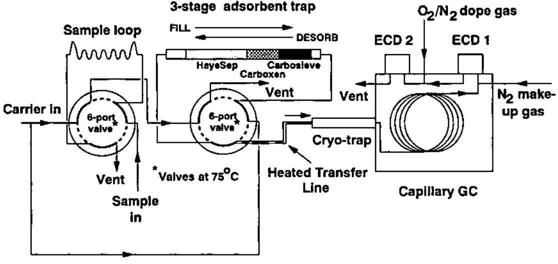

Fig. 2. Schematic of system used to test the design triple-stage adsorbent trap. The adsorbent trap was filled and flushed in one direction, and desorbed at elevated temperature in the reverse direction.

Further tests were conducted using a multiple stage adsorbent trap (Fig. 2). This was a 30 cm × 0.64 cm O.D. stainless-steel tube containing 3.5 ml HayeSep D_B, 1 ml Carboxen 1000 and 1 ml Carbosieve S-II, separated by deactivated pesticide-grade glass wool (Alltech, Waukegan, IL, USA). The tube exterior was coated with Omegabond 200 high-temperature epoxy (Omega, Stamford, CT, USA), which has a high electrical resistivity and thermal conductivity, close-wound with glass fiber double-insulated 30 gauge E-type thermocouple wire (Omega) as the heating element (largely achieved through the nickel-chromium conductor), and sheathed with Nextel sleeving (Omega).

Gas samples could be loaded on to the adsorbent trap by making loop injections into the nitrogen carrier gas used to flush the tube, and then analyzed by backflushing the trap with carrier gas at around 50 ml/min for 5–10 min while heating the tube. The compounds desorbed from the trap were refocussed on a 0.53 mm I.D. fused-silica capillary with a 10-μm thickness Al_2O_3/KCl wall coating (Chrompack column 7518, Raritan, NJ, USA) cooled to −165°C by liquid nitrogen with the cryogenic trapping section of a Chrompack Model 16400 purge-and-trap injector. This was in turn interfaced to a 30 m × 0.32 mm I.D. DB-1 (dimethylpolysiloxane) capillary column (J&W, Folsom, CA, USA), with a 5-μm film thickness, in a Shimadzu (Columbia, MD, USA) GC-9A chromatograph equipped with dual in-series ECD cells. ECD temperatures in the range 275–350°C, and standing currents of 0.2–1 nA, were used in different experiments (see Results section). UHP nitrogen, purified with a Supelco (Bellefonte, PA, USA) Model 3800 heated getter gas purifier, was used as the carrier and make-up gas, with the addition of 10% (v/v) of 2% oxygen-doped nitrogen (UHP grade, further purified with molecular sieve 13X) into the second ECD in series to improve the response of HCFC-22 and HFC-134a. A dramatic improvement in the HFC-134a response was observed, as will be reported in a later paper.

The cryogenic trap was desorbed at 200°C for 5 min at the column flow-rate (approximately 2 ml/min) on to the analytical column, which was held at

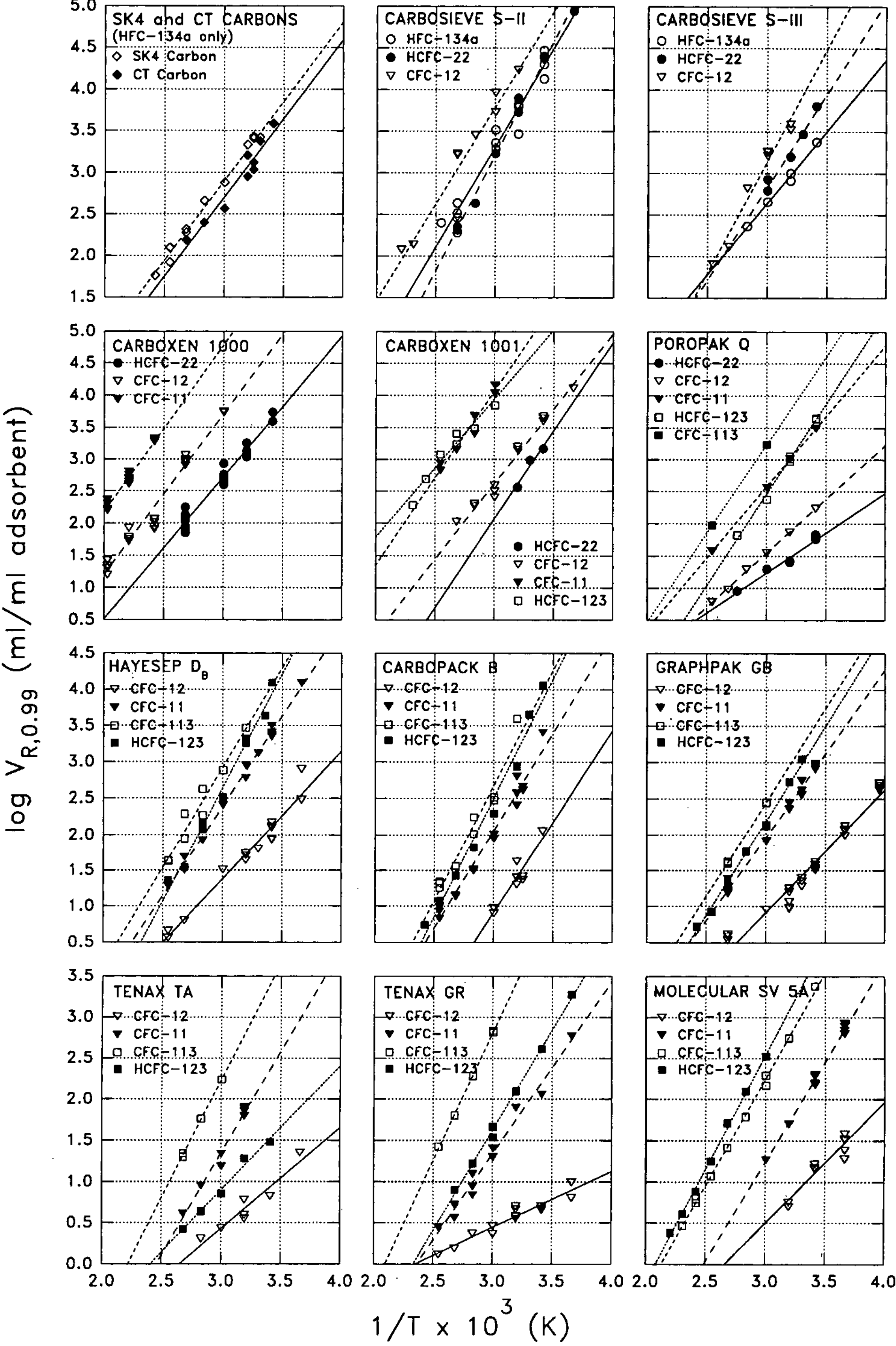

Fig. 3. Summary plots of log $V_{R,0.99}$ *versus* $1/T$ for all compounds tested with each adsorbent. Lines are linear regression fits to the data.

0°C. After a further 3 min, the column temperature was raised to 100°C at 8°C/min.

RESULTS

A technique that has commonly been used to estimate specific retention volumes for adsorbents at near or sub-ambient temperatures is to extrapolate linear fits of log V_R *versus* $1/T$, from data obtained at elevated temperatures, to the lower temperatures of interest [8]. Others have shown that this can lead to errors in the calculated retention volume due to non-linearity effects [9, 10]. Pankow [10] demonstrated a method of *estimating* and correcting for such errors in the extrapolation technique, but this requires a knowledge of the enthalpy of desorption from the adsorbent, and enthalpy of vaporization of the pure compound, and still may not account for all errors. We have therefore aimed to make measurements at temperatures close to ambient using short adsorbent-packed columns.

Fig. 3 shows plots of log $V_{R,0.99}$, normalized to a 1-ml volume of adsorbent, *versus* $1/T$ for all of the compound retentions that could be determined on the selected adsorbents, together with linear fits to

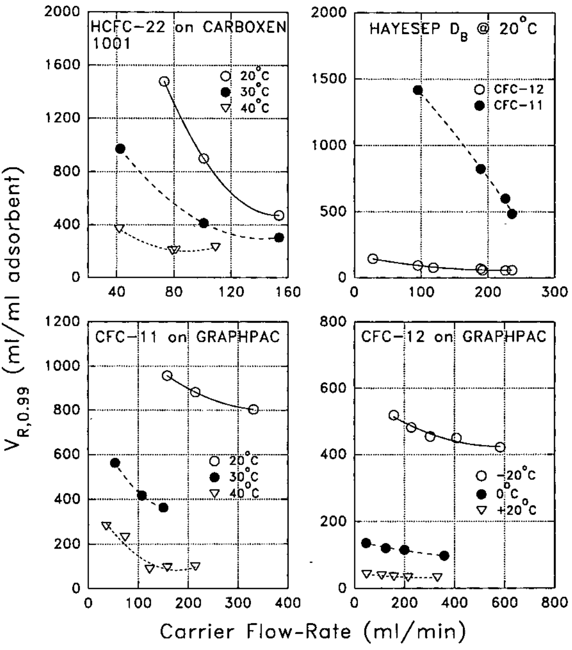

Fig. 4. Examples of variations in specific retention volumes with flow-rates through the tubes.

the data. Compounds that where either too strongly retained on an adsorbent and did not appear to elute within about 30 min, or were negligibly retained and consequently not separated from the oxygen peak, are not reported. A table of regression data for all of the plots in Figs. 3 and 5 can be obtained from the authors on request.

In most cases the plots showed a high degree of linearity. In some cases, however, there was a significant amount of scatter. Generally this scatter was related to differences in carrier flow-rates between experiments. Examples of variations in $V_{R,0.99}$ with flow-rate are shown in Fig. 4. Retention volumes generally increased with decreasing flow rate, but this varied between compounds, adsorbents, and column temperatures. The plots in Fig. 3 include only experiments with flow-rates $\leqslant 200$ ml/min since this is the range of flow-rates that we anticipate using in actual ambient air sampling. It is recommended that flow-rates below 100 ml/min be used to achieve retention volumes estimated from the linear fits.

It should also be noted that the values of $V_{R,0.99}$ in Fig. 3 are for ambient pressure in the adsorbent tube. This will normally be valid where samples are collected by drawing ambient air through an adsorbent tube. Where air is pumped through the tube, or where an adsorbent tube is used as a focussing trap on a GC system, then corrections should be made for internal tube pressure.

Of equal importance to the retention volume of a compound on a particular adsorbent is its desorption volume. An adsorbent may be a highly efficient sampling material, but will not be useable if the retained species cannot be thermally desorbed within a reasonable time and at a practical temperature. Fig. 5 shows plots of log $V_{D,0.99}$ *versus* $1/T$ for most of the adsorbents. Tenax TA, Tenax GR and molecular sieve gave desorption volumes that were too low to be determined at the higher temperatures. During desorption the tube will be at elevated pressure and temperature, and therefore corrections must be made (eqn. 1). For example, with a tube inlet pressure three times that of the outlet pressure, and a temperature of 200°C, a value of $V_{D,0.99}$ obtained from Fig. 5 must be increased by a factor of 1.25.

Table III summarizes retention and desorption values for ambient temperature sampling (25°C)

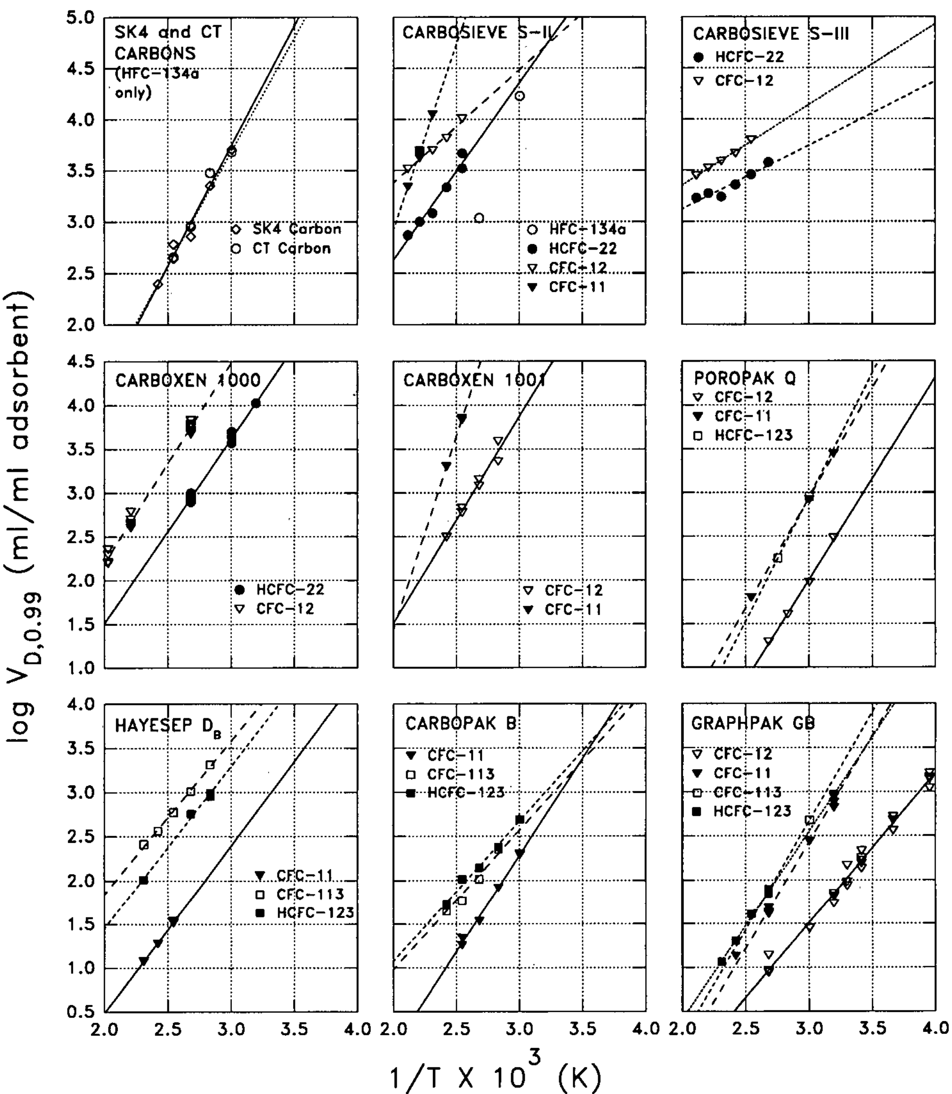

Fig. 5. Summary plots of log $V_{D,0.99}$ *versus* $1/T$ for all compounds tested with each adsorbent, except the two Tenax formulations and molecular sieve 5A (see text). Lines are linear regression fits to the data.

and desorption at 200°C. Note the different units for retention volumes (l/ml) and desorption (ml/ml). From the table it can be seen that retention volumes generally increase in the order (by halocarbon number): 134a < 22 < 12 < 11 < 113. This is in boiling point order for the CFC compounds, but the same is not true for the HHCs. HFC-134a, with a boiling point similar to that of CFC-12 (Table II), is less effectively trapped than CFC-12. The same effect will likely be seen for all HFC compounds due to the absence of chlorine atoms. HCFC-123 is not included in the above ranking as it shows some remarkable variations between adsorbents. Its boiling point is between that of CFC-11 and CFC-113 (Table II). All the porous polymers, except Tenax TA, and both graphitized carbons, gave HCFC-123 retention volumes that fell between those of CFC-11 and CFC-113. Carboxen 1001, however, gave values lower than that of CFC-11 at lower temperatures, whilst Tenax TA gave values between

CFC-12 and CFC-11, and molecular sieve 5A gave values which were actually higher than that of CFC-113. Caution should, therefore, be exercised in attempting to judge the probable efficiency of these adsorbents for other HHCs based solely on their boiling points.

All of the compounds except HFC-134a were so strongly retained on the two activated carbons that they could not be assessed for retention volume. SK4 Carbon was more efficient than CT Carbon for HFC-134a collection. In general, carbon molecular sieves are preferred over activated carbons because of the non-uniformity and humidity effects of the latter. Carbosieve S-II was found to be highly effective for trapping HFC-134a, and all the other compounds; more so than Supelco's newly introduced replacement, Carbosieve S-III. Curiously, despite the lower retention volumes measured with Carbosieve S-III, the desorption volumes were higher. This was apparent in the chromatograms as a pronounced tailing effect. Desorption volumes for HFC-134a could not be measured for either of the Carbosieve materials due to merging of the HFC-134a and HCFC-22 peak at elevated temperatures. It is assumed that the desorption volume of HFC-134a is lower than that of HCFC-22. This is supported by a few observations at lower temperatures (Fig. 3).

As with the Carbosieve materials, Carboxen 1001, the replacement for Carboxen 1000, was less efficient for trapping the compounds studied here than its predecessor. In both cases the replacement materials have lower specific surface areas (Table I) and different pore size distributions than the older versions.

The porous polymers were less effective for trapping the CFCs and HHCs than the activated carbons and carbon molecular sieves. Porapak Q was slightly more efficient for trapping CFC-12 and CFC-11 than HayeSep D_B, but the latter was more

TABLE III

SPECIFIC RETENTION AND DESORPTION VOLUMES FOR SAMPLE COLLECTION AT 25°C AND THERMAL DESORPTION AT 200°C, NORMALIZED TO NORMAL TEMPERATURE AND PRESSURE

Note the different scaling for $V_{R,0.99}$ and $V_{D,0.99}$. Italicized figures were obtained by interpolation of the linear fits in Figs. 3 and 5, the others are from extrapolation. Where there was insufficient data to establish a linear fit, an upper bound is indicated by a "$<$" prefix and the temperature of the measurement indicated.

Adsorbent	$V_{R,0.99}$ at 25°C (l/ml)						$V_{D,0.99}$ at 200°C (ml/ml)					
	Halocarbon						Halocarbon					
	134a	22	12	11	123	113	134a	22	12	11	123	113
SK4 Carbon	*3.8*						52					
CT Carbon	*2.4*						46					
Carbosieve S-II	*9.2*	*16*	41					*670*	*3200*	*2200*		
Carbosieve S-III	*1.8*	*4.2*	13					*1600*	*2800*			
Carboxen 1000		*3.2*	39	520				58	*290*	$<4\cdot10^3$ (180°C)		
Carboxen 1001		*1.1*	*2.8*	110	52				60	$<2\cdot10^3$ (140°C)	$<2\cdot10^3$ (180°C)	
Porapak Q		*0.048*	*0.14*	*2.3*	*3.0*	17		1	5	3	180	<180 (120°C)
HayeSep D_B			*0.10*	*1.9*	*5.7*	7.9				5	46	110
Carbopak B			*0.064*	*1.0*	*4.7*	6.4				2	18	15
Graphpac GB			*0.034*	*0.61*	*1.3*	2.4				2	4	<76 (100°C)
Tenax TA			*0.007*	0.17	*0.027*	1.8						
Tenax GR			*0.005*	*0.12*	*0.31*	8.1						<32 (120°C)
Molecular sieve 5A			*0.011*	*0.13*	3.3	*1.6*					<31 (160°C)	<13 (160°C)

efficient for HCFC-123 and CFC-113. HayeSep D_B has other desirable features over Porapak Q, including higher thermal stability (maximum temperature of 300°C *versus* 250°C for Porapak Q), and smaller desorption volumes for HCFC-123.

Carbopak B and Graphpac GB are both graphitized carbons of similar composition. Graphpac GB is in a coarse granular form designed to produce sampling traps of lower pressure drop. Carbopack B was somewhat more efficient than Graphpac GB for all of the compounds.

Tenax GR is manufactured by coprecipitating the same polymer used in Tenax TA with 30% graphitic carbon. Both Tenax forms had low collection efficiencies for all of the compounds except CFC-113. Efficiencies for CFC-12 and CFC-11 were similar, or slightly lower in the GR form. Given the higher density of GR (Table I), this equates to a significantly lower efficiency for GR on a per gram basis. For the heavier halocarbons, however, the situation was reversed with much higher retention volumes for HCFC-123 and CFC-113 on the GR form.

An important conclusion from Table III is that no one adsorbent is suitable to make ambient measurements of all of the listed compounds, due to the limitation of desorption volume. Two or more adsorbents need to be used in series to successively remove less easily trapped species from the sample air stream. This ensures that compounds do not penetrate to a large extent into an adsorbent from which they cannot be readily desorbed. The adsorbent tube must be desorbed by flowing carrier gas in the reverse direction to the sample flow.

DESIGN OF A MULTIPLE BED ADSORBENT TRAP

The information in Table III was used to design a trap that could be used to collect all of the compounds listed from a large enough air sample to permit low ambient concentrations (few ppt, v/v for the HHCs) to be determined. A portable automated multiple-tube sampling device is being developed in which the tube size is limited to about 30 cm × 0.64 cm O.D. The interior volume of a trap with these dimensions is about 6.7 ml.

In designing the trap, due attention must be paid to both the desorption and retention volumes. Some breakthrough of a species from its target adsorbent stage to the next can be tolerated since the molecules will be concentrated at the front of the next adsorbent stage and, during backflushing to desorb the trap, the whole volume of the adsorbent will not need to be desorbed to remove them again. However, to ensure rapid desorption, it is recommended that such breakthrough be kept to a minimum.

In the example given here the design sample volume was 5 l at ambient pressure and 25°C. For desorption we considered 200°C to be a safe temperature to avoid any thermal decomposition effects for these or any other halocarbons. A flow-rate of 50 ml/min can be achieved with the cryogenic refocussing system used, and 10 min was taken as a reasonable desorption time. Therefore desorption volumes should be less than 500 ml in total for the amount of adsorbent used.

CFC-11, HCFC-123 and CFC-113 are not readily desorbed from either the carbon molecular sieves or the activated carbons (Table III). The graphitic carbons and both Tenax formulations have relatively low retention volumes for CFC-11. Porapak Q has higher retention volumes than HayeSep for CFC-11 and CFC-113, but lower values for HCFC-123. HayeSep was selected to collect these three species due to its lower desorption volume for HCFC-123 relative to Porapak, and its higher thermal stability. A volume of 3.5 ml was selected to retain CFC-11 from volumes of up to 6.7 l, HCFC-123 from volumes up to 20 l, and CFC-113 from volumes up to 28 l. At 200°C all of the CFC-113 should be desorbed with 385 ml of carrier gas, and lesser volumes for the other two species. A 1-ml volume of Carboxen 1000 was selected to retain CFC-12 from up to 39 l of air and desorb with 290 ml of carrier at 200°C. A 1-ml volume of Carbosieve S-II was selected to retain HFC-134a from 9 l of air. The desorption volume could not be assessed, but is presumed to be less than that of HCFC-22 (670 ml), and within the 500 ml criterion. HCFC-22 will be collected partly on the Carboxen 1000 (up to 3.2 l), and partly on the Carbosieve S-II (up to a further 16 l). It is readily desorbed from the Carboxen (58 ml at 200°C). The given desorption volume for HCFC-22 on Carbosieve S-II is, at 670 ml, a little higher than the criterion, but it is considered that with a 5 l air sample, HCFC-22 will only penetrate part of the way into the Carbosieve S-II stage, and therefore be more readily desorbed.

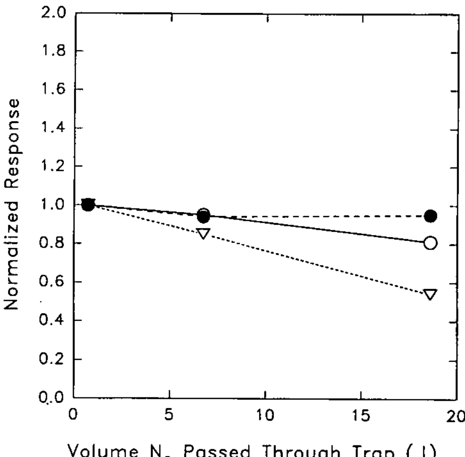

Fig. 6. Long period flushing tests of the triple-stage adsorbent trap: 10 ml standards in air were injected at 33°C, followed by nitrogen carrier gas at 114 ml/min to the volumes shown. Peak area responses were normalized to the experiment in which the trap was flushed with 300 ml nitrogen. ▽ = HFC-134a; ● = HCFC-22; ○ = CFC-12.

The design triple-stage trap was tested with large flushing volumes using the apparatus in Fig. 2 (see Methods section). A 10-ml loop injection of a standard was made into the nitrogen carrier flowing through the trap at 114 ml/min and 33°C. The carrier was allowed to continue flowing for a set time to give a total volume of nitrogen flushed through the trap. The trap was then desorbed at 200°C and analyzed. The results are shown in Fig. 6, normalized to the experiment in which only 300 ml of carrier were used to flush the trap. This experiment gives the "worst case" breakthrough since the sample is loaded at the start of the flushing period, whereas in the sampling of real air the analyte molecules enter the trap continuously through the sampling period. In addition the tube temperature was higher than the standard used in Table III (25°C), and the flow-rate was above the recommended maximum (100 ml/min). Nevertheless, the experiment demonstrated that the values used to design the tube are, for the most part, conservative. About 20% breakthrough (or non-recovery) of HFC-134a had occurred with 9 l of flushing. High collection efficiency of HCFC-22 was obtained up to almost 19 l of flushing, indicating that it was still being efficiently recovered after breaking through to the Carbosieve stage. A small reduction in CFC-12 efficiency was evident at the highest volume.

The triple stage trap was also used to determine accuracy and precision for the adsorbent trapping technique (Fig. 7). Loop injections (10 ml) of a series of CFC-12 gravimetric standards (accuracy better than 1% [11]) were made on to the trap, followed by 550 ml of nitrogen carrier. The trap was desorbed at 200°C. The same procedure was then used to determine the CFC-12 concentration in a cylinder of dried air filled at Niwot Ridge in the Colorado Rocky Mountains (40°02'N, 105°35'W, 3018 m above sea level). Two measurements gave results of 484 and 475 ppt, in good agreement with an earlier analysis (461 ppt CFC-12) of the same cylinder using direct loop injections into the GC system used by the National Oceanic and Atmospheric Administration (NOAA) Nitrous Oxide and Halocarbons group for their flask-sampling network [12].

Precision was determined by replicate measurements of 10-ml loop injections of a multiple standard mixture flushed on to the adsorbent tube with 400 ml of air at 30°C, and desorbed at 200°C. The results are shown in Table IV. The atmospheric concentrations that would yield the same CFC concentrations on the trap from a 5-l air sample can be obtained by dividing the concentrations in Table IV by 500. These resultant concentrations are well be-

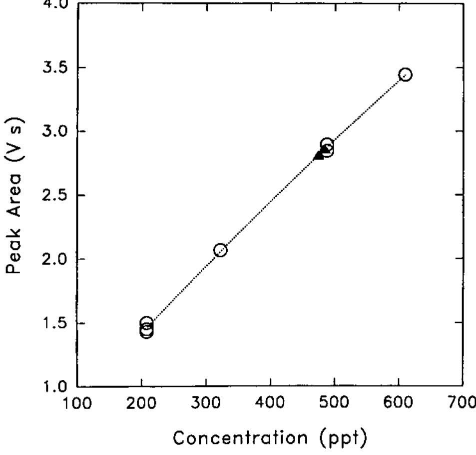

Fig. 7. CFC-12 calibration curve (second order polynomial) and analysis of dried air from Niwot Ridge using the triple-stage adsorbent tube. ○ = CFC-12 standards in air; ▲ = ambient air (Niwot Ridge, CO, USA).

TABLE IV

ANALYTICAL PRECISIONS FROM THREE ANALYSES OF 10-ml LOOP SAMPLES OF A MIXED HALOCARBON STANDARD DEPOSITED ONTO A TRIPLE-STAGE ADSORBENT TRAP WITH 400 ml N_2 CARRIER GAS AT 30°C AND DESORBED AT 200°C

Compound	Concentration (ppb)	Precision (sample standard error, n = 3) (%)	
		Peak area	Ratio to CFC-12
CFC-12	15.6	2.8	–
CFC-11	3.00	3.3	0.47
CFC-113	1.27	3.7	1.26
CH_3CCl_3	6.37	2.2	0.57
CCl_4	10.2	2.5	0.28
HCFC-22 (O_2-doped ECD)	4.99	4.3	0.86

low present-day background air values. Standard errors of the peak areas were 4% or less, and improved to 1% or less when ratioed to CFC-12. The ratio technique might be used to account for errors arising from sample volume measurement, and collection/recovery efficiency variations, at stations where independent CFC-12 measurements are available (*e.g.*, NOAA Baseline Monitoring Stations [12]).

It has been shown that ambient levels of CFC-12, CFC-11, CFC-113 and HCFC-22 can all be detected in 500-ml air samples using the Chrompack cryotrap/Shimadzu GC-9A system used in this work [12]. The question remains as to whether adequate sensitivity can be obtained for ultra-trace levels of HFC-134a and HCFC-123. Direct injections of HFC-134a and HFCF-123 standards were made into the cryotrap to assess detection limits. HFC-134a was measured with an ECD temperature of 275°C, a standing current of 0.5 nA, and oxygen doping to a final concentration of 0.2% within the ECD cell. A 10-ml loop injection of a 47.4 ppb HFC-134a standard gave a peak area 15.8 times the peak threshold; *i.e.* a detection limit of 3.0 ppb for a 10-ml sample. This implies a detection limit of 6 ppt in a 5-l air sample concentrated on an adsorbent trap. Fig. 8, however, indicates that at the cryotrapping temperature of −165°C, HFC-134a was inefficiently trapped, thus improved detection limits should be possible with a more efficient refocussing trap. We are presently addressing this issue.

The detection limit for HCFC-123 was determined with a 3-μl gas syringe injection of a 16.0 ppm HCFC-123 standard into the carrier gas entering the cryotrap. It was found that although oxygen doping enhanced the HCFC-123 signal it also enhanced the baseline noise. A lower S/N was obtained without oxygen doping. In this instance the ECD temperature was 325°C and the standing current was 0.5 nA. The peak area was 80.8 times the area threshold, giving a detection limit of 0.198 ppm for the 3-μl injection, equivalent to a detection limit of 0.12 ppt for a 5-l air sample.

These detection limits are, however, ideal in that no account has been made for interference from other compounds in real air samples. The presence of large amounts of water vapor or CO_2 can severely affect chromatographic separation. We noted that when ambient air samples of several liters volume were collected directly on to the traps, without

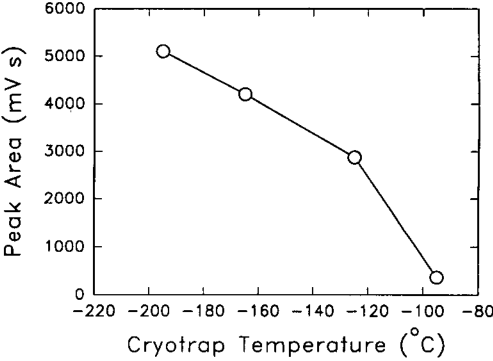

Fig. 8. Effect of cryotrapping temperature on HFC-134a peak size.

any attempt to remove water vapor or CO_2, the cryotrap became plugged during trap desorption. Both Carbosieve and Carboxen are carbon molecular sieves, and as such are strong adsorbers of CO_2. The carbon molecular sieves are hydrophobic, but the manufacturer's information for HayeSep D_B shows that it can be used to separate water vapor in near-ambient temperature chromatography, thus we suspect that some water vapor may be retained by this trap.

Flushing the trap with dry nitrogen prior to desorption should remove the water vapor, but it is unlikely that CO_2 can be similarly removed without also loosing some of the desired analytes. We have been able to prevent trap plugging by sampling air though a trap filled with Aquasorb (P_2O_5 on a vermiculite base: Mallinckrodt, Paris, KY, USA) and Ascarite (NaOH on a silicate base: Thomas Scientific, Swedesborough, NJ, USA) to remove water vapor and CO_2 respectively. CO_2 collection might also be avoided by selection of different adsorbents, although there appears to be no suitable alternative for Carboxen in the present application, or by refocussing with an adsorbent trap operated at a temperature above the freezing point of CO_2. We are currently investigating this latter option.

CONCLUSIONS

Adsorbents can be selected to perform sample concentration of volatile halocarbons and hydrohalocarbons at ambient temperature. Due to the large variations in boiling point and adsorbent affinity between the compounds of interest, it is necessary to use a multiple-stage adsorbent to be able to both trap and recover all of the species. Further work is needed to validate these findings in the field: in particular the effects of humidity and CO_2 adsorption on collection efficiency.

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Review

Solid-phase extraction in multi-residue pesticide analysis of water

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ABSTRACT

The determination of pesticides in water is fundamental to the solution of environmental problems as natural waters are usually contaminated with a large number of pesticides. The selection of an isolation and/or concentration technique depends largely on the class of pesticides to be determined. It is often necessary to determine simultaneously a wide variety of compounds in a water sample. Application of solid-phase extraction techniques offers a solution. The mechanisms of solid-phase extraction, types of sorbents and their application to multi-residue pesticide analysis are reviewed.

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1. INTRODUCTION

The determination of pesticide residues in water samples is necessary for solving various environmental and biological problems [1]. The accuracy and precision of analysis are dependent on both sample preparation and instrumental performance. The analysis is carried out using gas chromatography (GC) [2] or liquid chromatography (LC) [3]. These chromatographic techniques require efficient isolation and concentration procedures, such as liquid–liquid, supercritical fluid and solid-phase extraction [4,5].

Liquid–liquid extraction (LLE) is frequently used but it produces emulsions and different extraction efficiencies for various compounds; it also requires

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large amounts of solvent and is slow, laborious and difficult to automate [6,7].

Solid-phase extraction (SPE) is attracting increasing attention and constitutes an alternative to LLE. Desorption of retained organic compounds can be carried out by elution with a suitable solvent. SPE is widely used for the trace enrichment of very dilute solutions such as natural waters, where large sample volumes may have to be processed, to yield concentrations of analyte sufficient for detection. The technique has been reviewed in a variety of areas such as extraction of organic compounds [8–12], on-line precolumn techniques [13,14] and sample preparation in drug analysis [15]. Svoboda [16] reviewed the use of sorbents for the preconcentration of one or a few pesticides.

Supercritical fluid extraction (SPE) is in the early stages of development and so far has been used mainly with solid samples. However, it can readily be used with liquids if the sample is immobilized on a solid support. This means that the technique can be used to extract pesticides from water samples [8].

Although a single residue method is often used in analyses required by legislation or to confirm results, for the analysis of real environmental water samples, when nothing is known about the nature of possible contaminants, multi-residue methods are needed. Pesticides form a large group of compounds with widely differing structures and biological activities. Ideally, multi-residue methods should provide rapid identification and quantification of as many different pesticides as possible at the required sensitivity limit. This diversity poses problems for the analyst who is trying to develop methods that cover as many pesticides as possible [17–22].

Considering the above problems associated with this kind of analyte and matrix, we present here a detailed review of several multi-residue SPE procedures that have been proposed in the last 10 years for the determination of organochlorine, organophosphorus and organonitrogen pesticides in water.

2. MECHANIMS OF SOLID-PHASE EXTRACTION

The two major mechanisms of analyte retention on solid support are adsorption and partitioning. Extraction of trace amounts of organic compounds from water with solid sorbent is a method in which adsorption on a solid substance is used in order to isolate compounds dissolved in water. Sorbent extraction can also be based on the distribution of the dissolved compound between the solid sorbent and water. In these instances, provided that the sorbent has been selected correctly, the partition coefficient is shifted even more towards the sorbent than in water.

2.1. Adsorption

Pesticides have some affinity for binding on solid surfaces. Common adsorbents are charcoal and porous polymers. The adsorptive capacity of a given adsorbent depends in part on the treatment or manufacturing conditions and on the composition of the adsorbent (references are given in Table 1).

Charcoal was the first sorbent to be used for the extraction of organic compounds from water [10]. The advantage of this material was the high retention of low-molecular-mass polar pesticides and their metabolites [23–30].

Polymers have been used as alternative sorbents to carbon for trace enrichment since the late 1960s. Their homogeneous structure results in greater reproducibility of trace enrichment experiments. The most often used types of polymers are styrene–divinylbenzene copolymers (Polysorb S [31], Amberlite XAD-2 [32,35,36,39] and XAD-4 [33,34,37,38], PRP-1 [40–42]), acrylate polymers (Amberlite XAD-7 [39] and XAD-8 [36], Separon SE [43–45]), 2,6-diphenyl-*p*-phenylene oxide (Tenax GC [46–48]), ethylvinylbenzene–divinylbenzene (Porapak Q [40]), amide esters (polyurethane foam [49]) and organic polymeric sorbents without functional groups (Wolfatit Y77 [50]) (Table 2).

TABLE 1

ADSORPTION TECHNIQUES

Adsorbent	Ref.
Charcoal	23, 24, 25, 26, 27, 28, 29, 30
Porous polymers:	
Amberlites	32, 33, 34, 35, 36, 37, 38, 39
PRP-1	40, 41, 42
Separon SE	43, 44, 45
Tenax CG (polymide)	23, 46, 47, 48
Polyurethane foams	34, 49
Wolfatit	50

TABLE 2

DETERMINATION OF TRACES OF PESTICIDES IN WATER BY ADSORPTION ON CHARCOAL OR POROUS POLYMER SORBENTS

Extraction	Pesticides[a]	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
200 or 100 mg graphitized carbon black in glass column (GCB)	PCBs	30–40	10–50	GC with ECD and FID	–	23
50 ml hexane–diethyl ether (50:50)	Di-syston	95				
Samples: river water, sea water and drinking water	Malathion	97				
	Parathion	100				
	Ronel	90				
	α-Endosulfan	98				
	p,p'-DDD	92				
	α-BHC	101				
	Heptachlor	93				
	Aldrin	102				
	γ-BHC	102				
	β-Endsulfan	100				
	Heptachlor epoxide	99				
	Dieldrin	103				
	Endrin	104				
	p,p'-DDE	93				
	o,p'-DDD	95				
	o,p'-DDE	–				
GCB in glass column	Diclobenil	95.9	*ca.* 100	GC—ECD GC–MS	2–50 μg l^{-1}	24
6 ml light petroleum–toluene (2:1)	Trifluoralin	98.1				
	2,4-D ME	90.0				
	Propazine	76.1				
	Simazine	72.1				
	Atrazine	74.0				
	2,4,5-T ME	95.0				
	DCPA	98.4				
	Silvex ME	91.0				
50 mg GCB in catridges	α-BHC	94	1000	GC–FID GC–MS	10 ng l^{-1}	28
1 ml light petroleum–toluene (1:1)	β-BHC	96				
1 l drinking water	Heptachlor	87				
	δ-BHC	94				
	Aldrin	90				
	Heptachlor epoxide	97				
	p,p'-DDE	92				
	Dieldrin	97				
	Endrin	99				

(Continued on p. 138)

TABLE 2 (*continued*)

Extraction	Pesticides[a]	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
Compares with Tenax, Porapak P and C_{18}	*p,p'*-DDD	94				
	p,p'-DDT	95				
	2,4-DME	92				
	Trifluoralin	92				
	Simazine	97				
	Atrazine	93				
	Propazine	97				
	2,4,5-TME	92				
	DCPA	98				
50 mg GCB in cartridges 3 ml methanol–7 ml dichloromethane 50 ml pond water (pH 8)	$(DEDTP)^-$	82	1–50	GC–NPD GC–MS	20 μg l^{-1}	25
	$(DMDTP)^-$	86				
	$(DETP)^-$	59				
	$(DMTP)^-$	39				
	$(DEP)^-$	3				
	$(DMP)^-$	1				
50 mg GCB in cartridges 700 μl dichloromethane–methanol (60:40) 250 ml distilled, tap and surface water	Simazine	98.5	400	HPLC–UV GC–MS	2–4 ng l^{-1}	26
	Atrazine	97.9				
Double trap tandem system, 150 mg GCB, 150 mg SCX 5 ml dichloromethane–acetonitrile (60:40) 0.7 ml methanol–70 mmol/l KCl	Simazine	96.3–97.7	400	HPLC–UV	10 ng l^{-1}	29
	Simetryn	98.2–99.8				
	Atrazine	99.4–99.8				
	Prometon	98.6–100.3				
	Ametryn	97.3–99.4				
	Propazine	96.4–97.7				
	Prometryn	95.0–96.5				
	Terbutryn	95.7–97.2				
Same as above but 250 mg GCB eluted with 6 ml dichloromethane–methanol (95:5) 2 l drinking water	Fenuron	83–100	*ca.* 7000	HPLC–UV	1 ng l^{-1}	30
	Metoxuron					
	Monuron					
	Monolinuron					
	Fluometuron					
	Chlorturon					

Compares with C_{18}						
	Metobromuron					
	Difenoxuron					
	Isoproturon					
	Diuron					
	Linuron					
	Chlorbromuron					
	Chloruxoron					
	Neburon					
Granular activated carbon	Diazinon	55	1	FIA–HPLC–UV	0.5 mg l^{-1}	27
	Azinphos-methyl	60				
	Fenthion	>90				
Polysorb S	Aldrin	94.0	20–40	GC	0.1–0.2 μg ml^{-1}	31
	γ-HCH	98.7		HPLC		
	Heptachlor	97.4				
	Endrin	95.2				
	DDE	96.0				
	DDD	97.0				
	DDT	96.8				
	2,4-D	99.3				
	2,4,5-T	98.8				
100 ml XAD-2	Atrazine	–	105	HPLC, FD,	–	32
250 ml diethyl	Simazine			FAB		
ether and 250 ml	Linuron					
methanol	Metbromuron					
Drinking and	Monolinuron					
river water	Chlorotoluron					
	Buturon					
	Carbaryl					
	Phenmedipham					
	Diuron					
	Metoxuron					
	Chloroxuron					
	Methabezthiazuron					
	Fenuron					
	Cycluron					
50 mg XAD-4	Diazinon	75	1000	GC–FPD	–	33
0.5 ml ethanol	Malathion	97		GC–FID		
100–500 ml	Parathion	72				
water samples						
25–40 ml XAD-4	1,2,3,4-TCDD	53.3	1000	GC–ECD	0.25 ng l^{-1}	34
500 ml	2,8-DCDBF	66.5		GC–NPD	3	
dichloromethane–hexane	2,4-D	21.5			5	
	2,4-DME	–			113	

(Continued on p. 140)

TABLE 2 (*continued*)

Extraction	Pesticides[a]	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
16–19 l river water, agricultural drains (pH 8)	Amitraz	–			150	
	Aroclor 1232	51.4			–	
	Atrazine	71.2			20	
	Captan	–			–	
	Carbaryl	47.3			25	
	Diazinon	–			10	
	Diuron	51			10	
	Endosulfan	70.0			0.25	
	Ethion	77.6			10	
	Guthion	79.9			25	
	Molinate	68.0			25	
	Oryzalin	9.4			–	
	Tiobencarb	90.6			50	
100 ml XAD-2 500 ml acetone and 500 ml hexane 2 l sea water Compares with polyurethane foam and liquid–liquid extraction	PCB	–	–	GC–ECD	–	35
	BHC	–				
XAD-2, XAD-8 30 l distilled and river water 150 ml acetonitrile and 150 ml dichloromethane or 150 ml diethyl ether	Atrazine	95–100	105	GC–MS	1 $\mu g\ l^{-1}$	36
	Methyl atraton					
XAD-4 5 ml ethanol 1 l sample	Malathion	95	200	Spectrophotometric as phosphomolybdenum blue	150 $\mu g\ l^{-1}$	37
	Parathion	90				
	Phosalone	105				
1.5 g XAD-4 2 ml diethyl ether and 10 ml hexane 1 l water Compares with C_{18}	α-HCH	90–100	83	GC–ECD	–	38
	γ-HCH	75–100				
	Aldrin	80–90				
	Dieldrin	80–110				
	p,p'-DDE	55–90				
	p,p'-DDT	30–80				
	α-Endosulfan	80–100				
	β-Endosulfan	70–80				
	Azinphos-ethyl	80–120				

	Diclorphos	68–88				
	Disulfoton	24–45				
	Parathion	30–60				
XAD-2 and XAD-7	Alachlor	105.0	4000	GC–MS	0.005 μg l^{-1}	39
300 ml	Atrazine	83.0			0.005	
dichloromethane	Butylate	101.1			0.005	
2-l water sample	Captan	74.8			1.0	
	Carbaryl	97.9			0.05	
	Carbofuran	80.4			0.005	
	Chlorothalonil	90.9			1.0	
	Chlorpyriphos	86.7			0.005	
	Cyanazine	85.0			0.05	
	Diazinon	103.8			0.0005	
	Fenamiphos	113.0			0.05	
	Fonofos	108.6			0.005	
	Isofenphos	81.5			0.005	
	Linuron	98.7			0.05	
	Metolachlor	102.5			0.005	
	Metribuzin	91.3			0.005	
	Prathion	109.3			0.005	
	Pendimethalin	88.9			0.005	
	Simazine	81.0			0.005	
	Terbufos	84.3			0.05	
PRP-1 and Porapak Q	Propazine	90–100	250–1000	HPLC–UV	10–50 μg l^{-1}	40
1 ml acetonitrile	Atrazine					
250 ml water	Simazine					
	Prometryne					
	Prometone					
10 × 2 mm I.D. pre-column, PLRP-S	MCPA	>85	–	On-line HPLC–UV	0.05–0.1 μg l^{-1}	41
Acetonitrile–water	2,4-D					
25 ml tap water (pH 3)	MCPC					
	2,4-DP					
	Bentazone					
	2,4,5-T					
	MCPB					
	2,4-DB					
	2,4,5-TP					
15 mm PRP-1	Simazine		–	On-line HPLC–UV	–	42
2 ml water	Atrazine					
500 ml natural water (pH 6–8)	Propazine	25				
Completed with a cation exchanger	Terbutylazine	50				
0.22 g Separon SE	MCPA		100	GC–NPD		43

TABLE 2 (*continued*)

Extraction	Pesticides[a]	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
	Propazine					
0.5 ml acetone	Simazine					
50 ml river water	Ametryne					
	Atrazine	98.6–100.5	100	GC–NPD	8 μg l^{-1}	44
	Prometryne	98.6–100.9				
	Terbutryne	99.2–100.4				
	Ametryne	99.1–100.7				
	Desmetryne	99.5–100.9				
200 ml water	As above		400	GC–NPD HPLC–UV		45
Tenax GC	Diazinon	72	105	GC–ECD	0.01 μg l^{-1}	46
40 ml diethyl ether	Lindane	90		GC–FID		
1–10-l water samples	Heptachlor	63		HPTLC		
	Aldrin	10				
	Methyl parathion	75				
	Malathion	80				
	Dieldrin	72				
	Endrin	98				
	Phenmedipham	98				
	Carbaryl	71				
	Promecarb	77				
	Propham	100				
	Dinobuton	79				
0.75 g Tenax GC, two cartridges	α-HCH	59–75	500	GC–ECD	5 μg l^{-1}	47
15 ml light petroleum	γ-HCH	58–79		GC–MS		
1 l water	*p,p'*-DDE	73–84				
	p,p'-DDD	76–84				
	Heptachlor	53–93				
0.11 g Tenax-GC cartridges	α-HCH	113	–	GC–MS	–	48
Thermal on-line desorption for 5 min, 250°C	BHC	109				
1-l water samples.	*p,p'*-DDE	82				
	Dieldrin	86				

1 g polyurethane foam 50 ml acetone 0.5–3 l distilled or tap water	Dimethoate Azodrine Lannate	94–97 95–98 90–93	60	Spectro-photometry	–	49
0.2–10 g Wolfatit Y 77 25 ml methanol 1 l tap water	Methamidophos Dimethoate Trichlorfon Natrichlorfon acetate Fenuron Propachlor 2,4-D	74–93 90–96 84–98 22–100 79–94 98–100 68–99	40	Counting of ^{14}C	–	50

[a] ME = Methyl ester.

Desorption of the compounds from the concentration columns is mainly performed with a small volume of liquid. The partition coefficient in a given polymer–eluent system should favour the pesticide being studied shifted in favour of the eluent (hexane–diethyl ether [23,38], light petroleum–toluene [24,28], methanol–dichloromethane [25,26,29,30, 39], acetone [43–45,49], diethyl ether–methanol [32], dichloromethane–hexane [34], acetone–hexane [35], acetonitrile with dichloromethane or diethyl ether [36], diethyl ether [46], ethanol [33,37], acetonitrile [40], acetonitrile–water [41], water [42], light petroleum [47] and ethanol [50]).

Another system described is thermal desorption [48], which has been applied for the determination of some organochlorine pesticides. The extraction column is introduced inside the GC oven. The desorption process is similar to that in headspace analysis. Thermal desorption can fail as a result of the very strong interaction between the analyte and the sorbent, as a temperature sufficient to desorb the analyte might also destroy the sorbent, the analyte or both. If the analyte is thermally unstable, thermal desorption can invalidate quantification and introduce artefacts even if the analyte is only weakly adsorbed.

The use of supercritical carbon dioxide to accomplish the desorption can provide solutions to these last two problems. The application of this technique to desorb spiked γ-BHC, hexachlorobiphenyl and parathion from Tenax [51] and polymides [52] may become an attractive alternative to solvent and thermal approaches in the future.

2.2. *Partitioning*

The development of surface-modified materials for LC has opened up a new technology for applied research [53]. The bonded phases were originally introduced for use in LC to obviate the limitations of silica gel when used to separate mixtures of highly polar and ionic sustances [54,55]. These phases can be prepared by reacting silica gel with an appropriate organic mono-, di- or trichlorosilane, producing a surface coating of organic material that replaces the surface hydroxyl groups as the interacting moieties of the stationary phase. The interacting organic groups can be simple hydrocarbon chains, as with a reversed-phase material, a hydrocarbon chain with a terminal polar functional group, as with a polar bonded phase, or an ion-exchange moiety, as with an ion-exchange bonded phase [56]. The first attempts to use them as preconcentration media date back to 1971 [57], but the modern technique had its beginning in 1978 with the commercial introduction of Sep-Pak cartridges (Waters, Milford, MA, USA) [58].

Today, SPE has blossomed into a widely applied technique: more than 30 suppliers offer phases ranging from conventional HPLC phases, such as C_{18}, C_8, cyano and amino, to reactive particles that users can derivatize with a ligand of their choice [59]. The bonded silica mostly used is that with the octadecyl group [60–63].

Table 3 presents procedures for the preconcentration of various types of pesticides from water samples on bonded silicas.

It is well known that simple extraction, evaporation and other similar techniques share the disadvantage of a high risk of contamination from containers, solvents and laboratory surroundings, and also the risk of degradation on evaporation to dryness. Octadecyl-bonded SPE has been proposed by the US Environmental Protection Agency (EPA) in Method 525 [114].

Although an off-line SPE procedure usually shortens the time of sample handling, a certain amount of tedious labour remains. The means for reducing this time-consuming work is to automate the entire procedure as much as possible. The use of precolumns makes it possible to employ on-line concentration techniques on C_{18} or C_8 in conjunction with HPLC [66–69,71,74,75,103, 106–108] but also with GC [70].

Automation of sample preparation for pesticide analysis is essential when large water samples are required. Manufacturers have met the needs of residue chemists for sampling automation by offering laboratory robots with bonded silica cartridges. The Varian AASP (advanced automated sample processor) system has been proposed for pesticide analysis of water [115,116].

A new generation of SPE devices have recently emerged. Borrowing the disc configuration of membrane filters, these devices include flat discs with large cross-sectional areas that provide advantages for on-line preconcentration and clean-up methods with respect to sorption, capacity, back-pressure

TABLE 3

DETERMINATION OF TRACES OF PESTICIDES IN WATER BY PARTITIONING ON BONDED SILICAS

Extraction	Pesticides	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
200 mg C_8	α-HCH	105	200	GC–ECD	–	64
0.5 ml ethyl acetate	δ-HCH	105				
100 ml distilled, tap and sea water	β-HCH	104				
Compares with C_{18}, diphenyl, cyclohexyl, C_2, C_4, cyano, amino, benzenesulphonic acid and silica	Heptachlor	79				
	Aldrin	86				
	Endosulfan	102				
	Dieldrin	92				
	Zoolone	104				
	DDT	79				
500 mg C_{18}	TMF	99.2	20	HPLC–UV	$<5\ \mu g\ l^{-1}$	65
1.5 ml methanol	CDPA	85.3				
30 ml river, lake and distilled water (pH 4.0)	Bayer 73	91.8				
ACDA-Pt and C_{18} precolumn combination	Fenuron	–45	6	On-line HPLC–UV		66
1.7 ml methanol–water (60:40)	Metoxuron	92–98				
10 ml river and distilled water	Monuron	98–102				
	Fluometuron	92–103				
	Monolinuron	96–100				
	Buturon	0–				
	Chlortoluron	93–96				
	Metobromuron	93				
	Isoproturon	95				
	Difenoxuron	90				
	Diuron	91				
	Linuron	90				
C_{18} precolumn of 11 × 2 mm I.D. only	Fenuron	<90	6	On-line HPLC–MS	–	67
As above	Monuron					
	Diuron					
	Metobromuron					
	Linuron					
	Chlorbromuron					

(Continued on p. 146)

TABLE 3 *(continued)*

Extraction	Pesticides[a]	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
11 × 2 mm I.D	Fenuron	44	4	On-line	0.01 μg l^{-1}	68
with 10-μm	Metoxuron	98		HPLC–EC	0.02	
LiChrosorb RP-18	Monuron	98			0.02	
2.4 ml 0.02 *M*	Monolinuron	94			0.04	
phosphate buffer	Diuron	98			0.05	
(pH 7)–methanol	Chlorbromuron	94			0.4	
(45:55)	Linuron	96			0.3	
10 ml of water						
Methanol–0.02 *M*						
phosphate buffer						
(pH 7)(45:55)						
1 ml water						
20 × 2 mm I.D., 40 μm	Monuron	89–95	4	On-line	–	69
C_8 Analytichem	Monolinuron	89–104		HPLC–UV		
As above	Chlorotoluron	94–97				
	Diuron	95–96				
	Chlorobromuron	96–97				
LC micro-precolumn	α-HCH	97	12	On-line LC	0.3–0.5 ng l^{-1}	70
with C_8	HCB	99		with		
85 μl *n*-hexane	γ-HCH	94		GC–ECD		
1 ml water	Heptachlor epoxide	95				
	Aldrin	97				
	Heptachlor	107				
	o,p'-DDE	97				
	Endosulfan	97				
	p,p'-DDE	104				
	Dieldrin	68				
	o,p'-DDE	52				
	Endrin	–				
	*p,p'*DDD	44				
	o,p'-DDT	44				
	p,p'-DDT	28			1 ng $l^{-1}$for	
	Aroclor 1254	95–106			a	
					single	
					component	
[illegible]	Atrazine	84	4	On-line	0.1 μg l^{-1}	71
extraction discs,	Simazine	81		HPLC–UV	1 μg l^{-1}	
C_{18}	2,3,4-Trichlorophenol	89			0.1 μg l^{-1}	

2 ml acetonitrile– water (pH 3) (60:40) 10 ml water						
Sep-Pak C_{18} cartridges 4 ml diethylether and 8 ml methanol	Propachlor Alachlor Cycloate	–	–	TLC/^{14}C	–	72
Sep-Pak C_{18} cartridges 5 ml methanol 25 ml water (pH 4)	$(DMP)^-$ $(DMTP)^-$ $(DMDTP)^-$ $(DEP)^-$ $(DETP)^-$ $(DEDTP)^-$ Phosalone Azinphos-ethyl Azinphos-methyl Ultrazide Metasistox	2 29 14 15 85 64 76 91 77 98 96	4	GC–NPD derivatization by diazotation	2–3 $\mu g\ l^{-1}$	73
Sep-Pak C_{18} cartridge 8 ml tetrahydrofuran	*p,p'*-DDT 1,3,6,8-T_4CDD 1,3,6,7-T_4CDD 1,2,3,4,7-P_5CDD 1,2,3,4,7,8-H_6CDD 1,2,3,4,6,7,8-H_7CDD O_8CDD	–		On-line HPLC with scintillation counting	–	74
0.4 g C_{18} 0.1 *M* acetic acid–methanol (50:50) 2 ml water	2,4-D MCPA Dichlorprop Mecoprop	74–100 75–100 84–100 85–100	–	On-line HPLC–UV	5–10 $\mu g\ l^{-1}$	75
J. T. Baker 6 ml C_{18} 4 ml methanol 250 ml creek, river and pond water	Atrazine Simazine 2,4-D Silvex 2,4,5-T	70–88 70–88 93–100 93–100 93–100	2500	TLC–UV	1 $\mu g\ l^{-1}$	76

(Continued on p. 148)

TABLE 3 *(continued)*

Extraction	Pesticides	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
J. T. Baker C_{18} 500 mg 0.5 ml ethyl acetate 100–1000 ml water sample	Aldrin	Average 92%	2000	TLC–UV	0.06 $\mu g\ l^{-1}$	77
	p,p'-DDE					
	o,p'-DDT					
	p,p'-DDT					
	Dieldrin					
	α-Endosulfan					
	β-Endosulfan					
	Endrin					
	Heptachlor					
	Heptachlor epoxide					
	p,p'-Metoxychlor					
As above	Azinphos ethyl	83.5–96	2000	TLC–UV	0.4 $\mu g\ l^{-1}$	78
	Diazinon					
	Ethyl parathion					
	Fonofos					
	Malathion					
	Carbofenothion					
	Methyl parathion					
100 mg C_{18} 100 μl of ethyl acetate 100 ml water sample	Alachlor	91	1000	GC–MS	0.01 $\mu g\ l^{-1}$	79
	Atrazine	80				
	Chlordane	82				
	Cyanazine	92				
	p,p'-DDE	81				
	Endrin	106				
	Fonofos	96				
	Heptachlor epoxide	88				
	Lindane	98				
	Metolachlor	88				
	Metribuzin	88				
	Trifluoralin	90				
Same as above Compares with XAD-2	Carbaryl	83	1000	GC–MS	0.01 $\mu g\ l^{-1}$	80
	Carbofurane	92				
	Chlorpyrifos	82				
	Ethyl parathion	95				
500 mg RP-C_{18} 1 ml eluent 500–1000 ml water sample	Simazine	90–106	500–1000	GC–NPD HPLC–diode array	10–40 ng l^{-1}	81
	Atrazine	92–114				
	Propazine	93–114				
	Terbutylazine	93–120				

	Promethyne	93–106				
	Cyanacine	96–104				
	Metolachlor	94–104				
	Metazachlor	97–112				
500 mg C_{18}	Benodanil	–	1000	HPLC–UV	270–700	82
1 ml	Bentazon			and	$\mu g\ l^{-1}$	
water–methanol mixtures	Bromacil			fluorescence		
1 l river water	Chlorflurenol					
sample	Ethidiuron					
	Hexacinon					
	Napropamid					
	Thiazafluron					
100 mg C_{18}	PCB No. 149	110.9	5000	GC–ECD	–	83
2 ml *n*-hexane	PCB No. 153	95.4				
100–500 ml	PCB No. 151	97.8				
water	PCB No. 137	97.9				
	PCB No. 187	94.9				
	PCB No. 174	95.8				
	PCB No. 180	92.7				
	PCB No. 170	91.1				
	PCB No. 196	80.0				
1 g and 0.5 g	Sulfometuronmethyl	97.7	100	HPLC–UV	–	84
C_{18} column,	Chlorsulfuron	108				
J. T. Baker	AC 243,997	87.5				
9.5 ml methanol						
for eluted						
AC 243,997 and						
4.5 ml for the						
others						
500 ml water						
(pH 2) for AC						
243,997 and (pH)						
4.5 for the						
others						
As above	Picloram	98.2	100	HPLC–UV	–	85
	2,4-D	91.9				
C_{18} columns	α-HCH	94.7	500	GC–ECD	–	86
2 ml *n*-hexane	α-BHC	94.6				
100–1000 ml	Lindane	75.9				
river and	*p,p'*-DDE	77.3				
distilled water	*p,p'*-DDT	85.5				
sample						
Compares with C_8						

(Continued on p. 150)

TABLE III (*Continued*)

Extraction	Pesticides	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
500–800 mg C_{18}	Simazine	94.4–97	1000	HPLC	0.01 $\mu g\ l^{-1}$	87
1 ml methanol	Atrazine	102.8-103.5		GC		
1 l water sample	Propazine	100.0-102.8				
(pH 3)	Bentazon	97.3-102.4				
As above	Molinate	94.4–97.3	1000	HPLC	–	88
500 mg	Lindane	102.7	1000	GC–ECD	5 $ng\ l^{-1}$	89
Sep-Pak C_{18}	Methyl parathion	93.1			40	
1 ml ethyl	Malathion	94.7			20	
acetate	Metoxichlor	85.0			20	
1-l tap water						
samples						
Compares with						
liquid–liquid						
partitioning						
As above	Heptenophos	83–88	1000–10 000	GC–NPD	52 $ng\ l^{-1}$	90
Tap, lake and sea	Fonofos	78–96			20	
water samples	Disulfoton	72–95			15	
	Methyl parathion	90–108			39	
	Malathion	92–103			78	
	Sumition	95–102			15	
	Ethyl parathion	92–97			52	
	Phentoate	75–91			31	
	Ethion	90–93			18	
	Trithion	79–101			52	
500 mg C_{18} in	HCB	82	5000–50 000	GC–ECD	21 $ng\ l^{-1}$	91
glass column	Lindane	105			18	
5 ml light petroleum	Heptachlor	84			37	
1–10 l surface	Aldrin	80			39	
water samples	Heptachlor epoxide	101			26	
	o,p'-DDE	85			65	
	p,p'-DDE	85			47	
	o,p'-DDD	94			93	
	Endrin	100			71	
	p,p'-DDD	95			47	
	p,p'-DDT	88			69	
	Metoxychlor	98			92	

As above	Dichlobenil	77	5000	GC–ECD	3–60 ng l^{-1}	92
	Trifluoraline	94				
	Vegadex	74				
	Chloranil	60				
	Methyl-clorpyrifos	95				
	Propanil	80				
	Chlorpyrifos	91				
	Dacthal	99				
	Captan	104				
	α-Endosulfan	97				
	Folpet	102				
	Profenofos	104				
	Dieldrin	98				
	β-Endosulfan	101				
	Captafol	93				
	Tetradifon	105				
	Dicofol	99				
	Mirex	68				
	Dialifor	104				
As above but eluted with ethyl acetate	Prometryne	96.4–99.6	5000–50 000	GC–NPD	52 ng l^{-1}	93
	Propazine	95.2–98.3			37	
	Simazine	59.9–71.4			92	
	Cumaphos	82.4–98.4			45	
	Diazinon	89.1–90.4			89	
	Dimethoate	6.4–10.2			1	
	Formothion	60.9–64.2			16	
	Phorate	46.0–61.9			1	
	Piridafenthion	80.7–87.6			24	
	Pyrazophos	92.0–97.2			7	
	Quinalphos	81.3–90.0			2	
	Triazophos	84.0–87.0			48	
	Tetrachlorvinphos	84.7–88.7			10	
	Trichlorfon	5.5–6.3			729	
As above but eluted with *n*-hexane	2-PCB	91.8	5000–50 000	GC–ECD	0.6-53 ng l^{-1}	94
	2,2′-PCB	94.7				
	2,4-PCB	99.8				
	4,4′-PCB	92.6				
	2,4,5-PCB	108.5				
	3,3′,4,4′-PCB	91.9				
	2,2′,4,5,5′-PCB	85.7				
	2,2′,4,4′,5,5′-PCB	86.8				
	Decachlorobiphenyl	83.7				

(Continued on p. 152)

TABLE III (*Continued*)

Extraction	Pesticides[a]	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
Sep-Pak C_{18}	Ramrod	83.8	5	GC–ECD	0.38 $\mu g\ l^{-1}$	95
2 ml acetonitrile–isooctane	2,4-D methyl ester	75.0			0.22	
10 ml water	CIPC	83.6			2.6	
Compares with Florisil	Silvex methyl ester	83.8			0.032	
	2,4,5-T methyl ester	95.1			0.064	
	2,4-DB methyl ester	70.6			0.039	
	DEF	71.3			0.081	
200 mg C_{18}	TEPT	94.0	70	GC–FPD	–	96
1.0 ml of MTBE	Dichlorvos	56.9				
70 ml water	Ethoprop	92.0				
Compares with C_8	Phorate	54.6				
	Diazinon	89.3				
	Dimethoate	5.0				
	Methyl parathion	96.3				
	Parathion	94.3				
	Tokuthion	70.4				
	Fanphur	99.9				
	EPN	86.3				
	Azinphos-methyl	97.2				
200 mg C_{18} cartridges	Dicamba	62	1000	HPLC–diode array	–	97
1 ml methanol	2,4-D	101			10 $\mu g\ l^{-1}$	
1-l water samples	2,4,5-T	95			2 $\mu g\ l^{-1}$	
	Silvex	91			8 $\mu g\ l^{-1}$	
	Dinoseb	65			–	
C_8 cartridges	Malathion		450	GC–ECD	–	98
3 ml diethyl ether–isooctane	α-Endosulfan					
450 ml sea water	α;β-Endosulfan					
	β-Endosulfan					
	Fenvalerate					
6 ml C_{18} high-capacity cartridges	Carbofuran	85.9-105.3	50	HPLC–UV	0.4 mg l^{-1}	99
2 ml methanol–water (60:40), acidified	3-hydroxy-7-phenol carbofuran	101.7-110.0				
	3-Hydroxy carbofuran	103.8-112-9				
	3-Keto-7-phenol carbofuran	104.7-109.7				
	3-Ketocarbofuran	98.2-109.9				
		98.9-107.6				

100 ml distilled and rice water	7-Phenolcarbofuran					
C_8	2,4-D	93.8	50	HPLC–UV	6 $\mu g\ l^{-1}$	100
2 ml methanol	2,4-DP	103.0			6	
100 ml water	2,4-D IOE	92.0			20	
Compares with C_{18}	2,4-DP BEE	100.0			10	
	Dicamba	82.2			2.4	
	Pendimethalin	91.5			5	
	Chlorpyrifos	93.7			5	
500 mg Bond-Elut C_{18}	Atrazine	99–103	125	GC–NPD	–	101
2 ml ethyl acetate–isooctane (1:9)	Alachlor	96–105				
250 ml groundwater	Metolachlor	95–99				
500 mg C_{18}	Chlorpyrifos	96	1000	GC–NPD		102
5 ml dichloromethane	Isofenphos	96			0.1 ppb	
	Carbaryl	106			0.1	
1 l groundwater	Triadimefon	107			1	
	Iprodione	102			1	
ODS column	*p,p'*-DDT	59–63	–	On-line HPLC–UV	4 $\mu g\ l^{-1}$	103
methanol–water (75:25)	Aldrin	60–64			16	
	Dieldrin	64–77			7	
100 ml water	Heptachlor	63–69			10	
1 g C_{18}	Alachlor	95	500	GC–MS	–	104
2 × 3 ml dichloromethane	Atrazine	96				
	Cyanazine	97				
500 ml water samples	Metolachlor	95				
	Simazine	97				
330 mg C_{18} Sep-Pak plus cartridges	Propoxur	92	182	HPLC–UV	130 $ng\ l^{-1}$	105
	Carbofuran	91			140	
	Carbaryl	93			20	
0.75 ml acetonitrile	Propham	92			100	
	Captan	88			920	
100-ml water sample	Chloropropham	89			60	
	Barban	89			80	
	Dibutylate	84			300	

Continued on p. 154)

TABLE III (*Continued*)

Extraction	Pesticides[a]	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
3 cm ODS pre-column	As above			On-line HPLC–UV	Half the above values	106
Acetonitrile–water						
100 ml tap, distilled, deionized, commercial spring and HPLC-grade waters						
C_{18} pre-column, 3 cm × 4.6 mm I.D.	Carbedazin			On-line HPLC–UV	100 ng l^{-1}	107
	Aminocarb				65	
	Propoxur				65	
Water (pH 6.8)–acetonitrile	Carbofuran				70	
	Carbaryl				10	
100 ml water	Propham				50	
Compares with C_8	Captan				460	
	Chloropropham				30	
	Barban				40	
	Benomyl				500	
	Butylate				150	
C_8 precolumn	Aldicarb	104	–	On-line HPLC–UV	2.5-11 μg l^{-1}	108
Water–acetonitrile	Aldicarb sulphoxide	92				
	Aldicarb sulphone	102				
10 ml water	MBC	95				
	Benomyl	94				
C_8 Empore membrane disc, 47 mm diameter	Vernam	78.8-86.8	100	GC–NPD		109
	Atrazine	86.2–88.4		GC–ECD		
10 ml methanol	Diazinon	90.2–97.0				
1 l groundwater	Dyphonate	86.6–88.0				
	Metribuzim	17.0–25.2				
Compares with C_{18}	Alachlor	87.9–97.0				
	Sulprofox	62.5–115.8				
	Heptachlor	55.2–95.1				
	Aldrin	51.0–55.2				
	Endosulfan	66.8–99.2				

C_8 Empore membrane disc, 47 mm diameter	Alachlor	103	1000	GC–MS		110
10 ml ethyl acetate and 10 ml dichloromethane	Aldrin	99–114				
1 l water sample (pH < 2)	Atrazine	129–139				
	α-Chlordane	68–82				
	γ-Chlordane	82–85				
	trans-Nonachlor	18–37				
	Endrin	126–128				
	Heptachlor	116–131				
	Heptachlor epoxide	122–141				
	BHC	27–60				
	Lindane	113–122				
	Metoxychlor	48–95				
	2-PCB	78–112				
	2,3-PCB	101–125				
	2,4,5-PCB	90–108				
	2,2′,4,4′-PCB	97–144				
	2,2′,3′,4,6-PCB	106–118				
	2,2′,4,4′,5,6′-PCB	95–131				
	2,2′,3,3′,4,4′,6-PCB	15–30				
	2,2′,3,3′,4,5′,6,6-PCB	45–102				
	Simazine	110–112				
	Toxaphene mixture	304				
Sep-Pak C_{18} cartridge 2 ml ethyl acetate–isooctane	Atrazine	–	625	HPLC–UV	0.04–0.1 μg l^{-1}	111
250 ml groundwater	Alachlor			Immunoassay	0.1 μg l^{-1}	
	Carbofuran					
C_8 Empore disc, 47 mm diameter	α-HCH	65		GC–NDP	0.02–0.5 μg l^{-1}	112
15 ml ethyl acetate	HCB	92				
1 l water	β-HCH	73				
	γ-HCH	75				
	δ-HCH	68				
	Endosulfan ether	90				
	heptachlor	113				
	Aldrin	65				
	Heptachlor epoxide	85				
	o,p′-DDE	82				
	α-Endosulfan	86				
	Dieldrin	103				
	p,p′-DDE	87				
	o,p′-DDD	80				
	Endrin	82				
	β-Endosulfan	87				
	p,p′-DDE	74				
	o,p′-DDT	80				

TABLE III (*Continued*)

Extraction	Pesticides	Recovery (%)	Concentration ratio	Determination method	Detection limit	Ref.
	p,p'-DDT	75				
	Metamidophos	46				
	Dichlorvos	60				
	Trichlorfon	48				
	Heptenophos	85				
	Phorate	75				
	Diazinon	95				
	Dimethoate	52				
	Chlorpyrifos-methyl	84				
	Parathion-methyl	75				
	Chlorpyrifos-ethyl	87				
	Parathion-ethyl	72				
	Quinalphos	72				
	Profenofos	75				
	Ethion	75				
	Vanmidothion	34				
	Phosalone	50				
	Azinphos-methyl	75				
	Azinphos-ethyl	62				
	EPTC	54				
	Molinate	78				
	Cycloate	67				
	Trietazine	78				
	Alachlor	70				
	Propazine	78				
	Terbutylazine	83				
	Atrazine	82				
	Prometryn	75				
	Terbutrine	75				
	Simazine	68				
	Ametryn	78				
	Propachlor	60				
	Trifluoraline	65				
	Benfluoraline	75				
	Propyzamide	95				
	Metribuzin	72				
	Metolachlor	85				
	Chlorthal-dimethyl	92				
	Isopropalin	50				
	Pendimethalin	60				
	Dichlofop-methyl	63				

Sep-Pak C_{18} cartridges 10 ml ethyl acetate–10 ml dichloromethane 1 l water	α-HCH	81.1	1000	GC–ECD GC–NPD	0.5 μg l^{-1}	113
	β-HCH	79.8			0.3	
	Lindane	82.3			0.2	
	δ-HCH	81.0			0.3	
	Endrin	79.2			0.4	
	Dieldrin	78.1			0.4	
	Aldrin	80.0			0.04	
	Heptachlor	82.2			0.05	
	Heptachlor epoxide	78.8			0.2	
	Endosulfan A	80.3			0.4	
	Endosulfan B	78.2			0.3	
	p,p'-DDT	83.4			0.3	
	p,p'-DDE	82.2			0.4	
	p,p'-DDD	80.5			8	
	Chlorbenside	75.2			15	
	Chlorfenson	78.9			1	
	PCND	81.4			0.3	
	HCB	79.5			20	
	PCB2	83.2			9	
	PCB7	78.2			3	
	PCB28	78.3			0.3	
	PCB52	79.3			0.2	
	PCB101	81.2			0.1	
	PCB138	82.1			0.08	
	PCB153	80.7			0.05	
	PCB180	79.4			0.01	
	PCB209	81.9			0.002	
	Fenitrothion	83.2			2	
	Parathion-ethyl	85.6			4	

Abbreviations: ACDA = 2-amino-1-cyclopenteno-1-dithiocarboxylic acid; IOE = isooctyl ester; BEE = butoxyethanol ester.

and stability after repeated use [71,109,110,112].

Immobilized liquid membranes for separations have been developed in recent years. They have most frequently been used for separations of metal ions by facilitating transport mechanisms [117], but also for separations of organic molecules [118]. Audunsson [119] used immobilized liquid membranes in a flow system for the determination of amines in aqueous samples. A similar system was used by Nilve and co-workers [120,121] for enriched phenoxyalkanoic acids and sulphonyl urea herbicides prior to on-line determination by HPLC. With the liquid membrane technique sample preparation is performed in a flow system, which is easily automated.

3. FACTORS AFFECTING SOLID-PHASE EXTRACTION

The extraction recovery of pesticides from water samples depends on a number of factors such as the type of water samples (presence of particulate matter, ionic strength of the water), pH and sorbent treatment.

3.1. Type of water

Unfortunately, experiments are usually carried out on aqueous samples with low ionic strength and free from colloidal particles, such as distilled, deionized, tap or finished waters, representing a matrix that is different from natural waters and particularly from sea water [122]. Significant losses in recovery tests on pesticides have been observed with SPE when water samples with high contents of organic matter have been analysed owing to competition for the actives sites of the adsorbent between the chlorinated hydrocarbons and other hydrophobic groups present in the sample [64,92,94,99].

On analyses of marine and surface waters containing solid particles forming suspensions, the recoveries from unfiltered waters were found to be substantially lower than expected for some pesticides [48]. Humic substances in water can increase the apparent solubility of these compounds, bind organic compounds either with covalent bonds, as charge-transfer complexes, by hydrogen bonding or by Van der Waals interactions. These substances are adsorbed on the suspended solid particles [123–125].

Detergents diminish the retention of the pesticides in the solid phase, an effect probably due to an increase in the solubility of the pesticides in water [64,92–94].

An increase in the ionic strength of aqueous samples leads to weakening of the interaction between undissociated molecules and water, resulting in an increase in the extraction efficiency. A positive salting-out effect on adsorption on octadecylsilica has been observed for some herbicides [75,104] and pyrazone [126] and on Wolfatit Y77 for organophosphorus compounds [50]. In another report [126], an increase in ionic strength improved the retention for hydrophobic organic solutes in the water–Amberlite XAD-8 system [127]. However, the addition of NaCl or KCl had no significant effect on the extraction of a wide range of organic compounds on C_{18} [64,90,92].

3.2. Sample volume

The effect of sample volume on SPE recovery is of crucial importance for samples of environmental interest. Extraction of a sample volume of 200 ml–1 l is necessary in order to determine low levels of pollutants. In SPE, the solvent in which the solute is dissolved (*i.e.*, water for environmental samples) is capable of eluting the solute from the column; the solute of interest has some finite capacity factor in the sample solvent itself. If the number of column volumes of water required to elute the solute from the column, plus one column volume (the volume in the column when the sample was introduced), is exceeded, then the solutes begin to elute from the column as more sample is being continuously added to the head of the column; this results in decreased recoveries. The maximum sample volume from which 100% recovery can be achieved and beyond which the solute of interest begins to elute from the column is called the breakthrough volume. The breakthrough volume is determined by the capacity factor of the solute in the sample solvent, that is, the sample solvent strength. For reversed-phase sorbents, the breakthrough volume is a function of the hydrophobicity of the solute and the mass of sorbent used [45,84].

3.3. pH

The effect of pH on the retention of compounds on a solid phase can only be studied with stable and non-ionic pesticides [45,49,84,90,94]. In addition, it may be necessary to adjust the pH of the sample to ensure that the compound is in the appropiate form to achieve the efficient retention by the solid phase [41–43,73,82–84,87].

Most synthetic polymers are unaffected by extreme pH values, but some acrylates may be hydrolysed at high pH. Extreme pH values can change the nature of bonded phases; the recommended pH values are between 2 and 8.

3.4. Sorbent treatment

A typical sorbent treatment sequence involves the following steps: activation of the sorbent (wetting); washing for bonded phases; elution of concentrated pesticides; and regeneration of the column.

3.4.1. Activation

A requirement for effective adsorption is perfect mutual contact between the solid and liquid phases. The type of carbon generally used for pesticide enrichment is granular activated carbon with a large surface area (300–2000 m^2 g^{-1}) and a wide pore diameter distribution, which does not require previous treatment. As more than 99% of the surface area of a polymer sorbent is the internal area of the pores, the need for penetration of liquid phase into the pores is obvious. Complete permeation of the water into all these pores of the hydrophobic polymer is usually ensured by wetting the polymer first, with an organic water-miscible solvent, which is then replaced with water. Prewetting of chemically bonded silicas causes opening of the hydrocarbon chains, thus increasing its surface area.

3.4.2. Washing

After the sample has been extracted, potential interferents can be removed by washing the column with solvents of various strengths. For most non-polar phases, water can be used to remove many of the polar constituents of water samples without eluting pesticides. Less polar contaminants may be removed by adding relatively weak solutions of methanol or acetonitrile in water. However, to ensure that no breakthrough or loss of analytes occurs during washing steps, preliminary analyses should be carried out.

3.4.3. Desorption

Desorption is usually accomplished by the use of solvents. When the extraction is finished, a small volume of a liquid for which the partition coefficient in a given solid phase–eluent system favours the eluent is allowed to pass through the column [90–92,95,110]. Experimental results from adsorption and partitioning TLC and LC can be applied to the selection of the appropiate eluent [64].

The SPE column can be used more than once, provided that it is regenerated with the solvent used for its activation.

Preliminary studies with C_{18} SPE columns revealed the presence of interferents that co-eluted with the analytes of interest. Plasticizers have been reported to be frequent interferents [91,94,128]. Although the size of the interferent peaks was reduced by the precleaning procedure, they could not be completely eliminated. This phemomenon has also been observed with other polymeric sorbents [129].

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CHROMSYMP. 2747

Evaluation of graphitized carbon black as a selective adsorbent for extracting acidic organic compounds from water

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ABSTRACT

By a simple and rapid treatment, graphitized carbon black (GCB) can be made to act as both a reversed phase and an anion exchanger. The base–neutral/acid fractionation of organic species extracted from water with a GCB cartridge can be easily achieved by differential elution, provided that the pK_a values of the acidic species are not higher than about 7. A tetramethylammonium hyroxide-basified solvent mixture is the most effective eluent for the rapid elution from a GCB extraction cartridge of any kind of organic acid. The subfractionation of acidic species can be easily performed on the basis of their acidity strength by passing sequentially through a GCB cartridge various solvent mixtures containing suitable phase modifiers. The ability of a GCB extraction cartridge to retain organic acids was unaffected by the ionic strength of the water sample. The influence of fulvic acids dissolved in water on the capability of GCB to extract acidic compounds and isolate them from co-extracted base–neutral species was evaluated. The advantage of using a GCB cartridge over that containing a chemically bonded silica for accurately determining acidic compounds is demonstrated by two practical applications.

INTRODUCTION

Graphitized carbon blacks (GCBs) are adsorbing media that are produced by heating carbon blacks at 2700–3000°C in an inert atmosphere. GCBs are essentially non-specific, non-porous sorbents with surface areas ranging about between 8 and 100 m^2/g, depending on the type of starting material submitted to the graphitization process. GCBs have proved to be useful stationary phases for gas–liquid–solid chromatography [1–3]. In the last 10 years, GCB with the highest surface area, commercially referred to as Carbopack B or Carbograph 1, has been successfully used for the liquid–solid extraction (LSE) of analytes of both clinical [4–6] and environmental [7–11] interest.

Some years ago, on studying the nature of the chemical heterogeneities contaminating the GCB surface, we obtained experimental evidence [12] for the presence of an oxygen complex having a chromene-like structure, that is a burnt-off residue left over from the heating of carbon blacks [13] in producing graphitic carbons. In the presence of water this surface group is rearranged to form benzpyrylium salts, according to

$$\text{(chromene-like structure)} \xrightarrow[+H_2O]{+O_2} \text{(benzpyrylium structure)} \quad \text{(I)}$$

The presence of these positively charged chemical impurities on the GCB surface enables it to act as both an anion exchanger and a non-specific sorbent. By using GCB extraction cartridges and differential elution, the singular feature has been empirically exploited for fractionating estrogens and their conjugated forms in body fluids [14,15] and for

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isolating acidic pesticides from co-extracted base–neutral pesticides present in aqueous environmental samples [16,17]. In these reports, several solvent systems have been proposed for desorbing from the GCB surface particular target acidic compounds. Moreover, no systematic investigation on the efficiency and reliability of GCB in specifically adsorbing acidic species from various aqueous matrices has yet been performed.

This study was devoted to the systematic characterization of GCB as an anion exchanger for establishing whether GCB extraction cartridges can be advantageously included in analytical schemes elaborated for determining acidic organic compounds at trace levels in natural waters. The effects that the pH of the aqueous matrix, its ionic strength and the presence in it of fulvic acids and acidic surfactants can have on the capability of a GCB cartridge to extract quantitatively sixteen selected acidic model compounds and to isolate them from base–neutral species by selective desorption were studied. The possibility of subfractionating co-extracted acidic compounds on the basis of their acid strength by stepwise desorption was also investigated.

EXPERIMENTAL

Reagents

For this study, the model compounds used were selected with the criterion of choosing, among the acidic organic compounds of environmental interest, those having different pK_a values in order to cover a large range of acidity. The test compounds selected were as follows: 2,4-dichlorophenol (pK_a 8.0) (2,4-DCPh); *p*-nitrophenol (pK_a 7.2) (*p*-NPh); 2,4,6-trichlorophenol (pK_a 7.0) (2,4,6-TCPh); 4-hydroxy-3(3-oxo-1-phenylbutyl)coumarin (pK_a 5.7) (warfarin); 4-(2,4-dichlorophenoxy)butyric acid (pK_a 4.8) (2,4-DB); pentachlorophenol (pK_a 4.7) (PCP); 2-*sec.*-butyl-4,6-dinitrophenol (pK_a 4.6) (dinoseb); 2,4-dinitro-*o*-cresol (pK_a 4.4) (DNOC); 2,4-dinitrophenol (pK_a 4.1) (2,4-DNPh); 4-hydroxy-3,5-diiodobenzonitrile (pK_a 3.9) (ioxynil); 4-chloro-2-methylpropanoic acid (pK_a 3.7) (mecoprop); 3-isopropyl-1*H*-2,1,3-benzothiadiazin-4-one 2,2 dioxide (pK_a 3.5) (bentazone); 2,4-dichlorophenoxyacetic acid (pK_a 2.6) (2,4-D); 2,4,5-trichlorophenoxyacetic acid (pK_a 2.2) (2,4,5 T); 3,6-dichloro-2-methoxybenzoic acid (pK_a 1.9, (dicamba); and octylbenzenesulphonic acid (pK_a 0.8) (C_8-LAS). These compounds were supplied by Riedel-de Haën (Hannover, Germany) and Aldrich (Milwaukee, WI, USA). The pK_a values in water reported above were taken from various sources. Individual standard solutions were prepared by dissolving 100 mg of each compound in 100 ml of acetonitrile. A composite working standard solution was prepared by mixing 0.2–0.4 ml of each standard solution and diluting to 10 ml with acetonitrile.

Fulvic acids were kindly donated by Dr. A. Piccolo, who prepared them as described elsewhere [18]. A commercial C_{10}–C_{13} linear alkylbenzesulphonate (LAS) mixture (Marlon A) was supplied by Chemische Werke Hüls (Marl, Germany).

Trifluoroacetic acid (TFA), tetramethylammonium hydroxide (TMAOH), tetrapropylammonium bromide (TPABr) and formic acid were purchased from Aldrich. For HPLC, distilled water was further purified with an Elgastat UHQPS apparatus (Elga, Bucks, UK). Methanol and acetonitrile of gradient grade were obtained from Riedel-de Haën. All other solvents were of analytical reagent grade (Carlo Erba, Milan, Italy) and were used as received.

Graphitized carbon black (120–400 mesh size), commercially referred to as Carbograph 1, and the other materials used for preparing the extraction cartridges were kindly supplied by Alltech (Deerfield, IL, USA). GCB cartridges (300 mg) were prepared as reported previously [16,17]. The trap was fitted into a side-arm flask and liquids were forced to pass through the cartridge by vacuum from a water pump.

The measurements of the anion-exchange capacities of both GCB and its parent material were conducted by passing through the 300-mg sorbent cartridges 10 ml of a CH_2Cl_2-CH_3OH mixture (50:50, v/v) containing 2 g/l of C_8-LAS. The fraction of this acid unspecifically adsorbed was removed by washing the cartridge with 12 ml of CH_2Cl_2–CH_3OH (80:20, v/v). The C_8-LAS fraction specifically sorbed on the benzpyrylium ions were desorbed by passing 8 ml of CH_2Cl_2–CH_3OH (80:20, v/v) basified with TMAOH (20 mmol/l). After solvent removal, the residue was reconstituted by a suitable acidified aqueous methanolic mixture and a portion of it was injected into the HPLC apparatus.

Procedure

Recovery studies were performed by pretreating the GCB cartridge with 7 ml of water acidified with HCl (pH 1). After extracting the water samples, the cartridge was washed with an aqueous solution containining 20 mmol/l of $KHCO_3$. The major part of water was eliminated by room air drying for 30 s. Thereafter, residual water was eliminated by passing 1 ml of methanol. Before desorbing acids, the cartridge was washed with 7 ml of CH_2Cl_2–CH_3OH (90:10, v/v). This solvent mixture is able to remove from the cartridge any non-acidic compounds. Unless indicated otherwise, acidic compounds were eluted with 6 ml of CH_2Cl_2–CH_3OH (90:10, v/v) basified with 20 mmol/l of TMAOH.

When using TMAOH as an eluent modifier, the extract was acidified with 100 μl of 10% TFA in methanol, before evaporating the eluate. This was done in order to avoid the decomposition of 2,4-DNPh and DNOC that occurs under alkaline conditions. In all instances, the extracts were evaporated to a volume of *ca.* 100 μl using a stream of nitrogen at 30°C. To this, 150 μl of acidified water (pH 1) were added and, after measuring the exact volume, 50 μl of this solution were injected into the HPLC apparatus.

HPLC analysis

Liquid chromatography was carried out with a Varian (Walnut, Creek, CA, USA) Model 5000 chromatograph equipped with a Rheodyne Model 7125 injector having a 50-μl loop and with a Model 2550 UV detector (Varian). A 25 cm × 4.6 mm I.D. column filled with 5-μm LC-18 packing (Supelco, Bellefonte, PA, USA) was used. For separating the acidic compounds selected, gradient elution was performed. Solvent A was water acidified with 0.05% TFA; solvent B was acetonitrile–methanol (80:20, v/v) acidified with 0.01% TFA. The initial mobile phase composition was 47% solvent B, linearly programmed to 85% after 25 min. The eluted compounds were monitored with the UV detector set at 220 nm. The flow-rate of the mobile phase was 1.5 ml/min.

The concentrations of the acidic compounds in the final extract were calculated by measuring the peak heights for each compound and comparing them with those obtained from standard solutions. These were prepared by taking known and appropritate volumes of the working standard solution, evaporating the solvent and reconstituting the residue with 100 μl of methanol and 150 μl of water acidified to pH 2.

RESULTS AND DISCUSSION

The anion-exchange capacity of GCB was measured by packing 300 mg of it in a cartridge and pretreating the adsorbent with various solutions. The results are reported in Table I. According to the proposed reaction mechanism (I), conversion of chromene-like structures to benzpyrylium ions was much more readily achieved by washing the sorbent column with aicidifed water. Moreover, only a sligth increase in the number of exchange sites was obtained by both increasing the volume of the acidified water and decreasing the pH of the water. This indicates that chromene groups are rapidly rearranged to give benzpyrylium salts in the presence of acidified water.

It has been reported [19] that benzpyrylium salts are promptly reduced bo benzpyrans under mild conditions according to

+ $NaBH_4$ →

In order to obtain additional evidence about the nature of the exchange sites populating the surface framework of GCB, this material was first washed

TABLE I

THE EFFECT OF VARIOUS PRETREATMENTS ON THE ANION-EXCHANGE CAPACITY OF GCB

Pretreatment	Capacity, μequiv./g[a]
None	0.89
TFA in CH_2Cl_2–MeOH	3.5
14 ml Water (pH 6)	3.1
7 ml Water (pH 2)	7.5
14 ml Water (pH 2)	7.4
7 ml Water (pH 1)	7.8
7 ml Water (pH 0)	7.8
7 ml Water (pH 1) followed by $NaBH_4$	0.84

[a] Mean values from duplicate experiments.

with an acidic aqueous solution and then treated with sodium tetrahydroborate dissolved in acetonitrile. As can be seen in Table I, after this treatment the anion-exchange capacity of the GCB material decreased abruptly, confirming that benzpyrylium ions are responsible for binding organic anions via electrostatic forces. Interestingly, the exchange site concentration of the $NaBH_4$-treated GCB was very similar to that measured for untreated GCB. This finding suggests that, in addition to chromene groups, the GCB surface might also be contaminated by a small number of chemical heterogeneities of different nature, even those capable of exchanging anions.

The ion-exchange capacity of non-graphitized carbon black was also measured and it wâs found to be about twice that of the GCB surface. This seems surprising considering that carbon blacks are graphitized at very high temperatures at which any surface organic chemical groups should be decomposed. Probably chemical heterogeneities are partially reformed during cooling of the carbonaceous material after the graphitization process.

When water contaminated by organic compounds passes through a GCB column, base–neutral compounds are adsorbed on the graphitic framework of the sorbent, whereas adsorption of acidic compounds takes place on benzpyrylium ions. In this situation, the base–neutral/acid fractionation can be easily achieved by first eluting base–neutral species with a neutral organic solvent mixture and then passing a basified or acidified solvent system to desorb acidic compounds. For conciseness, from this point onwards the solvent systems designed to elute non-acidic and acidic adsorbates will be called eluent A and eluent B, respectively.

This scheme succeeded in isolating acidic from base–neutral pesticides [16,17]. The acidic pesticides considered had pK_a values ranging between about 2 and 5. We evaluated whether even weaker acidic compounds could still be isolated from base–neutral compounds by stepwise desorption. For this purpose, 1 l of distilled water was spiked with the sixteen acidic model compounds selected and with a neutral compound, such as linuron, which is a well known phenylurea herbicide. This artificially contaminated aqueous sample was then extracted with the 300-mg GCB cartridge. After extraction, the cartridge was washed with 7 ml of an aqueous solution containing 20 mmol/l of $KHCO_3$ (pH 8.1).

Differential elution was carried out as described under Experimental. The results reported in Table II shows that both p-NPh and 2,4,6-TCPh, having pK_a values of about 7, were found quantitatively in the acid-containing extract. When the step of washing the cartridge with water slightly basified with $KHCO_3$ was omitted, *ca.* 30 and 15% of p-Nph and 2,4,6-TCPh, respectively, were eluted by eluent A. It can be assumed that the undissociated fraction of ân acidic compound present in water is, as such, unspecifically adsorbed on the GCB surface. This fraction is probably ionized on passing through the sorbent bed a basified aqueous solution and then it moves from unspecific adsorption sites to those ones bearing a positive charge.

Under the experimental conditions selected, 2,4-DChPh, having a pK_a about ten times higher than those of the two phenols mentioned above, was al-

TABLE II

BASE–NEUTRAL/ACID FRACTIONATION AFTER EXTRACTION FROM WATER OF SELECTED COMPOUNDS WITH A GCB CARTRIDGE

Compound	Recovery (%)[a]	
	Eluent A[b]	Followed by eluent B[c]
Linuron	97	–
2,4 DCPh	80	12
p-NPh	–	98
2,4,6-TCPh	–	95
Warfarin	–	99
2,4-DB	–	101
PCPh	–	96
Dinoseb	–	99
DNOC	–	90
2,4-DNPh	–	98
Ioxynil	–	100
Mecoprop	–	100
Bentazone	–	101
2,4-D	–	99
2,4,5-T	–	98
Dicamba	–	100
C_8-LAS	–	97

[a] Mean values obtained from triplicate measurements.
[b] 7 ml of CH_2Cl_2–CH_3OH (90:10, v/v).
[c] 6 ml of CH_2Cl_2–CH_3OH (90:10, v/v) basified with TMAOH (20 mmol/l).

most completely washed out by the neutral mobile phase. Increasing the pH of the aqueous washing phase decreased only to a slight extent the percentage of 2,4-DChPh found in the neutral extract. Probably methanol contained in eluent A is able to compete with very weakly acidic compounds for adsorption on benzpyrylium ions. On the other hand, it has been reported [16] that solvent systems not containing methanol are ineffective for rapidly eluting from GCB cartridges any kind of neutral adsorbate. In fact, when eluent A was composed of methylene chloride or methylene chloride–acetonitrile, more than 85% of linuron was found in the second extract containing the acidic species. Definitively, the goal of isolating an acidic compound from co-extracted base–neutral compounds can easily be achieved by differential elution, provided that the pK_a value is not higher than about 7.

In the past, several solvent mixtures composed of various amounts of methanol in chloroform or methylene chloride and containing various concentrations of different acid-displacing agents, such as tetrapropylammonium bromide (TPABr) [14], tetramethylammonium hydroxide (TMAOH) [8,20], potassium hydroxide [16] and trifluoroacetic acid (TFA) [17], have been used empirically for eluting particular acidic analytes from a GCB cartridge. By varying both the nature of the displacing agent and the composition of the solvent mixture in which they were dissolved, the abilities of various eluent systems to re-extract rapidly acidic adsorbates from a 300-mg GCB extraction cartridge were compared. These experiments were conducted by passing through the cartridge 1 l of distilled water spiked with the acidic model compounds considered at individual concentrations of 0.5 μg/l. The adsorbates were eluted by passing through the GCB bed eight 2-ml aliquots of the eluent system selected and analysing each individual aliquot. The concentration of any phase modifier considered was 20 mmol/l. The results are reported in Table III.

Both TPABr and KOH were effective phase modifiers for rapidly removing from the GCB surface organic anions, except for some phenol derivatives. In general, their effect was enhanced when dissolved in solvent systems rich in methylene chloride. TFA

TABLE III

VOLUMES OF VARIOUS ELUENT SYSTEMS NEEDED FOR RE-EXTRACTING ACIDIC COMPOUNDS FROM THE GCB CARTRIDGE

Compound	Eluent system volume (ml)[a]											
	TPABr			KOH			TFA			TMAOH		
	90:10[b]	80:20	60:40	90:10	80:20	60:40	90:10	80:20	60:40	90:10	80:20	60:40
p-NPH	6	4	4	4	4	4	4	4	4	4	4	4
2,4-DNPh	6	12	>16	10	12	14	6	10	12	4	4	4
Dicamba	4	4	4	4	4	4	4	4	4	4	4	4
Bentazone	4	4	4	4	4	4	4	4	4	4	4	4
C_8-LAS	4	4	4	4	4	4	>16	>16	>16	4	4	4
2,4-D	4	4	4	4	4	4	4	4	4	4	4	4
DNOC	14	16	>16	14	10	>16	12	16	>16	6	6	6
Ioxynil	8	8	12	8	6	10	4	4	4	4	4	4
Mecoprop	4	4	4	4	4	4	4	4	4	4	4	4
Warfarin	4	4	4	4	4	4	4	4	4	4	4	4
2,4,5-T	4	6	16	4	4	16	4	4	4	4	4	4
2,4-DB	4	4	6	4	4	6	4	4	4	4	4	4
TCPh	4	4	4	4	4	4	4	4	4	4	4	4
Dinoseb	4	6	16	8	4	10	4	6	6	4	4	4
PCP	12	12	>16	10	8	>16	12	16	>16	6	6	6

[a] Volume of the eluent system needed to elute at least 90% of the adsorbate. Mean values obtained from duplicate experiments.
[b] CH_2Cl_2 to CH_3OH volume ratio in the solution containing the acid-displacing agent.

was unable to elute C_8-LAS quantitatively from the GCB cartridge. This can be accounted for by considering that the sulphonated compound has a higher acidity than TFA. Regardless of the composition of the solvent system in which TMAOH was dissolved, this organic base was capable of rapidly eluting from the GCB cartridge even those phenol derivatives which exhibited a particularly high affinity for binding with benzpyrylium ions. This ability is probably the result of a synergic action played by the OH^- ion, which competes with organic anions for adsorption on the exchange sites, and the "naked" tetraalkylammonium cation able to form with organic anions stable ion pairs, which are readily soluble in organic solvents.

Recently, we reported [17] that 8 ml of CH_2Cl_2–CH_3OH (80:20, v/v) acidified with TFA sufficed to elute quantitatively from a 300-mg cartridge eighteen acidic pesticides, including DNOC and PCP. This appears to be in contrast to data obtained in this study. For this reason, we evaluated the extent to which batch-to-batch variations in the physico-chemical characteristics of the GCB surface could affect the re-extraction of acidic compounds from the GCB cartridge when eluting them with a TFA-containing solvent mixture. Two distilled water samples were spiked with the selected acidic compounds considered at different individual concentrations, namely 0.25 and 2.0 μg/l. Each artificially contaminated water sample was analysed six times by extracting 1-l aliquots with cartridges containing GCB from six distinct batches. The re-extraction of the acidic compounds was performed by using both TFA and TMAOH as eluent modifiers. The results are reported in Table IV.

With TFA as modifier, unsatisfactorily low mean recoveries of DNOC, 2,4-DNPh and PCPh with values scattered over a wide range were obtained when analysing the water sample containing the lowest concentration of acids. Under the same conditions, the recovery and precision for the three phenols mentioned above were also measured by extracting water with cartridges filled with GCB from one individual batch. The recovery and the

TABLE IV

RECOVERY OF SELECTED ACIDS FROM GCB CARTRIDGES PREPARED FROM SIX DISTINCT SORBENT BATCHES BY PASSING THROUGH TWO SELECTED ELUENT SYSTEMS

Compound	Recovery (%)							
	8 ml CH_2Cl_2–CH_3OH (90:10, v/v)–20 m*M* TFA				6 ml CH_2Cl_2–CH_3OH (90:10, v/v)–20 m*M* TMAOH			
	0.25 μg/l[a]		2.0 μg/l[a]		0.25 μg/l[a]		2.0 μg/l[a]	
	Mean[b]	Range	Mean[b]	Range	Mean[b]	Range	Mean[b]	Range
p-NPH	99	96–103	99	96–99	99	98–101	100	97–101
2,4-DNP	79	70–93	96	90–99	98	94–103	98	96–100
Dicamba	99	96–103	99	96–98	99	98–102	99	96–100
Bentazone	100	98–104	99	97–100	100	99–102	100	99–100
2,4-D	97	91–102	98	96–99	98	96–100	98	96–99
DNOC	62	50–85	91	85–95	95	91–98	95	90–99
Ioxynil	99	96–102	100	97–101	98	96–100	100	99–100
Mecoprop	97	95–101	99	98–100	101	98–102	98	97–100
Warfarin	99	97–102	98	96–100	100	99–103	100	99–100
2,4,5-T	98	96–103	98	95–100	99	98–102	99	99–100
2,4-DB	100	97–104	99	97–101	101	99–104	100	99–100
2,4,6-TCPh	99	98–103	98	98–100	100	98–102	100	99–100
Dinoseb	94	92–100	97	95–99	99	97–103	99	97–100
PCPh	83	70–91	90	86–95	98	96–105	99	98–100

[a] Concentration in water of the model compounds.
[b] Average recovery calculated from six determinations.

range were 84% (79–86%), 75% (72–79%) and 82% (78–84%) for 2,4-DNPh, DNOC and PCPh, respectively. By comparing these values with those ones reported in Table IV, it can be concluded that batch-to-batch variations of the GCB material were responsible for large variations in the recovery of these three phenols. Conversely, the re-extraction efficiency of the TMAOH-basified organic mixture was not dependent on the particular GCB batch.

This finding is not thoroughly understood. However, one could speculate that the mobilities along the GCB column of particular non-ionized phenol derivatives are influenced by additional interactions occurring between these eluates and unknown active sites present on the GCB surface. The surface concentration of these chemical heterogeneities is very low, as their action is evident only when a few hundred nanograms of the three phenol derivatives are adsorbed on the GCB surface. Variations in the surface concentration of these active sites could arise from insufficient control of all the factors affecting the quality of the graphitization process.

With respect to the past, the multi-component determination of organic contaminants in environmental waters is nowadays made more difficult as a much larger number of target compounds have to be monitored and the demand is for methods of greater sensitivity. Therefore, the use of a single sorbent cartridge able not only to extract quantitatively a large number of pollutants having a broad range of physico-chemical properties but also to fractionate them by differential elution is highly desirable. As shown above, base–neutral/acid fractionation can be easily accomplished by the use of a GCB extraction cartridge.

The possibility of subfractionating acids on the basis of differences in their acid strengths by a multi-stage desorption process was also investigated. For these experiments, a water sample spiked with the acidic compounds considered was passed through the GCB cartridge. After washing the cartridge with the solvent mixture designed to elute base–neutral compounds, stepwise desorption of acids was performed by passing sequentially three different, selected eluent systems. The results reported in Table V show that, except for a slight carryover between DNPh, DNOC, PCPh and C_8-LAS, subfractionation of acidic compounds was accomplished on the basis of their pK_a values by properly selecting the eluotropic strength of the eluent system. In particular, a 5 mmol/l acetic acid-containing mobile phase was able to remove from the GCB surface only those acids having pK_a values between 7 and 5. The addition of 0.25 mol/l of formic acid to the CH_2Cl_2–CH_3OH mixture succeeded in eluting acids with pK_a values between about 5 and 2. Finally, by passing TMAOH in CH_2Cl_2–CH_3OH, even the compound bearing a sulphonic group was quantitatively recovered in the third fraction. Formic acid was preferred to TFA as a displacer of medium-strength acidic compounds because with TFA a certain, variable amount of C_8-LAS was found in the second fraction. More interestingly, it was observed that by replacing TFA with formic acid the re-extraction efficiency of even traces of 2,4-DNPh, DNOC and PCPh was unaffected by batch-to-batch variations in the surface characteristics of GCB.

It is known that one serious drawback discourag-

TABLE V

GROUP SEPARATION OF SELECTED ACIDIC COMPOUNDS BY DIFFERENTIAL ELUTION FORM A GCB CARTRIDGE

Compound	Recovery (%)[a]		
	Eluent B_1[b]	Eluent B_2[c]	Eluent B_3[d]
p-NPH	97	–	–
2,4-DNPh	–	96	5
Dicamba	–	101	–
Bentazone	–	101	–
C_8-LAS	–	4	95
2,4-D	–	98	–
DNOC	–	93	5
Ioxynil	–	100	–
Mecoprop	–	100	–
Warfarin	96	–	–
2,4,5-T	–	99	–
2,4-D B	98	–	–
2,4,6-TChPh	99	–	–
Dinoseb	–	98	–
PCPh	–	96	4

[a] Mean values obtained from triplicate measurements.
[b] 6 ml of CH_2Cl_2–MeOH (90:10, v/v) containing acetic acid (4 mmol/l).
[c] 8 ml of CH_2Cl_2–MeOH (90:10, v/v) containing formic acid (250 mmol/l).
[d] 6 ml of CH_2Cl_2–MeOH (90:10, v/v) containing TMAOH (20 mmol/l).

ing the use of conventional anion exchangers for the selective determination of acidic compounds in environmental waters is that the extraction efficiency of these materials is dependent on the ionic strength of the aqueous matrix. The influence of inorganic anions present in water on the ability of a 300-mg GCB cartridge to extract organic acids and isolate them from co-extracted base–neutral compounds was evaluated and compared with that of a cartridge containing the same mass of a high-capacity resin-based strong anion exchanger, such as Amberlite CG-400-II. Aliquots of 1 l of distilled water were spiked with the acidic model compounds and 35 g of sodium chloride were added to simultate a sea-water specimen. Before extraction with the conventional anion exchanger, the pH of the water sample was adjusted to 9 to increase the dissociation of the weakest acids.

The recovery data reported in Table VI show that, in contrast to that of the resin-based anion exchanger, the extraction efficiency of GCB was unaffected by the presence in water of a large amount of inorganic anions. Also, no significant amount of any acidic compounds was lost by washing the GCB cartridge with eluent A designed to remove base–neutral compounds from the sorbent surface. With respect to inorganic anions, the higher affinity that organic ions have for specific adsorption on the benzpyrylium ions of the GCB surface can be explained by assuming that non-specific interactions taking place between the hydrophobic part of an ionized organic compound and the graphitic surface framework of the sorbent make a significant contribution to the overall heat of adsorption. An analogous situation takes place on conventional ion exchangers but, in this instance, the contribution mentioned above is far more reduced as the number of sites for the ion exchange greatly predominate over those for non-specific adsorption. From a practical point of view, the GCB cartridge appears to be suitable for determining acidic pollutants in sea water.

It has been reported [21] that about 80% of the dissolved organic content (DOC) in environmental waters is due to the presence of fulvic acids (FA). Owing to their nature. FA can compete with acidic analytes for adsorption on benzpyrylium ions present on the GCB surface. The effect of the presence of FA in water on the extraction and isolation of acidic compounds by a GCB cartridge was evaluated. For these experiments, simulated surface water samples were prepared by adding to distilled water samples various amounts of FA, from 2.5 to 20 mg/l, after adjusting the pH of the water to 8. Each of these samples was spiked with known amounts of the model compounds and 0.5-l aliquots were analysed. The re-extraction of the acidic compounds was performed by passing the neutral eluent A followed by the basified eluent B (see Experimental).

The results reported in Table VII show that there was an incresing tendency of the model compounds to be eluted from the GCB cartridge by the neutral eluent A as the FA content in water increased. This was probably due to the fact that the ion-exchange sites on the GCB surface were progressively saturated by FA with the result that the adsorption of the ionized model compounds partially occurred also on the predominant unspecific surface sites of the sorbent.

Another negative effect induced by the presence in water of large amounts of FA was that some of

TABLE VI

RECOVERY OF SELECTED ACIDIC COMPOUNDS FROM A WATER SAMPLE WITH A HIGH IONIC STRENGTH (0.6 mol/l) BY EXTRACTING WITH THE GCB CARTRIDGE AND WITH A CARTRIDGE FILLED WITH A CONVENTIONAL ANION EXCHANGER

Compound	Recovery (%)[a]	
	Amberlite CG-400-II	GCB
p-Nph	38	97
2,4 DNPh	99	98
Dicamba	2	96
Bentazone	96	100
C_8-LAS	98	99
2,4-D	12	98
DNOC	97	94
Ioxynil	98	97
Mecoprop	10	96
Warfarin	81	95
2,4,5-T	38	97
2,4-DB	30	100
2,4,6-TChPh	95	100
Dinoseb	97	96
PCPh	98	96

[a] Mean values obtained from duplicate determinations.

TABLE VII

RECOVERY OF SELECTED ACIDIC COMPOUNDS FROM 0.5 l OF WATER SAMPLES CONTAINING VARIOUS AMOUNTS OF FULVIC ACIDS BY EXTRACTION WITH GCB CARTRIDGES

Compound	Recovery (%)[a]									
	2.5 mg/l FA		5 mg/l FA		10 mg/l FA		20 mg/l FA		10 mg/l FA[b], B[c]	20 mg/l FA[b], B[c]
	A[c]	B[c]	A[c]	B[c]	A[c]	B[c]	A[c]	B[c]		
p-NPh	–	98	16	80	18	65	23		90	84
DNPh	–	95	–	96	12	85	30	74	100	95
Dicamba	–	94	–	93	10	52	20	2	87	75
Bentazone	–	97	–	97	4	92	66	33	95	90
C_8-LAS	–	98	–	98	–	97	8	90	101	96
2,4-D	–	103	–	97	5	88	16	80	100	101
DNOC	–	95	–	94	–	93	–	96	100	96
Ioxynil	–	100	–	97	7	88	8	85	98	98
Mecoprop	–	98	–	93	5	88	15	73	93	88
Warfarin	–	96	–	91	3	80	7	47	94	79
2,4,5-T	–	100	–	95	–	90	–	92	99	95
2,4-DB	–	96	–	92	5	88	5	68	95	99
TCPh	–	98		96	–	92	25	–	96	88
Dinoseb	–	96	–	94	–	94	6	86	97	96
PCP		98		97		96	4	90	96	96

[a] Mean values from duplicate measurements.
[b] Water acidified to pH 2.2.
[c] The compositions of mobile phases A and B are the same as reported in Table II.

the acids were partially lost in the water effluent. Two different causes may be responsible for this loss. One is that the graphitic framework of the GCB surface is scarcely able to adsorb from water very polar organic anions. Another source of loss might be traced to some kind of association occurring between FA and some of the acidic compounds considered. Binding of organic molecules with FA in water has already been observed [22,23]. If this occurs, a sorbent column is unable to retain solutes associated with that fraction of FA passing unretained through it. At concentrations of FA in water $\geq$ 10 mg/l, we observed that the water samples after extraction had a brownish colour, indicating the presence of FA in the effluent. In our experience, river waters contain FA at concentrations lower than 10 mg/l. Anyway, when acidic analytes have to be monitored in a river water sample suspected to contain an anomalously high content of FA (a brownish colour of the sample is a valid indication), in order to avoid loss of the analytes and as an alternative to the obvious expedient of sampling smaller water volumes, the aqueous sample could be acidified before extraction. By doing so with water samples having high contents of FA, only a moderate loss of the water effluent of some of the acidic compounds was observed. By suppressing ionization, the adsorption of acids mainly occurs on the unspecific sites of the GCB surface, which are much larger in number than specific sites and are not readily saturated by FA. This result was obtained, however, at the expense of selectivity, because on passing through the sorbent bed the neutral eluent A designed to elute non-acidic compounds, variable fractions of most of the acidic model compounds considered were washed out from the cartridge.

Linear alkyl benzenesulphonates (LAS) are anionic surfactants of major use in detergent formulations. Commercial LAS materials are mixtures of various alkyl homologues that vary from C_{10} and C_{13} and phenyl positional isomers. Because of their widespread use, they occur in many different environmental compartments. In our experience,

typical concentrations of LAS in surface water samples range from 5 to 50 μg/l and only rarely do they occur in higher concentrations. When extracting with a GCB cartridge surface water samples contaminated by LAS, these compounds can interfere with the analysis or target acidic compounds in two ways. One is that, being strongly adsorbed on the benzpyrylium ions present on the GCB surface, they tend to saturate them. Moreover, LAS co-extracted with target acidic compounds may interfere with the subsequent HPLC analysis of the latter as the former produce large and broadened peaks. The effect of the presence in water of LAS on the quality of the analysis of acidic analytes compounds was evaluated by artificially contaminating 0.5 l of pure water samples with both the model acidic compounds and various amounts of a mixture composed of C_{10}–C_{13} LAS and analysing. The recovery data showed that the quantitative extraction by the GCB cartridge of acidic compounds and their successive isolation from base–neutral compounds by stepwise desorption were unaffected by the presence of LAS even when they were present in water at a total level of 300 μg/l. In addition, using the formic acid-containing solvent mixture mentioned above, we were able to elute completely from the GCB cartridge the model compounds, except C_8-LAS, and to determine them by HPLC without any interference by LAS, as the latter compounds were not desorbed from the GCB surface by the selected eluent.

For monitoring acidic compounds in natural waters, the advantage of using a GCB extraction cartridge over the popular octadcyl-bonded silica (C_{18}) cartridge is that the analysis can be made more selective by a suitable choice of both the washing and the eluent phases. Practical evidence for this statement was obtained by performing two experiments. In the first, a river water sample was spiked with 0.2 μg/l of PCPh and 2.0 μg/l of a polychlorinated biphenyl (PCB) mixture (Arochlor 1254). It is known

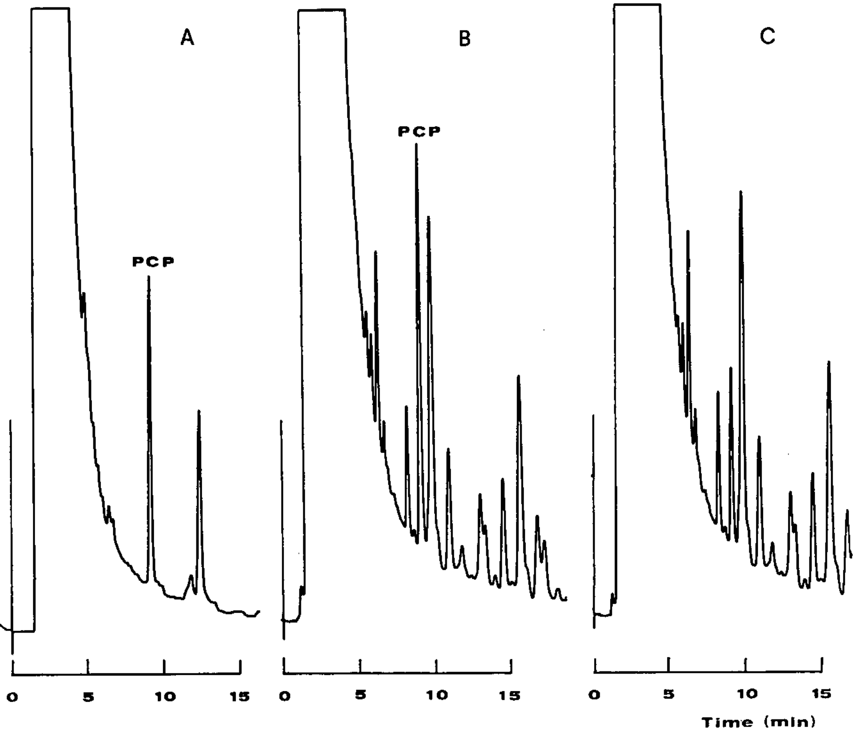

Fig. 1. Chromatograms obtained on analysing 1-l aliquots of river water (Tevere) spiked with 0.2 μg/l of pentachlorophenol and 2.0 μg/l of a mixture of polychlorobiphenyls (Arochlor 1254) by two procedures involving the use of (A) the GCB extraction cartridge and (B) a 0.5-g C_{18} extraction cartridge. Chromatogram C was obtained by the latter procedure on analysing 1 l of the same water sample spiked only with 2 μg/l of Arochlor 1254.

that, as a consequence of their widespread use, PCBs are present in all compartments of the natural environment. A 1-l aliquot of the artificially contaminated water sample was analysed as such by extracting PCPh with the 300-mg GCB cartridge and, after washing PCBs from the GCB surface by passing 7 ml of CH_2Cl_2–CH_3OH (90:10, v/v), PCPh was eluted with the formic acid-containing mobile phase. Before extracting with the 0.5-g C_{18} cartridge (Supelco), the pH of a 1-l aliquot of the same water sample was adjusted to about 2.5. After the water had passed through the C_{18} cartridge, PCPh was eluted by passing through it 6 ml of methanol. The two eluates were dried and the residues reconstituted with 0.25 ml of an acidified aqueous methanol mixture. Volume of 50 μl of each of two extracts were then injected into the HPLC system. The column was operated isocratically with a water–methanol mixture (20:80, v/v) acidified with TFA (0.05%, v/v). The resulting chromatograms are shown in Fig. 1, which also shows a third chromatogram obtained by analysis with the procedure involving the use of the C_{18} extraction cartridge the surface water sample spiked only with PCBs. From comparison of the chromatograms, it appears that the use of a non-selective analytical procedure, such

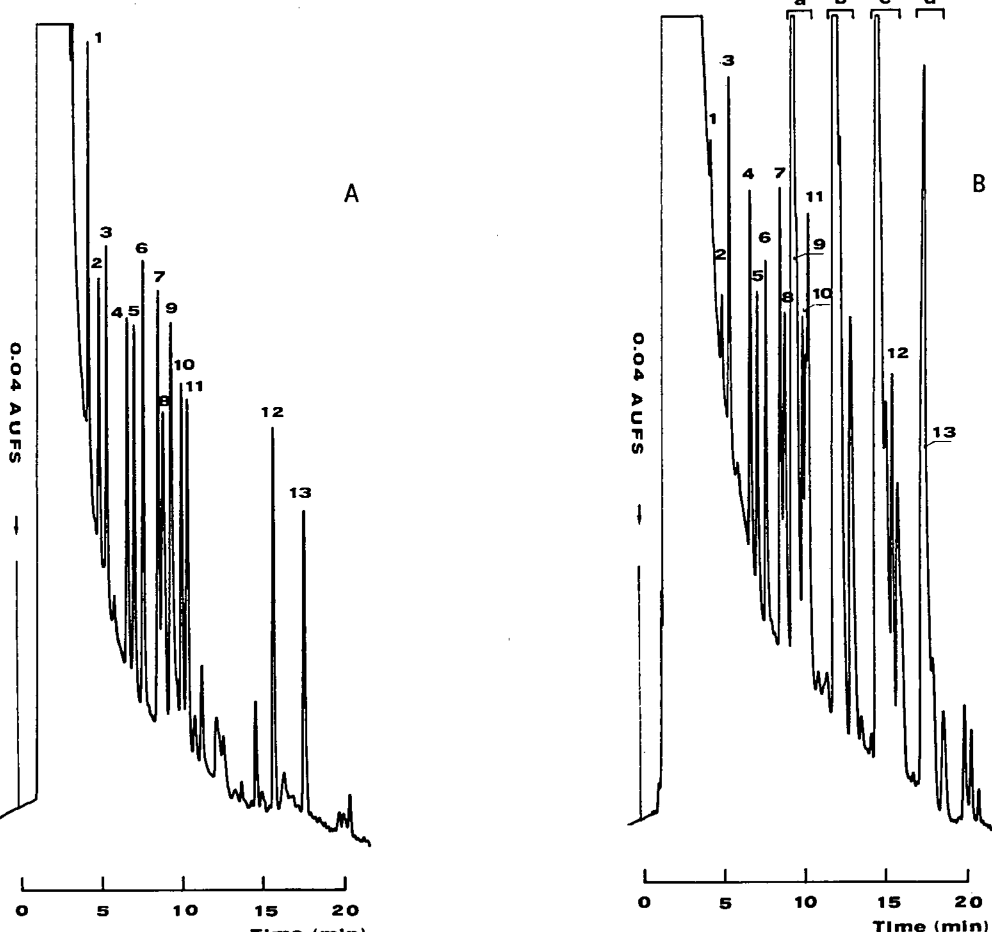

Fig. 2. Chromatograms obtained on analysing 0.5 l aliquots of river water (Tevere) spiked with 1–2 μg/l of thirteen selected acidic compounds and with 100 μg/l of a C_{10}–C_{13} LAS mixture by two procedures involving the use of (A) the GCB extraction cartridge and (B) a 0.5-g C_{18} cartridge. 1 = 2,4-DNPh; 2 = dicamba; 3 = bentazone; 4 = 2,4-D; 5 = DNOC; 6 = ioxynil; 7 = mecoprop; 8 = warfarin; 9 = 2,4,5-T; 10 = 2,4-DB; 11 = 2,4,6-TCPh; 12 = dinoseb; 13 = PCPh; a = C_{10} LAS; b = C_{11} LAS; c = C_{12} LAS; d = C_{13} LAS.

as that involving the C_{18} extraction cartridge, resulted in overestimation of PCPh, because one peak for PCBs overlapped that for the phenol derivative.

In the second experiment, the determination of thirteen of the acidic compounds considered in a surface water sample contaminated by LAS was simulated. This was done by artificially contaminating a surface water sample with both the thirteen acidic compounds at the individual level of 1–2 μg/l and with 100 μg/l of the C_{10}–C_{13} LAS mixture, and assaying 0.5-l aliquots of the sample by the same two procedures described above. On comparing the resulting two chromatograms shown in Fig. 2, it appears that only the procedure involving the use of the GCB cartridge was suitable for determining all of the thirteen target compounds present in a water sample containing LAS. This result was achieved by selecting as the phase modifier formic acid, which is able to elute only those acids having pK_a values higher than that of sulphonated compounds. In fact, when using TMAOH as phase modifier for eluting acidic compounds from the GCB cartridge, a chromatogram similar to that obtained by the analytical procedure making use of the C_{18} extraction cartridge was obtained. Finally, we observed that the GCB extraction cartridge was able to retain quantitatively both 2,4-DNPh and dicamba. These compounds were almost completely lost in the water effluent when using the C_{18} extraction cartridge.

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Selective determination of phenols in water by a two-trap tandem extraction system followed by liquid chromatography

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ABSTRACT

The ability of a two-trap tandem system, one containing 300 mg of graphitized carbon black (GCB) and the other filled with 50 mg of Sephadex QAE A-25 strong anion exchanger (SAX), to extract trace amounts of phenols from environmental waters and isolate them from base–neutral species was evaluated. After the water sample had passed through the GCB cartridge, the latter was connected to the SAX cartridge and base–neutral species were removed from the GCB surface by a neutral eluent. Co-eluted very weakly acidic phenols were selectively readsorbed on the SAX surface. Still maintaining the two cartridges in series, an acidified eluent was allowed to flow through the two cartridges to recover the most acidic phenols from the GCB cartridge and the least acidic phenols from the SAX cartridge. After partial removal of the solvent, the final extract was submitted to reversed-phase high-performance liquid chromatography with UV detection. Recoveries of seventeen phenols of environmental concern added to 2 l of drinking water at levels between 0.2 and 2 μg/l were higher than 90%. The effect of the presence in water of fulvic acids on the efficiency of the extraction device was assessed. In term of recovery, the two-trap tandem system was compared with other two single extraction cartridges, one containing a chemically bonded siliceous material (C_{18}) and the other SAX material. The limits of detection of the analytes considered were well below 0.1 μg/l.

INTRODUCTION

Phenols are toxic substances frequently occurring in the aquatic environment as a result of contamination from a variety of sources. Both the European Economic Community (EEC) and the US Environmental Protection Agency (EPA) include many phenol derivatives in the list of priority pollutants that should be monitored in environmental waters. This has prompted the development of various methods making use of gas chromatography [1–5] or liquid chromatography (LC) [6–10] for their separation and determination. Most of these methods, however, are not sufficiently sensitive to comply with a recent EEC Directive which sets maximum admissible individual concentrations of 0.1 μg/l for organic contaminants in drinking water. Also, many proposed analytical procedures are inadequate for monitoring traces of phenols in complex aqueous matrices, as they lack selectivity.

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Liquid–liquid extraction (LLE) is usually the technique of choice for extracting phenols from water samples. In recent years, in order to eliminate some well known drawbacks of the LLE technique, liquid–solid extraction (LSE) of phenols by various reversed-phase adsorbing materials [6,10,11–13] has been included in many analytical schemes. For determining phenols in complex aqueous matrices, binding the target analytes under alkaline conditions to an anion-exchange material has been proposed [14,15] for enhancing selectivity.

Graphitized carbon black (GCB) has proved to be a valuable adsorbing material for the LSE of pesticides [16,17] in aqueous environmental samples. GCB cartridges proved to be more efficient than commonly used octadecyl (C_{18})-bonded silica

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cartridges for the LSE of polar compounds, such as phenols [10], chloroanilines [18] and certain very hydrophilic pesticides [19]. Although GCB is known to behave as a natural reversed phase, it contains on its surface chemical heterogeneities able to bind anions via electrostatic forces [20]. In previous papers [16,17,21] we have shown that this singular feature of the GCB material can be advantageously exploited for rapidly and simply isolating acidic analytes from co-extracted base–neutral compounds by differential elution. When applied to phenols, however, base–neutral/acid fractionation by stepwise desorption failed to isolate from base–neutral species phenols having pK_a values higher than 7 [21].

Recently, selective determinations of triazine herbicides [22] and chloroanilines [19] in water have been achieved by a two-trap tandem system, one containing GCB and the other filled with a strong cation exchanger. The object of this work was to evaluate the ability of a two-trap tandem system, with one cartridge filled with a GCB and the other containing a conventional anion exchanger, to achieve in a simple and rapid manner the simultaneous extraction and isolation of phenolic compounds from water samples.

EXPERIMENTAL

Reagents and chemicals

Authentic phenols were obtained from various sources. Individual standard solutions were prepared by dissolving 100 mg of each phenol in 100 ml of methanol. For recovery studies, we prepared a working composite standard solution, whose composition was as follows with concentrations of the analytes in mg/l (in parantheses): guaiacol (20); *p*-nitrophenol (15); *p*-cresol (30); *o*-chlorovanillin (10); *o*-chlorophenol (25); 2,4-dichlorophenol (15); *o*-nitrophenol (15); 2,4-dimethylphenol (40); 2,4-dichlorophenol (20); 3,5-dibromo-4-hydroxybenzonitrile (bromoxynil) (15); 4,6-dinitro-*o*-cresol (25); 3,5-diiodo-4-hydroxybenzonitrile (ioxynil) (15); 2,4,6-trichlorophenol (30); 3,4,5-trichlorosyringol (25); 2,3,4,6-tetyrachlorophenol (25); 2-*sec*.-bytyl-4,6-dinitrophenol (dinoseb) (30); and pentachlorophenol (30).

Fulvic acids were kindly donated by Dr. A. Piccolo, who prepared them as reported elsewhere [23].

For HPLC, distilled water was further purified by passing it through a Norganic cartridge (Millipore, Bedford, MA, USA). Acetonitrile and methanol of gradient grade were obtained from Riedel-de Haën, Selze, Germany. All other solvents and reagents were of analytical-reagent grade (Carlo Erba, Milan, Italy). Trifluoroacetic acid (TFA) and tetramethylammonium hydroxide pentahydrate (TMAOH) were obtained from Aldrich (Milwaukee, WI, USA).

Phenols were desorbed from the two in-line traps by passing sequentially through them two suitable eluent systems, which will be called eluents A and B; eluent A was CH_2Cl_2–CH_3OH (60:40, v/v) and eluent B was 0.25 mol/l formic acid in CH_2Cl_2–CH_3OH (90:10, v/v).

Apparatus

A 300-mg GCB extraction cartridge, commercially referred to as Carbograph 1 (Carbochimica, Rome, Italy), was prepared and pretreated as reported elsewhere [16,17]. A 50-mg amount of Sephadex QAE A-25 (particle size 40–120 μm) (Aldrich) was packed in a plastic tube (6 cm × 0.5 cm I.D.) (Supelco, Bellefonte, PA, USA). The upper polyethylene frit (Supelco) was located about 2 mm above the exchanger bed to allow it to swell on passing basified water. The connection between the two cartridges was made with a suitable plastic adapter (Supelco). The strong anion-exchange (SAX) material was converted from the Cl^- to the OH^- form by washing it with 15 ml of 0.1 mol/l sodium hydroxide in water. The excess amounts of OH^- anions and water were eliminated by washing the exchanger bed with 2 ml of methanol.

The GCB cartridge was fitted into a side-arm filtration flask and liquids were forced to pass through the cartridge by vacuum (water pump).

Procedure

Aqueous samples were fortified with known volumes of the working composite standard solution of phenols. When analysing hypochlorite-containing tap water samples, hypochlorite was reduced in advance by adding 0.4 g/l of sodium sulphite to prevent oxidation of the analytes. Water samples were then shaken for 1 min and after about 10 min were poured into a glass reservoir that was connected to the GCB cartridge. Unless they contained algae and

debris, which were eliminated by filtering the water sample through Whatman GF/C glass-fibre pads (pore size 10 μm), river water samples were extracted as collected after adding phenols to them. Water was forced to pass through the cartridge at flow-rates of 110–130 ml/min. Just after the sample had passed through the column, the cartridge was filled with 7 ml of HCl-acidified water (pH 3), which was allowed to pass through the cartridge at a flow-rate of 5–7 ml/min.

Following the passage of large volumes of water, some shrinkage of the sorbent bed may occur. In such an event, before washing with acidified water, the upper frit was pushed against the top of the sorbent bed. This expedient facilitates the subsequent removal of water from the extraction cartridge and improves the effectiveness of the eluent systems as they can permeate the sorbent bed more homogeneously.

After the acidified water had passed through the trap, most of it was removed by reducing to the minimum pressure in the flask for 30 s. The water pump was disconnected, 0.5 ml of methanol was poured into the cartridge, the pump was linked to the flask again and methanol was passed slowly through the sorbent bed to eliminate residual water. Thereafter, the GCB cartridge was connected to the SAX cartridge and 8 ml of the eluent phase A were poured into the GCB extraction cartridge (upper cartridge) and allowed to pass through the two cartridges at a flow-rate not exceeding 2.5 ml/min.

Eluent A leaving the SAX cartridge (lower cartridge) was discharged. If desired, this liquid phase could be submitted to a solvent reduction step for assaying non-acidic target compounds co-extracted with phenols from the water sample. As a consequence of the passage through the two cartridges of eluent A, phenols were distributed between the two cartridges. In particular, the most acidic phenols remaind still adsorbend on the GCB surface, while the least acidic phenols, washed away from the GCB cartridge together with non-acidic compounds, were readsorbed by the SAX cartridge. All of the phenols were eluted from the two sorbent beds by pouring 8 ml of eluent B into the GCB cartridge and allowing it to percolate through the two cartridges at a flow-rate not exceeding 2.5 ml/min. The last drops of eluent B were forced out of the two in-line traps by vacuum. The 8 ml of eluate was collected in a centrifuge tube with of *ca.* 1.4 cm I.D. The extract was basified by adding 0.52 ml of 3 mol/l TMAOH in methanol and then concentrated to about 320 μl in a water-bath at 30°C under a gentle stream of nitrogen. The methanolic solution of TMAOH, which served to basify the eluate prior to solvent reduction, was prepared weekly and stored in the dark at 4°C. When these precautions were not taken, some artifacts originated that produced chromatographic peaks, one of which overlapped that of bromoxynil. If the eluate evaporation step is terminated at volumes larger than that reported above, some methylene chloride may still be contained in the final extract. This solvent interferes with the subsequent separation and quantification by HPLC. After acidifying the solution with 170 μl of 6 mol/l HCl, the exact final extract volume was measured and 80 μl of it were injected into the HPLC system.

HPLC apparatus

A Model 5000 liquid chromatograph (Varian, Walnut Creek, CA, USA) equipped with a Rheodyne Model 7125 injector with a 100-μl loop and a Model 2550 variable-wavelength UV detector (Varian) was used with a 25 cm × 4.6 mm I.D. column filled with 5-μm LC-18 reversed-phase packing (Supelco). Phenols were chromatographed with premixed methanol–acetonitrile (10:90, v/v) containing 0.015% of TFA and water acidified with TFA (0.05%, v/v). TFA was stored in the dark at 4°C in order to avoid the formation of an artifact that produced a peak disturbing the determination of pentachlorophenol. The initial eluent composition was 34% organic modifier + 66% acidified water, which was increased linearly to 75% organic modifier after 24 min. The flow-rate was 1.5 ml/min. Phenols were detected with the UV detector set initially at 280 nm and then at 230 nm after 7.8 min.

The concentrations of the phenols in water samples were calculated by comparing the heights of the peaks obtained with the sample and with a standard solution. The latter was prepared by taking known and appropriate volumes of the working composite standard solution, by evaporating methanol and reconstituting the residue with 0.5 ml of water–methanol (60:40, v/v) acidified with HCl (pH 2).

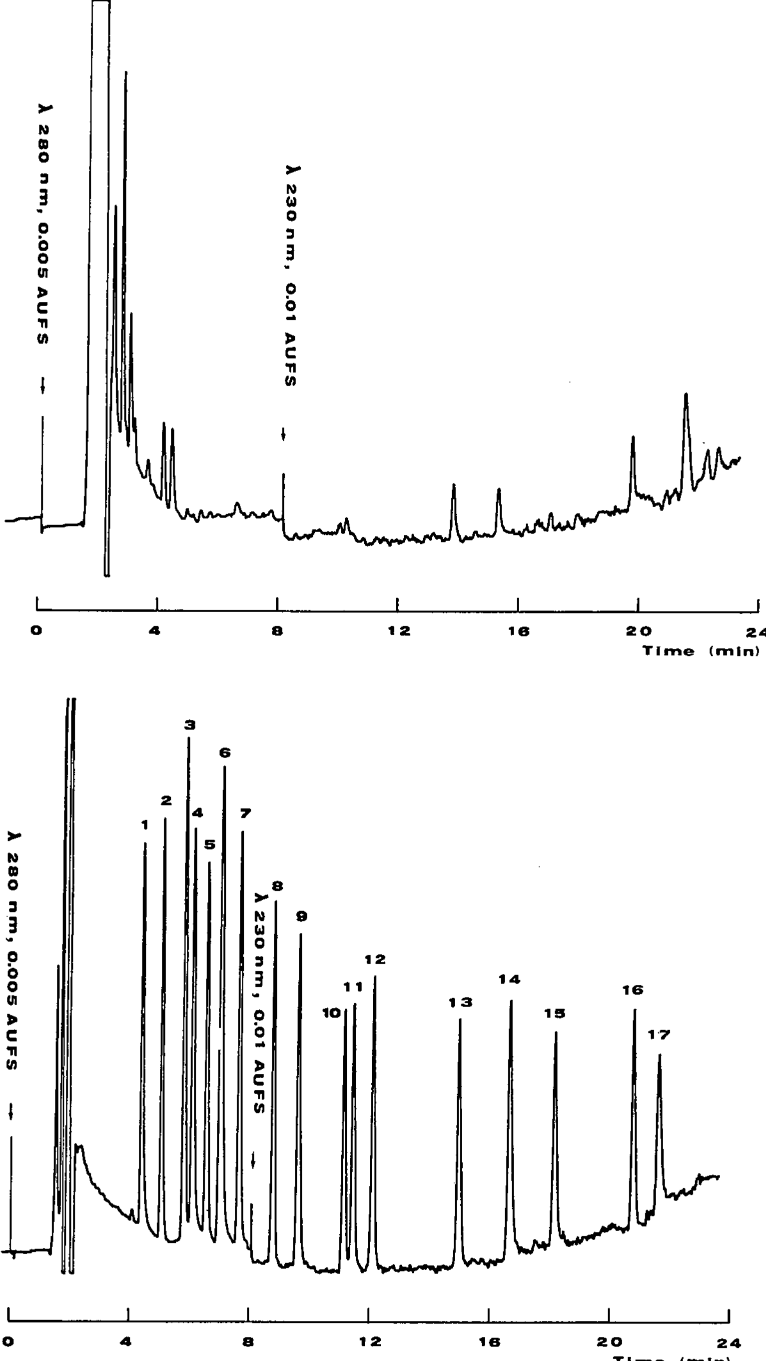

Fig. 1. Chromatograms of a typical overall blank procedure (top) and the working composite standard solution of phenols (bottom). Elution order (with amounts in ng of each phenol injected in parentheses): 1 = guaiacol (32); 2 = p-nitrophenol (24); 3 = *p*-cresol (48); 4 = *o*-chlorovanillin (16); 5 = *o*-chlorophenol (40); 6 = 2,4-dinitrophenol (24); 7 = *o*-nitrophenol (24); 8 = 2,4-dimethylphenol (64); 9 = bromoxynil (24); 10 = 2,4-dichlorophenol (32); 11 = 4,6-dinitro-*o*-cresol (40); 12 = ioxynil (24); 13 = trichlorophenol (48); 14 = trichlorosyringol (40); 15 = tetrachlorophenol (40); 16 = dinoseb (48); 17 = pentachlorophenol (48).

RESULTS AND DISCUSSION

Critical analytical variables

Originally, according to a previously reported method [21], the GCB cartridge was washed with slightly basified water (pH 8.1) after the water sample had passed through it. Under these conditions, however, about a 40% loss of 2,4-dimethylphenol was observed. In addition to benzpyrylium ions, we had experimental evidence for the existence of a few quinone groups on the GCB surface [20]. The partial loss of the 2,4-dimethylphenol may be explained by the fact that it is first oxidized by quinones under alkaline conditions and then added to them, according to a reaction mechanism reported elsewhere [24]. This problem was eliminated by replacing the alkaline wash with an acidic wash (pH 3).

Sorption of very weak acids on an anion exchanger under strictly anhydrous conditions probably takes place via salt formation or hydrogen bonding [25,26]. However, similarly to classical ion-exchange chromatography, a relatively long equilibration time is needed for the adsorption of eluates on the exchanger beads and their subsequent desorption. As a consequence, the broadening of the chromatographic band is strongly influenced by varying the flow-rate of the eluent phase. In order to avoid losses of the least acidic phenols, it is necessary that the flow-rates at which eluents B and especially A pass through the exchanger cartridge do not exceed 2.5 ml/min.

Fig. 1 shows chromatograms obtained by injecting an aliquot of the final extract relative to a blank overall procedure and an aliquot of the working composite standard solution. The extent of background interferences was calculated. When analysing 2 l of drinking water, the background for guaiacol was 25 ng/l whereas for the other phenols the background interferences were less than 4 ng/l.

Recovery studies

The ability of the GCB cartridge to retain phenols quantitatively on passing through the GCB cartridge increasing volumes (0.5, 1 and 2 l) of tap water spiked with the phenols considered at individual levels of 0.5–2 μg/l was evaluated. The recoveries obtained from three determinations for each water volume considered showed that only when 2 l of tap water were extracted was about 10% of guaiacol lost in the water effluent.

Fulvic acids (FA) represent up to 80% of the soluble organic carbon in environmental waters [27]. It has been reported [28] that the presence of relatively large amounts of humic substances in aqueous samples can make LSE cartridges less efficient in extracting target compounds. Saturation of sorptive sites by FA or formation of chemical complexes occurring between hydrophobic analytes and FA, which are scarcely retained by LSE cartridges, may be responsible for this failure.

The extent to which the efficiency of the proposed device for selectively extracting phenols was affected by the presence of fulvic acids in aqueous samples was assessed. For these experiments, two portions of a pure water sample were fortified with two different concentrations of FA, 5 and 10 mg/l, and with phenols at individual levels of 1–4 μg/l aliquots of 0.5 and 1 l of these two water samples were then analysed in triplicate. The results reported in Table I show that, among the phenols considered, the extraction efficiency of guaiacol, *p*-cresol and *o*-chlorophenol was to some extent affected by the presence of FA in the water samples. In order to account for the loss observed, both the water effluent from the GCB cartridge and eluent A which had passed through the Sephadex QAE cartridge were analysed. The amounts of the three phenols found in the water effluent completely accounted for the loss observed. Amog the phenols considered, the three phenols mentioned above have the highest mobilitis on the GCB cartridge. Therefore, it is conceivable that the incomplete adsorption of the three phenols was a result of the decrease in the number sorptive sites due to adsorption of FA rather than some kind of association occurring between phenols and FA with the formation of a complex having a low affinity for adsorption on the GCB surface. In our experience, surface water samples contain concentrations of FA lower than 10 mg/l. Anyway, when the GCB cartridge is used under field conditions, we recommend extracting volumes of water not larger than 0.5 l to monitor low-molecular mass phenols accurately.

Selectivity

Using the proposed extraction device, only acidic organic compounds can interfere with the subsequent HPLC analysis of phenols.

Linear alkyl benzenesulphonates (ALS) are wide-

TABLE I

RECOVERY OF PHENOLS FROM 0.5- AND 1.0-l ALIQUOTS OF WATER SAMPLES CONTAINING TWO DIFFERENT CONCENTRATIONS OF FULVIC ACIDS

Compound	Recovery (%)[a]			
	0.5 l		1 l	
	5 mg/l FA	10 mg/l FA	5 mg/l FA	10 mg/l FA
Guaiacol	93	86	81	69
p-Nitrophenol	98	100	101	98
p-Cresol	93	85	88	77
o-Chlorovanillin	97	97	99	87
o-Chlorophenol	98	85	93	76
2,4-Dinitrophenol	102	99	100	94
o-Nitrophenol	98	98	97	97
2,4-Dimethylphenol	97	98	98	97
Bromoxynil	99	99	100	100
2,4-Dichlorophenol	98	97	96	100
4,6-Dinitro-*o*-cresol	95	96	97	98
Ioxynil	99	100	100	101
2,4,6-Trichlorophenol	100	99	96	98
3,4,5-Trichlorosyringol	100	101	99	101
Tetrachlorophenol	100	101	99	101
Dinoseb	98	98	98	97
Pentachlorophenol	99	97	99	100

[a] Mean values obtained from triplicate measurements.

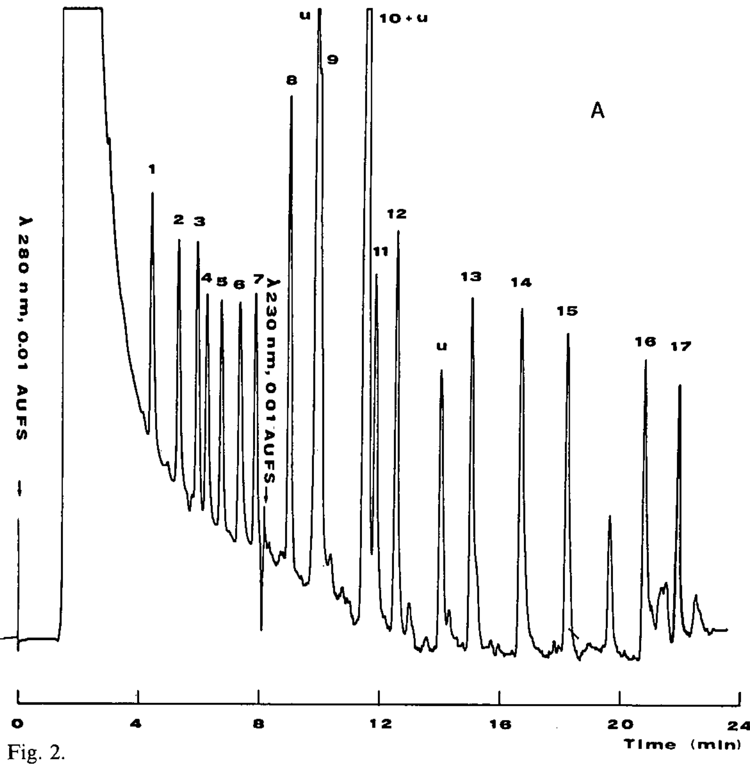

Fig. 2.

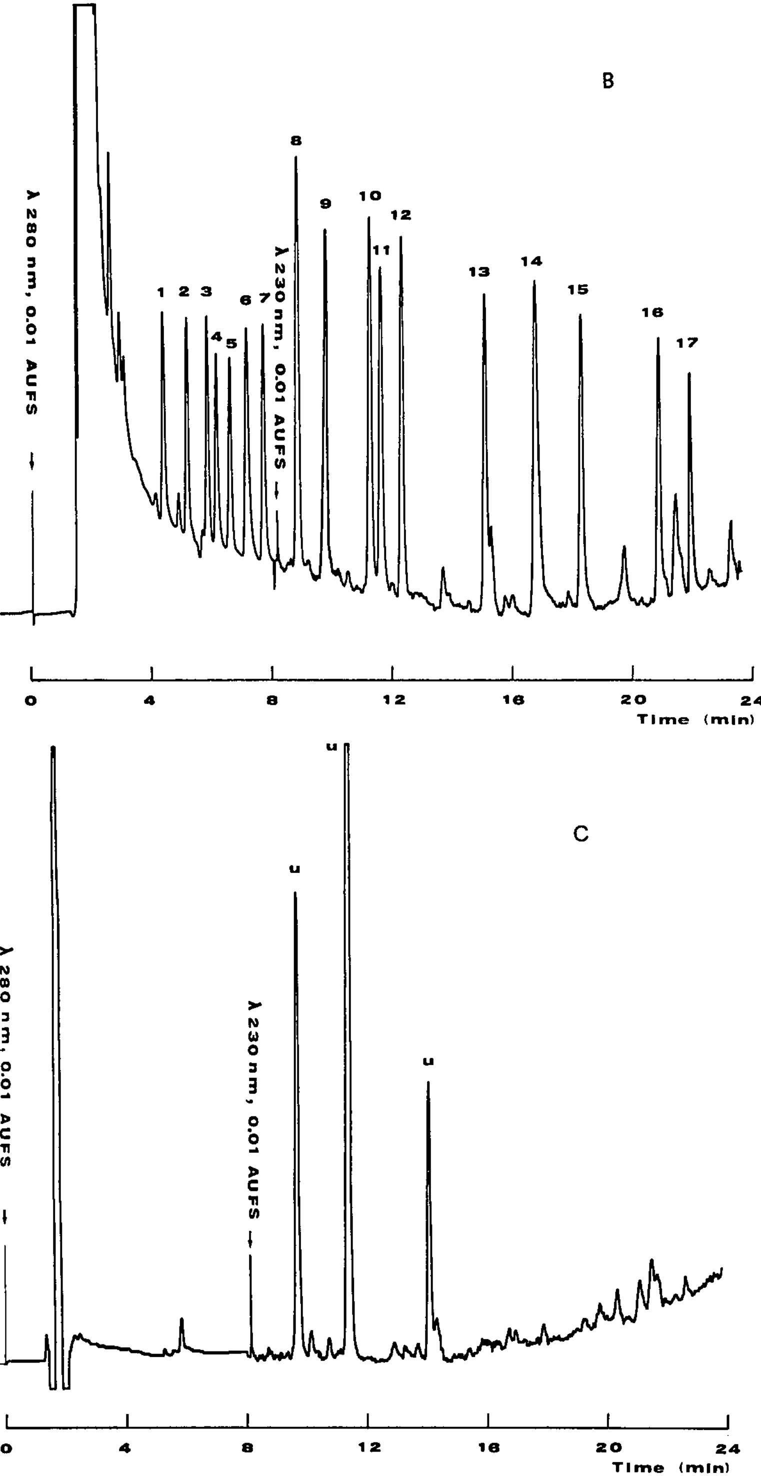

Fig. 2. Chromatograms obtained in analysing 2-l aliquots of a drinking (tap) water sample spiked with phenols at individual concentrations of 50–200 ng/l. Water was extracted with (A) the GCB cartridge alone and (B) the proposed method. The third chromatogram (C) resulted from injecting an aliquot of the base–neutral-containing extract. Peak numbers as in Fig. 1; u = unknown neutral compound.

ly used anionic surfactants and they frequently occur in aqueous environmental samples at levels ranging between 1 and 50 μg/l. LAS are co-extracted with phenols by the GCB cartridge, but the former are not eluted from it by eluent B, because formic acid contained in the mobile phase is unable to displace LAS from the positively charged sorption sites populating the GCB surface. Moreover, as reported elsewhere [21], LAS do not decrease the extraction efficiency of the GCB cartridge appreciably unless present in water at levels higher than 300 μg/l.

Among the most popular acidic pesticides, namely phenoxy acid derivatives, bentazone and dicamba, only the last compound can interfere with the determination of *o*-nitrophenol as these two compounds produced overlapping peaks.

As an example and to show the potential of this method in terms of selectivity and sensitivity, a tap water sample was spiked with phenols at individual concentrations of 50–200 ng/l and 2-l aliquots were analysed by the proposed method and by a conventional, non-selective method, such as that involving the use of a single GCB extraction cartridge for preparing the sample. In this case, the adsorbates were eluted from the GCB column by using only eluent B. Fig. 2 shows chromatograms obtained by the two procedures. As can be seen, the proposed method is less prone to positive bias than that involving the use of a single extraction cartridge.

Method comparison

The extraction efficiency of the two-stap assembly was compared with those obtained by using both a 0.5-g octadecyl-bonded silica (C_{18}) disposable cartridge (Supelco) and a 300-mg anion-exchange cartridge. The latter material was the same as that used with the GCB cartridge. The anion exchanger was converted into the OH^- form prior to use. For these experiments, 1-l aliquots of both a tap water sample and a sea-water sample were supplemented with phenols at individual concentration of 1–4 μg/l. Before extraction with the C_{18} cartridge, the pH of the water samples was adjusted to 2.5. Phenols trapped by the C_{18} cartridge were eluted with 6 ml of methanol. When using the conventional ion-exchange cartridge, the pH of the water was adjusted to 10.5 and the precipitated calcium salts were eliminated by filtration with a glass-fibre filter. After the water sample had passed through the ion-exchange cartridge, the latter was washed with 1 ml of methanol and phenols were eluted with 12 ml of eluent B, whose composition is reported under Experimental. The solvent reduction step was performed in the same way as that adopted in our method. No pH adjustment of the water samples was necessary when extracting with the GCB cartridge.

The recoveries reported in Table II show that the C_{18} cartridge failed, in both instances, to retain many of the phenols considered. Except for guaiacol, *p*-cresol and 2,4-dimethylphenol, the 300-mg anion-exchange cartridge exhibited a good capability to extract the other phenols considered from 1 l of water having a relatively low content of inorganic anions. In contrast, owing to competition of chloride ions with phenols for adsorption on the surface of the anion exchanger, poor recoveries of eight phenols were obtained when on 1 l of sea water. The extraction efficiency of the GCB cartridge was not affected by the ionic strength of the water sample. This positive feature of the GCB material has been extensively discussed elsewhere [21]. When extracting very weakly acidic phenols, it appears that the role played by GCB, when associated with a conventional high-capacity anion-exchange material, is mainly that of eliminating any inorganic ions so that the unique feature of the latter material can be fully exploited.

Recovery and precision

The recovery and the within-run precision of the proposed method with various concentrations of the seventeen phenols considered were assessed. A sample of tap water made 0.4 g/l in sodium sulphite was divided into two portions, which were spiked with the analytes at levels of 3–12 and 0.1–0.4 μg/l. Each portion was divided into six 2-l aliquots, each of which was analysed by the procedure. The results showed that the recovery of all the phenols was independent on their concentrations, demonstrating the absence of any adverse effect of irreversible adsorption by the materials composing the extraction apparatus. The relative standard deviations (R.S.D.s) at concentrations of 0.1–0.4 μg/l ranged from 2.36% for *p*-nitrophenol to 6.45% for guaiacol, and at 3–12 μg/l the R.S.D.s ranged from 0.63% for *p*-nitrophenol to 1.97% for *p*-cresol.

TABLE II

RECOVERY OF PHENOLS FROM 1 l OF TAP WATER AND SEA WATER BY THE PROPOSED METHOD COMPARED WITH THOSE FROM TWO OTHER EXTRACTION METHODS

Compound	Recovery (%)[a]					
	Anion exchanger		C_{18}		This method	
	Tap	Sea	Tap	Sea	Tap	Sea
Guaiacol	12	5	3	8	98	93
p-Nitrophenol	96	56	6	9	99	98
p-Cresol	27	7	8	10	97	92
6-Chlorovanillin	94	33	20	28	100	99
o-Chlorophenol	97	14	6	11	98	95
2,4-Dinitrophenol	98	95	7	13	96	98
o-Nitrophenol	93	47	7	9	97	97
2,4-Dimethylphenol	44	14	20	36	98	97
Bromoxynil	97	89	68	84	101	101
2,4-Dichlorophenol	94	73	20	32	99	100
4,6-Dinitro-*o*-cresol	98	93	40	56	95	95
Ioxynil	102	96	101	98	100	100
2,4,6-Trichlorophenol	98	89	88	85	98	98
3,4,5-Trichlorosyringol	99	90	93	94	96	97
Tetrachlorophenol	100	93	98	98	99	100
Dinoseb	99	94	96	97	101	101
Pentachlorophenol	101	97	99	100	97	97

[a] Mean values obtained from triplicate measurements.

Application to environmental samples

The affects that unknown compounds and FA dissolved in surface water samples can have on the recovery of the seventeen phenols considered were evaluated. Aliquots of 0.5 l of eight different river water samples (3.5–7.4 mg/l dissolved organic carbon) collected from various rivers flowing between Florence and Rome were spiked with phenols at individual levels of 1–4 μg/l and analysed. For the sake of conciseness, in Table III we reported accuracy data relating only to those three phenols (see above) whose recoveries are affected by the content of FA in water.

TABLE III

ACCURACY OF THE PROPOSED METHOD FOR THE DETERMINATION OF SELECTED PHENOLS IN RIVER WATER SAMPLES (n = 8)

Spike level: 2–3 μg/l.

Compound	Recovery (%)	Range (%)	R.S.D. (%)
Guaiacol	98.3	91–103	
p-Cresol	93.2	91– 98	
o-Chlorophenol	97.8	93–103	3.93

Limit of detection

Under the chromatographic conditions selected and extracting 2 l of drinking water, the limits of detection (signal-to-noise ratio = 3) for the seventeen phenols considered were between 2 ng/l (chlorovanillin) and 15 ng/l (pentachlorophenol).

Reusability of the LSE cartridge

The reusability of the SAX cartridge was evaluated by carrying out repeated analyses of phenols in tap water by making use of the extraction device that was renewed each time by changing only the GCB cartridge. The SAX cartridge was reactivated each time as reported under Experimental. After twenty such analyses the recovery of the phenols considered was unchanged within the precision of the method. The same experiments were conducted

with a GCB cartridge by repeatedly extracting phenols from 2-l aliquots of tap water. After each extraction, the GCB bed was restored with 3 ml of methanol and 5 ml of water. After five such extractions, the recovery of the analytes considered had not charged significantly, but the permeability of the columns was decreased to some extent, probably owing to suspendend particles in the water that plugged the upper frit. Such experiments were not performed with surface water samples.

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Re-evaluation of solid-phase adsorption and desorption techniques for isolation of trace organic pollutants from chlorinated water

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ABSTRACT

Amberlite XAD resin and activated carbon columns were tested for their abilities to concentrate trace organic pollutants in chlorinated water. Both XAD-2 and XAD-7 resin columns (20 ml) were capable of adsorbing about 30% of total organic halogen (TOX) present in 20 l of drinking water (pH 7) containing about 100 μg/l of TOX, whereas the carbon column (10 ml) adsorbed over 90% of TOX. The adsorption capacity of XAD-7 resin was found to be strongly dependent on the solution pH, as compared with those of XAD-2 and carbon adsorbents. Soxhlet and sonication extractions were also evaluated for their abilities to recover the adsorbed organics from the adsorbents, by measurements of TOX, chromatographable compounds and mutagenicity in the eluates. Soxhlet extraction gave higher recoveries than sonication, as measured with the above indices, but these differences were generally small (*ca.* 20%), with exception of the carbon extracts. The XAD-2 and XAD-7 extracts of drinking water also showed about 3–4 times higher mutagenic activity than the carbon extracts.

INTRODUCTION

Chlorination is widely used in wastewater and water treatment plants to control harmful microorganisms in water, and in several bleaching steps in the pulp and paper industry. However, this is now considered as a major source of organic halogens, one class of which is called as purgeable organic halogens (POX), including trihalomethanes (THMs), and another non-purgeable organic halogens (NPOX), found in drinking water [1,2] and natural waters [3,4]. Although THMs are of concern, because of both their effects on health [5–7] and their significant levels in water, recent work has shown that the even higher levels of NPOX can be formed from chlorination of the organic compounds in natural water [8–13]. Because it has also been shown that the mutagenic character of drinking water is more closely associated with the concentrations of NPOX [7,14–16], it seems reasonable to consider not only the occurrence of THM but also of NPOX in chlorinated water. However, no simple and accurate method exists for the identification and determination of the major individual NPOX that are formed as the result of chlorination.

The potential health effects of organic contaminants in chlorinated water can be studied only after they have been isolated from the water and identified. However, because most of these compounds are present at micrograms per litre (ppb) levels or less in water, a concentration step is necessary prior to the identification of individual contaminants. Conventional techniques used for isolating organic compounds from the water for analytical purposes include liquid–liquid extraction [17–20], carbon adsorption [16,21–25] and resin adsorption [21,25–32]. These methods, like other concentration procedures, fail to provide a totally representative concentrate as one or more groups of organic compo-

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nents are not recovered effectively. Consequently, systematic evaluation of different extraction methods for several group parameters in water is important in developing an effective concentration procedure.

The aim of this study was to re-evaluate common solid-phase extractions for isolating trace organic pollutants from chlorinated water, using XAD-2 and XAD-7 resins and activated carbon. The adsorption capacity of these water pollutants on each adsorbent and the desorption efficiency of these compounds using Soxhlet and sonication extraction were evaluated by measurements of different indices such as TOX, chromatographable compounds and mutagenicity.

EXPERIMENTAL

Materials

Organic solvents (acetone, *n*-hexane, and methanol) were of analytical-reagent grade for pesticide residue analysis (Wako, Osaka, Japan). They were checked for purity by evaporating 100 ml to 100 μl followed by gas chromatographic (GC) and TOX analyses. High-purity water obtained from a Milli-Q purifier system (Millipore) was used throughout. Chlorinated humic solution as humus-rich model water was prepared by treatment with hypochlorite of humic acid (5 mg/l as total organic carbon; Fluka, Buchs, Switzerland) at a Cl:C molar ratio of 5 and at pH 7 for 24 h. The residual chlorine was removed by addition of an equivalent volume of sodium thiosulphate solution. The concentrations of TOX in the chlorinated humic solution were determined by with a TOX analyser.

The adsorbents, XAD-2 and XAD-7 resins (20–50 mesh) (Rohm and Hass, Philadelphia, PA, USA) and activated carbon (AMF QUNO), were commercially available. Fines were removed by decanting after slurrying in water. The resin and carbon adsorbents were washed in a Soxhlet extractor with acetone–*n*-hexane (50:50, v/v) for 24 h, in order to remove interferences from the adsorbents. During the cleaning, a portion of the solvent was evaporated and checked for interferences by GC. If necessary, the solvent washing in the Soxhlet extractor was repeated. When the blank chromatogram showed no interferences, the adsorbents were removed from the extractor. The solvent remaining on the adsorbents was then evaporated completely in a vacuum desiccator for 24 h. The purified adsorbents were stored under methanol.

Adsorption and desorption tests

The cleaned XAD-2 (20 ml), XAD-7 (20 ml) and carbon (10 ml) materials were separately packed in small-scale glass columns (15 cm × 2 cm I.D.) equipped with a No. 2 glass filter and a PTFE stopcock. Before processing a 20-l water sample, the column was washed with a 2 l of water. Drinking water or the chlorinated humic solution was introduced bottom-to-top into the column, which was connected directly to the water tap in the laboratory or to the water tank (20 l). Water samples were continuously passed through the column at room temperature at a flow-rate of 2 bed volumes/min. An SF-160 fraction collector (Toyo, Tokyo, Japan) was used for collection of water fractions to measure breakthrough curves. The adsorption efficiencies of the adsorbents were investigated by monitoring the concentrations of TOX in the effluent fractions.

After processing a 20-l sample of the chlorinated water, the column was washed with 500 ml of 0.08 *M* sodium nitrate solution to remove residual chloride ions on the adsorbent. The remaining water in the column was gently evaporated at room temperature under a light stream of dry nitrogen. Each adsorbent was removed from the column and the organic substances on the adsorbent were then extracted by sonication with 20 ml of acetone–*n*-hexane (50:50, v/v) for the small-scale column. The sonication extractions were repeated twice more with a fresh 20-ml volume of the solvent mixture. Soxhlet extractions were also performed using the same mixed solvent for 4 h, in order to remove the organic substances from each adsorbent. These extracts were dried over anhydrous sodium sulphate and evaporated to dryness by means of a rotary evaporator at 40°C. The dry concentrate was dissolved, as rapidly as possible, in 2 ml of diethyl ether or methanbol, producing a concentration factor of 10^4, and stored in a refrigerator at 4°C until the subsequent analyses were performed.

A large-scale glass column (16 cm × 4 cm I.D.) equipped with a No. 2 glass filter and a PTFE stopcock, which was packed with 200 ml of each cleaned adsorbent, was also used for the isolation of trace organic pollutants in drinking water [33], in order

to study their mutagenic activities and chemical characteristics.

Organic halogen determination

The activated carbon–microcoulometric method was carried out with a Mitsubishi Chemical TOX-10 organic halogen analyser to determine the TOX in the water samples and in the acetone–hexane extracts. The analytical procedures for TOX determinations in these samples were essentially the same as in a previous study [3]. The corresponding detection limits were *ca.* 5 μg/l for the carbon adsorption method and 2.5 μg/l for the mixed solvent extraction method.

Gas chromatography

The Soxhlet- and sonication-extracted organics were analysed using a Shimadzu GC-6A gas chromatograph equipped with a flame ionization detector [33,34]. A glass column (2 m × 2 mm I.D.) packed with 2% OV-1 on Uniport HP (60–80 mesh) was employed. The temperature of the column oven was increased from 80 to 260°C at 5°C/min. The carrier gas (nitrogen) flow-rate was 40 ml/min. A Shimadzu Chromatopac-1A data system was used to determine the retention times and peak areas on the chromatograms.

Mutagenicity tests

The mutagenicity of the samples was tested according to the method of Ames *et al.* [35] with minor modifications. *Salmonella typhimurium* strain TA 100 was used through the experiments in the absence of metabolic activation, because most of the extracts of chlorinated water have been shown to be active in this system [25]. The samples were dissolved in methanol and pre-incubated with the strain at 37°C for 30 min (prior to plating). After addition of the test samples, the plates were incubated at 37°C for 2 days. The assay was performed in triplicate for each sample. The mutagenic activity is expressed as the mean value of mutagenicity ratios (revertants of sample/revertants of control).

RESULTS AND DISCUSSION

Comparison of the adsorption capacities of XAD resins and carbon

The adsorption of organic compounds from aqueous solution on XAD resins and activated carbon has been extensively used for the study of volatile organic compounds. Compounds evaluated in previous studies include pesticides [36–39], polycyclic aromatic hydrocarbons [40–43], polychlorinated biphenyls [44] and chlorophenols [45–47]. Although the recovery efficiency of these volatile organics by resin adsorption has been well established, little information is available on the non-volatile organics in chlorinated water, which are not amenable to GC. The capacities of XAD-2, XAD-7 and activated carbon to adsorb the non-volatile organics were therefore determined by TOX measurements in aqueous samples, because the concentrations of TOX have been shown to be closely associated with the mutagenicity of chlorinated drinking water [15,16].

Fig. 1 shows the breakthrough curves of TOX in chlorinated humic solution (pH 7) and drinking water (pH 7) on small-scale resin and carbon columns at a flow-rate of 2 bed volumes/min. The greater capability of the carbon column to adsorb TOX from the chlorinated humic solution (1000 μg/l of TOX) and drinking water (100 μg/l of TOX) was observed even after passing water sample of 20 l. In contrast, only 30% of the original TOX (100 μg/l) present in drinking water could be adsorbed on the two resin columns when a 20-l sample of water was passed through.

Because the XAD resins are non-ionic in nature, ionic organic compounds are adsorbed more efficiently on the resins at a pH at which ionization is suppressed, while neutral compounds are adsorbed independent of pH. Therefore, at normal drinking water pH, which is generally neutral, acidic organic compounds are ionized, whereas at pH 2, ionization is suppressed and they are retained more efficiently. In order to confirm this fact, subsequent experiments were conducted to determine the capacity of the resins and carbon columns to adsorb the organic halogens under the various pH conditions and using chlorinated waters containing 100 and 1000 μg/l of TOX.

Table I shows the influence of solution pH on the adsorption on the small-scale XAD-2, XAD-7 and carbon columns of TOX in 2 l of a chlorinated water sample after passing it through the column at a flow-rate of 2 bed volumes/min. A decrease in the solution pH resulted in an increase in the amounts

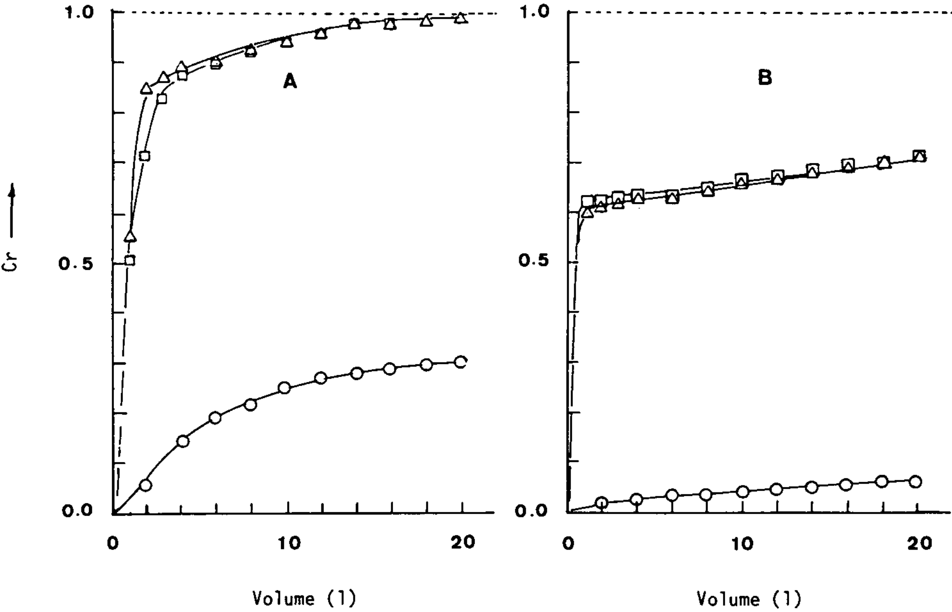

Fig. 1. Breakthorough curves of total organic halogen (TOX) on activated carbon (10 ml) and XAD resins (20 ml), measured as relative concentrations of TOX in aqueous effluent fraction. (A) Chlorinated humic solution (TOX = 1000 μg/l and pH 7); (B) drinking water (100 μg/l and pH 7). $C_r = C_e/C_i$, where C_e and C_i are the concentrations of TOX in the effluent and influent, respectively. △ = XAD-2; □ = XAD-7; ○ = carbon.

of adsorbable TOX on these adsorbents. Especially the capability of the XAD-7 column to adsorb TOX in drinking water was found to be strongly dependent on the solution pH, in contrast to those of XAD-2 and carbon adsorbents. About 90% of the original TOX present in the 2-l water sample was adsorbed on the XAD-7 resin at pH 2, whereas at pH 7 only 50% of the TOX was retained on this resin. This phenomenon agrees with the observation that certain mutagenic substances could be adsorbed on resins after acidification of water samples [26,30,32,48]. It is also known that XAD-2 has a

TABLE I

EFFECT OF THE SOLUTION pH VALUES ON ADSORPTION ON XAD RESIN AND ACTIVATED CARBON COLUMNS OF TOTAL ORGANIC HALOGEN (TOX) IN WATER

Volume of 2 l of chlorinated water samples were separately passed through each column at a flow-rate of 2 bed volumes/min at room temperature. The amounts of TOX adsorbed on each packing material were calculated from measurements of TOX concentrations in the effluent and influent.

Water sample	Packing material	Adsorption efficiency of TOX (μg per column)			
		pH 2	pH 4	pH 6	pH 7
Drinking water (100 μg/l of TOX)	XAD-2 (20 ml)	127 (63.5%)	119 (59.5%)	112 (56.0%)	100 (50.0%)
	XAD-7 (20 ml)	179 (89.5%)	153 (76.5%)	120 (60.0%)	100 (50.0%)
	Carbon (10 ml)	200 (100%)	198 (99.0%)	194 (97.0%)	190 (95.0%)
Chlorinated humic solution (1000 μg/l of TOX)	XAD-2 (20 ml)	1090 (54.5%)	1040 (52.0%)	920 (46.0%)	800 (40.0%)
	XAD-7 (20 ml)	1350 (67.5%)	1260 (63.0%)	1170 (58.5%)	960 (48.0%)
	Carbon (10 ml)	2000 (100%)	1980 (99.0%)	1940 (97.0%)	1900 (95.0%)

high affinity for non-polar compounds [26], whereas XAD-7 has been found to be more efficient in the recovery of polar compounds such as humic acids [49]. Hence it seems that 40% of the XAD-7 resin-adsorbable TOX present in drinking water at pH 2 consists of ionic organic halogens and about 50% non-polar halogenated organics.

Comparison of Soxhlet extraction and sonication techniques for isolation of organic substances from the adsorbents

The most popular method for the elution of adsorbed compounds is direct addition of diethyl ether to the wet column followed by a 10-min penetration period before drawing off the solvent. However, our experience showed that the in-column elution procedure with any organic solvent did not give uniformly high recoveries of organic materials from the adsorbents. Therefore, the classical Soxhlet elution method and sonication extraction for isolating the adsorbed organics on the XAD resins and carbon were performed using acetone-*n*-hexane (50:50, v/v) as the eluting solvent. The desorption efficiencies were evaluated for both extraction methods by measurements of TOX, chromatographable compounds and Ames mutagenicity of the eluates.

TOX. Table II shows the efficiency of recovery of the adsorbed organics on XAD-2, XAD-7 and activated carbon using the Soxhlet and sonication techniques, evaluated by measurements of TOX in the eluates. Before Soxhlet and sonication extractions are performed, 20 l of chlorinated humic solution containing 1000 μg/l of TOX were separately passed through the column at a flow-rate of 2 bed volumes/min. A low recovery efficiency of $<10\%$ for the adsorbed TOX from these adsorbents was observed with both extraction systems. In particular, a poor recovery of the adsorbed TOX from the carbon adsorbent was observed when sonication extraction was performed. However, Soxhlet extraction was more effective in recovering the adsorbable TOX than the sonication technique, with the highest efficiency from XAD-7 resin. These results are in agreement with those reported previously [50].

Chromatographable compounds. On the basis of TOX measurements, subsequent investigations were performed to determine the chromatographable compounds in the Soxhlet and sonication extracts, using a packed column and flame ionization detection (FID). Fig. 2 shows the gas chromatograms of the sonication extracts from XAD-2, XAD-7 and carbon adsorbents after processing 20 l of the chlorinated humic solution containing 1000 μl/g of TOX. Over 40 compounds that respond to FID were detected in both resin extracts. In contrast, small numbers of compounds were found in the carbon extract even though the highest TOX content, most of which is likely to the chlorinated organic acids, had been detected in this extract (see Table II). Of note is the higher recovery of XAD resin-extractable organics with longer retention times in sonication extraction, as can be seen in Fig. 2.

Because of the complexity and the very low concentrations of these organic compounds, the exact nature of the individual compounds corresponding to each peak on the chromatograms was not estab-

TABLE II

EXTRACTION EFFICIENCY OF TOTAL ORGANIC HALOGEN (TOX) FROM XAD-2 (20 ml), XAD-7 (20 ml) AND ACTIVATED CARBON (10 ml) BY SONICATION AND SOXHLET TECHNIQUES AFTER PROCESSING OF 20 l OF CHLORINATED HUMIC SOLUTION (pH 7) CONTAINING 1000 μg/l OF TOX

The values are averages of three determinations.

Packing material	Amount of TOX (mg/per adsorbent)		
	Retained on the material	Sonication extractable	Soxhlet extractable
XAD-2	1.50 (100%)	0.05 (3.33%)	0.08 (5.33%)
XAD-7	1.60 (100%)	0.07 (4.37%)	0.12 (7.50%)
Carbon	12.80 (100%)	0.09 (0.70%)	0.34 (2.65%)

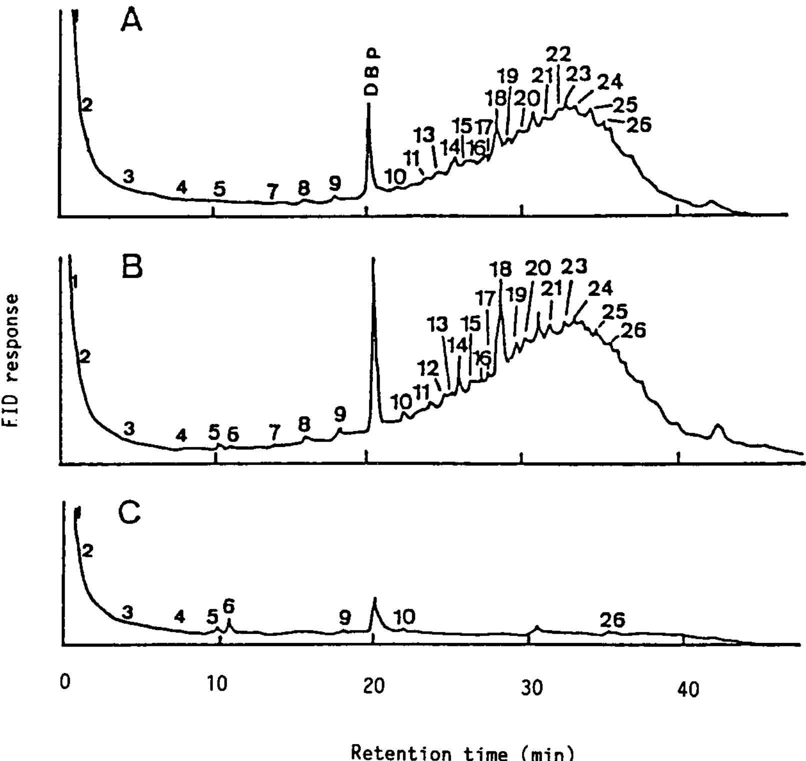

Fig. 2. Gas chromatograms (flame ionization detection) of acetone–n-hexane (50:50, v/v) extracts of chlorinated humic solution (1000 μg/l of TOX and pH 7) obtained through (A) XAD-2, (B) XAD-7 and (C) activated carbon adsorption and subsequent sonication extraction. Before the extraction, 20 l of the water sample were passed through the column at a flow-rate of 2 bed volumes/min. The temperature of the GC column (packed with 2% OV-1 on Uniport HP) was raised from 80 to 260°C at 5°C/min. A 5-μl volume of the extract, equivalent to 5 ml of chlorinated humic solution, was injected into the column. The numbered peaks were used for evaluation of each extract with respect to the concentrations of organic compounds in water.

lished. Consequently, the individual peak areas of compounds appearing on the chromatograms were measured using a Shimadzu Chromatopac-1A integrator and the amount of each compound in the extracts was then evaluated from the GC peak area, relative to the area of corresponding compounds in the XAD-7 extract.

Table III shows the extraction efficiency of the chromatographable compounds in chlorinated humic solution using XAD resin or activated carbon adsorption and subsequent Soxhlet or sonication desorption. The total amounts of chromatographable compounds in the XAD-7 extracts obtained by both Soxhlet and sonication extraction were found to be about 2 and 100 times greater than those observed for the XAD-2 and carbon extracts, respectively. Soxhlet extraction resulted in an increase in the amounts of chromatographable compounds, but the differences between Soxhlet and sonication extraction were not very large (*ca.* 20%), with the exception of the carbon extracts. Hence it seems that over 70% of the adsorbed organics obtained by Soxhlet extraction can be recovered from the XAD resins using sonication extraction.

Mutagenicity. On the basis of TOX and chromatographable compound analyses of the water concentrates, further experiments were conducted to determine the mutagenic acitvities of XAD-2, XAD-7 and activated carbon extracts obtained through Soxhlet and sonication extractions. Be-

TABLE III

EXTRACTION EFFICIENCY OF CHROMATOGRAPHABLE COMPOUNDS FROM XAD RESIN (20 ml) AND ACTIVATED CARBON (10 ml) COLUMNS

Before extraction, 20 l of chlorinated humic solution (pH 7) containing 1000 μg/l of TOX were passed through the column at a flow-rate of 2 bed volumes/min. The amount of each compound was calculated from the GC peak area relative to the area of corresponding compounds in the XAD-7–Soxhlet extract.

Compound		Amount detected (%)					
Peak no. in Fig. 2	Retention time (min)	XAD-7		XAD-2		Carbon	
		Soxhlet	Sonication	Soxhlet	Sonication	Soxhlet	Sonication
1	0.52	100	0	0	0	2	0
2	1.26	0	0	0	0	100	0
3	3.72	100	0	341	0	2655	0
4	7.77	100	0	100	0	146	0
5	10.16	100	63	65	17	70	15
6	10.87	100	23	132	5	58	43
7	13.96	100	33	77	25	59	0
8	15.79	100	100	79	76	69	0
9	17.96	100	81	84	57	21	3
10	22.06	100	18	100	29	44	6
11	23.89	100	77	69	41	14	0
12	24.56	100	0	0	0	71	0
13	25.19	100	100	97	61	0	0
14	25.73	100	88	54	39	12	0
15	26.42	100	89	67	56	8	0
16	26.86	100	93	61	50	9	0
17	27.46	100	77	73	40	43	0
18	28.56	100	86	48	47	1	0
19	29.12	100	94	41	29	9	0
20	29.76	100	96	40	35	1	0
21	31.39	100	100	54	54	0	0
22	32.26	100	89	53	30	2	0
23	32.89	100	100	70	68	0	0
24	33.33	100	100	40	40	1	0
25	34.39	100	92	100	90	0	0
26	35.26	100	48	100	52	59	15

TABLE IV

COMPARISON OF THE AMES MUTAGENIC ACTIVITY, TOX CONTENT AND GAS CHROMATOGRAPHABLE (GC) COMPOUNDS IN DRINKING WATER CONCENTRATES OBTAINED THROUGH SOLID-PHASE ADSORPTION AND SUBSEQUENT SONICATION EXTRACTION

A large-scale column packed with each adsorbent was used for the isolation of organic pollutants from 2000 l of drinking water at a flow-rate of 2 bed volumes/min. The values are averages of three determinations.

Packing material	Mutagenicity (revertants/l)	TOX content (μg/l)	GC compounds (GC counts/l)
XAD-2	138	2.17	84.7
XAD-7	134	3.25	97.2
Carbon	43	1.27	38.6

cause the extracts of the chlorinated humic solution showed a poor or scattered mutagenicity in the test strain, only drinking water concentrates obtained with a large-scale column were tested for their mutagenicities.

The Ames mutagenic assays and TOX and chromatographable compound determinations in the resins and carbon extracts of drinking water obtained by both Soxhlet and sonication extractions are summarized in Table IV. The recovery of mutagenicity from drinking water using XAD-2 and XAD-7 adsorption and subsequent sonication extraction was about 3–4 times greater than that of the carbon adsorption. The use of Soxhlet extraction provided an efficient recovery of mutagenicity from the activated carbon. However, the efficiency of recovery from the resins by Soxhlet extraction was not as high as that observed with the sonication technique. This can be explained in terms of the substantial destruction of mutagenic compounds on heating [25] and of the co-extraction of compounds that exhibits toxicity to the test strain during Soxhlet extraction procedure.

In connection with mutagenic activity in the drinking water concentrates, these extracts were also chromatographed using a packed column and FID. Fig. 3 shows the gas chromatograms of XAD resin and carbon extracts obtained through sonication extraction. A large difference between the compositions of chromatographable organics present in drinking water and chlorinated humic solution is seen in the chromatograms in Figs. 2 and 3. Several compounds with relatively shorter retention times were detected on the chromatograms of the resin

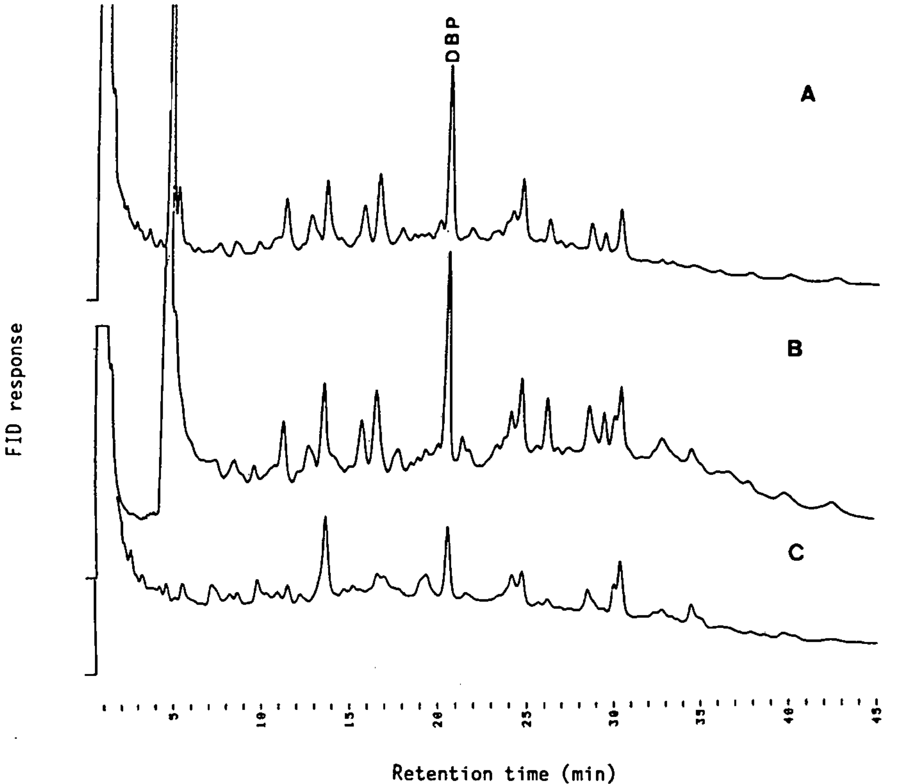

Fig. 3. Gas chromatograms (flame ionization detection) of acetone–n-hexane (50:50, v/v) extracts of drinking water (TOX = 100 μg/l and pH 7) obtained through (A) XAD-2, (B) XAD-7 and (C) activated carbon adsorption and subsequent sonication extraction. GC column temperature programme as in Fig. 2. A 5-μl of volume of the extract, equivalent to 5 l of drinking water, was injected into the column.

extracts of drinking water, whereas compounds with relatively longer retention times were present in the resin extracts of the chlorinated humic solution. This indicates the presence of not only naturally occurring organics, similar to humic acids, but also man-made chemicals in drinking water. The presence of synthetic compounds such as phthalate esters and polyaromatic hydrocarbons in the mutagenic resin extracts of drinking water has been reported previously [33].

In this work, common solid-phase extractions for the isolation of trace organic pollutants in chlorinated water were compared by measurements of some group parameters. Because various organic halogen compounds with a wide polarity range are present in chlorinated water, it is not surprising that low recoveries of TOX from the resin and carbon adsorbents by both Soxhlet and sonication extractions was obtained [51]. Recent studies [52–55] have demonstrated that chemically bonded silica and graphitized carbon black are effective for the solid-phase extraction of trace organic substances from water. Therefore, a further investigation will be conducted to determine and compare the extraction efficiencies of these adsorbents.

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Evaluation of a solid-phase extraction system for determining pesticide residues in milk

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ABSTRACT

A simple and rapid procedure based on reversed solid-phase extraction with octadecylsilica was developed for determining chlorinated pesticide residues in milk. The need for an agent that breaks the fat globules was first investigated and the method variables were optimized on whole homogenized milk (3.6% fat content). Recovery experiments performed for 26 organochlorine pesticides at levels of 3–40 μg/l gave >80% recoveries for all the compounds. The method was validated and evaluated by comparison with two widely used liquid–liquid extraction methods. The performance was checked by analysis of a certified standard with natural low endogenous levels (CMR 187) and different kinds of milk (skimmed, 2%, powdered, evaporated and condensed) spiked with the 26 pesticides. The procedure was used to analyse 45 commercial milks in which the presence of organochlorine residues had been detected. The proposed method offers advantages such as low cost and simplicity and the fact that the extracts obtained do not require a purification step.

INTRODUCTION

Two off-line steps are usually necessary for isolating organochlorine pesticides (OCPs) from milk: (1) extraction of the organochlorine pesticides from interfering matrix components, which involves liquid–liquid partitioning with organic solvents [1–6] or solid-phase extraction [7–9]; and (2) separation of the pesticides from co-extracted matrix constituents by means of column chromatographic separation (*e.g.*, alumina [10], Florisil [11], gel permeation [12,13] or HPLC [14]) or with reagents such as sulphuric acid [15,16] or potassium hydroxide [17].

These traditional methods are time consuming and expensive because of the high cost of the solvents and adsorbents. In contrast to such conventional extraction procedures, on-line extraction and clean-up procedures have been described using normal solid-phase extraction [18], which allows an important decrease in the number of manual operations involved but has the disadvantage of requiring large amounts of solvents.

Reversed solid-phase extraction reduces the cost of the analysis, because it requires smaller volumes of solvents and the amounts of solid phase needed are small. The use of octadecylsilica to extract organochlorine pesticide residues in milk has only been partially investigated for homogenized milk (fat globules of diameter 1 μm) [19] and skimmed and 2% milk [20].

In order to enhance the advantages of reversed solid-phase extraction, we have developed a optimized method for the determination of organochlorine residues in different kinds of milk.

EXPERIMENTAL

Reagents

Dichloromethane, *n*-hexane, acetonitrile, 2-propanol, ethanol, isobutanol, methanol, toluene and light petroleum (b.p. 40–60°C) were of pesticide grade and sodium lauryl sulphate, Triton 100-X, cetrimide, sodium chloride, sulphuric acid, sodium oxalate and anhydrous sodium sulphate were analytical-reagent grade.

Preparative octadecylsilica (55–105 μm) was obtained from Waters–Millipore. A C_{18} Empore disc

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(47 mm diameter and 8 μm particle size) was purchased from Analytichem International.

Organochlorine pesticide standards were purchased from Promochem, Riedel de Haën and Aldrich. A certified standard of powdered milk (CRM 187) was supplied by the EEC Community Bureau of Reference (BCR).

Apparatus

The GC analyses were carried out on a Konik 3000 chromatograph equipped with a ^{63}Ni electron-capture detector and two fused-silica columns. One was the primary column (30 m × 0.25 mm I.D.), cross-linked with BP-5 (Scientific Glass Engineering), and the other was used as a confirmation column (30 m × 0.24 mm I.D.), coated with DB-17 (J & W Scientific). In both columns, 3 μl of sample were injected in the splitless mode and the splitter was opened after 0.7 min.

The temperature programme was 0.8 min at 50°C, increased at 30°C min^{-1} to 140°C, which was held for 2 min, then increased at 5°C min^{-1} to 280°C, the final temperature being held for 10 min.

Procedure

A 1-g amount of octadecylsilica was transferred into a 100 mm × 9 mm I.D. glass column fitted with a coarse frit (No. 3) and covered with a plug of silanized glass-wool. The microcolumn was treated with 10 ml of methanol and 10 ml of distilled water.

A 5-ml volume of milk plus 5 ml of water added of 10 ml of methanol were mixed by sonication in an erlenmeyer flask and passed through the microcolumn; a vacuum was applied. The microcolumn was washed twice with 10 ml of distilled water and the washings were discarded. The adsorbed residues were then eluted with 10 ml of organic solvent. The extract was concentrated at 45°C and 3 μl were injected into the gas chromatograph.

For milk powder, 1 g of the powder was reconsti-

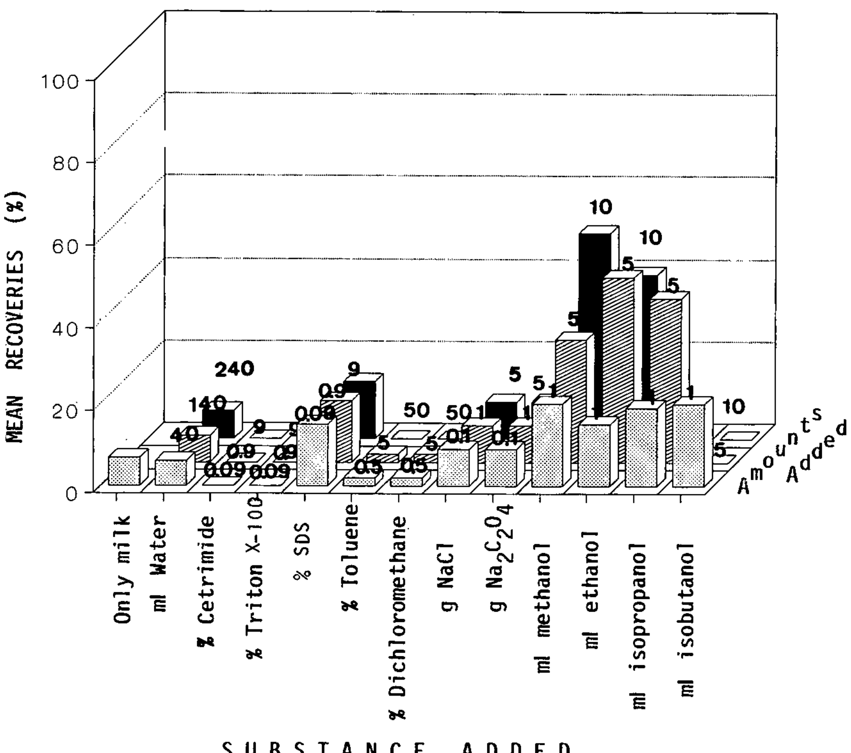

Fig. 1. Effect of different substances that can induce the rupture of fat globules. Experiments were performed using 10 ml of milk as sample, 0.5 g of C_{18} and eluting with *n*-hexane. SDS = Sodium dodecyl (lauryl) sulphate.

TABLE I

RECOVERY [MEAN ± R.S.D. (%) (n = 5)] OF ORGANOCHLORINE PESTICIDES FROM 10 ml OF MILK USING 0.5 g OF C_{18}, DIFFERENT VOLUMES OF METHANOL AND 10 ml OF n-HEXANE

Compound	Concentration in milk (μg l^{-1})	Volume of methanol (ml)						
		1	3	5	7	10	13	15
Aldrin	5	8 ± 10	22 ± 12	31 ± 11	49 ± 9	51 ± 6	50 ± 12	29 ± 14
Captafol	14	13 ± 13	18 ± 14	29 ± 8	54 ± 9	50 ± 5	49 ± 8	40 ± 10
Captan	7	14 ± 12	30 ± 11	36 ± 11	37 ± 10	35 ± 9	35 ± 4	14 ± 12
o,p'-DDD	6	12 ± 9	39 ± 12	42 ± 14	49 ± 12	47 ± 7	47 ± 12	28 ± 14
p,p'-DDD	9	10 ± 13	25 ± 12	38 ± 11	42 ± 12	42 ± 7	43 ± 14	33 ± 12
o,p'-DDE	7	7 ± 15	32 ± 13	41 ± 11	59 ± 10	64 ± 8	60 ± 12	46 ± 15
p,p'-DDE	7	5 ± 12	14 ± 10	23 ± 10	34 ± 11	33 ± 7	32 ± 9	19 ± 12
o,p'-DDT	9	25 ± 14	34 ± 12	44 ± 9	54 ± 12	55 ± 9	45 ± 12	25 ± 11
p,p'-DDT	9	12 ± 13	25 ± 10	34 ± 9	42 ± 16	59 ± 8	50 ± 9	35 ± 9
Dicofol	40	5 ± 10	12 ± 14	25 ± 16	44 ± 12	54 ± 8	53 ± 11	33 ± 10
Dieldrin	6	30 ± 14	40 ± 11	47 ± 12	60 ± 10	71 ± 9	70 ± 10	49 ± 15
α-Endosulfan	15	17 ± 14	30 ± 15	40 ± 12	47 ± 11	48 ± 10	48 ± 13	33 ± 13
β-Endosulfan	6	16 ± 14	36 ± 13	42 ± 9	50 ± 12	60 ± 8	60 ± 10	54 ± 12
Endosulfan sulphate	11	7 ± 9	26 ± 12	40 ± 11	49 ± 13	51 ± 7	42 ± 9	39 ± 12
Endrin	13	33 ± 10	50 ± 12	57 ± 12	74 ± 12	63 ± 8	62 ± 12	45 ± 13
Heptachlor	5	21 ± 12	25 ± 11	30 ± 10	35 ± 9	38 ± 9	39 ± 13	35 ± 12
Heptachlor epoxide	5	12 ± 13	23 ± 13	43 ± 12	47 ± 10	52 ± 10	55 ± 15	47 ± 11
α-HCH	5	49 ± 10	44 ± 11	41 ± 12	46 ± 11	50 ± 7	49 ± 10	36 ± 11
β-HCH	5	43 ± 14	44 ± 11	62 ± 11	65 ± 9	66 ± 7	60 ± 10	55 ± 12
δ-HCH	5	42 ± 12	46 ± 13	49 ± 11	49 ± 10	49 ± 8	49 ± 10	45 ± 12
γ-HCH	3	45 ± 12	44 ± 11	46 ± 14	48 ± 11	46 ± 10	44 ± 11	35 ± 12
BHC	3	19 ± 15	35 ± 10	40 ± 12	45 ± 13	49 ± 8	44 ± 14	30 ± 13
Isodrin	10	8 ± 12	27 ± 12	37 ± 14	42 ± 10	45 ± 9	39 ± 12	23 ± 14
Metoxichlor	16	22 ± 13	36 ± 12	43 ± 12	46 ± 11	50 ± 10	49 ± 12	34 ± 11
Mirex	8	12 ± 14	18 ± 15	21 ± 11	40 ± 15	50 ± 8	29 ± 10	12 ± 9
Tetradifon	8	15 ± 10	24 ± 10	39 ± 11	54 ± 13	61 ± 6	48 ± 12	25 ± 10

tuted with distilled water (1:9). For evaporated milk, 2 ml of the milk were mixed with 6 ml of distilled water. For condensed milk, 7 g were reconstituted with 18 ml of distilled water.

For recovery experiments, 0.1 ml of an ethyl acetate solution containing OCPs was added to milk and the mixture was left to stand overnight at 4°C. The sample was equilibrated to room temperature before proceeding with the above procedure.

RESULTS AND DISCUSSION

In order to optimize the extraction conditions, we first tested the follow mechanisms that can produce the rupture of the fat globule membrane: addition of water to 40, 240 and 490 ml; addition of surfactant, using different kinds of detergents, *viz.*, anionic (sodium lauryl sulphate), non-ionic (Triton 100-X) and cationic (cetrimide), and different concentrations in milk, *viz.*, 0.09, 0.9 and 9.9% (w/v); addition of different amounts (0.5, 5.0 and 50.0%, v/v) of non-polar organic solvents, such as toluene and dichloromethane, to milk; modification of the saline concentration, using different salts (NaCl and $Na_2C_2O_4$), and different concentrations (1, 10 and 50%, w/v) in milk; and addition of polar organic solvents, using methanol, ethanol, 2-propanol, isobutanol, acetonitrile, acetone and tetrahydrofuran, to different volumes (1, 5 and 10 ml).

Fig. 1 shows the results obtained as average recoveries of 26 organochlorine pesticides.

There are two mechanisms that do not provide

TABLE II

EFFECT OF SAMPLE SIZE ON RECOVERIES [MEAN ± R.S.D. (%) (n = 5)] FROM 0.5 g OF C_{18} USING 10 ml OF METHANOL AND 10 ml OF n-HEXANE AS ELUENT

Compound	Milk sample (ml)		
	2.5	5	10
Aldrin	53 ± 5	53 ± 6	51 ± 6
Captafol	42 ± 4	43 ± 6	40 ± 5
Captan	50 ± 6	42 ± 2	45 ± 9
o,p'-DDD	65 ± 8	65 ± 7	47 ± 7
p,p'-DDD	60 ± 10	55 ± 8	42 ± 7
o,p'-DDE	70 ± 5	66 ± 5	64 ± 8
p,p'-DDE	60 ± 8	56 ± 6	33 ± 7
o,p'-DDT	72 ± 7	65 ± 8	62 ± 9
p,p'-DDT	68 ± 10	69 ± 7	59 ± 8
Dicofol	90 ± 9	78 ± 10	62 ± 8
Dieldrin	83 ± 6	82 ± 9	71 ± 9
α-Endosulfan	78 ± 8	69 ± 9	48 ± 10
β-Endosulfan	81 ± 9	65 ± 2	60 ± 8
Endosulfan sulphate	72 ± 6	64 ± 7	51 ± 7
Endrin	79 ± 9	78 ± 9	63 ± 8
Heptachlor	52 ± 8	46 ± 9	38 ± 9
Heptochlor epoxide	80 ± 8	74 ± 9	52 ± 10
α-HCH	70 ± 9	64 ± 8	50 ± 7
β-HCH	89 ± 9	80 ± 6	66 ± 7
δ-HCH	78 ± 10	70 ± 9	49 ± 8
γ-HCH	67 ± 8	65 ± 6	46 ± 10
BHC	67 ± 8	66 ± 8	49 ± 8
Isodrin	70 ± 9	68 ± 10	45 ± 9
Metoxichlor	76 ± 11	69 ± 9	50 ± 10
Mirex	74 ± 9	68 ± 8	50 ± 8
Tetradifon	80 ± 10	73 ± 7	61 ± 6

better recoveries than those obtained when milk alone is used: addition of water and modification of the saline concentration. The former probably does not give good recoveries because the dilution of milk with water does not alter the fat globule structure. The latter destroys the fat globules when the liquid–liquid extraction method [6] is used but, in our case, did not give good results.

Although the addition of non-polar solvents has been proposed for C_{18} extraction from homogenized milks by Barcarolo *et al.* [19], our assays showed that it actually decreases the recoveries. One explanation for this could be that the diameter of the fat globules is larger in our case than in the previous work and the organic solvents cannot break them up, and moreover the residues are probably more soluble in the organic solvents than in octadecylsilica sorbent. With concentrations of 50% the mechanism cannot operate because two layers form.

Addition of surfactants yields the most variable results. With non-ionic and cationic surfactants, no organochlorine pesticides were recovered. With an anionic surfactant the recoveries were slightly better than without it.

The use of polar organic solvents has been reported for the selective extraction of OCPs from milk [1,9,20]. Acetone, acetonitrile and tetrahydrofuran are not suitable for solid-phase extraction because they yield a precipitate. Although alcohols increase the recoveries, the results are better when the

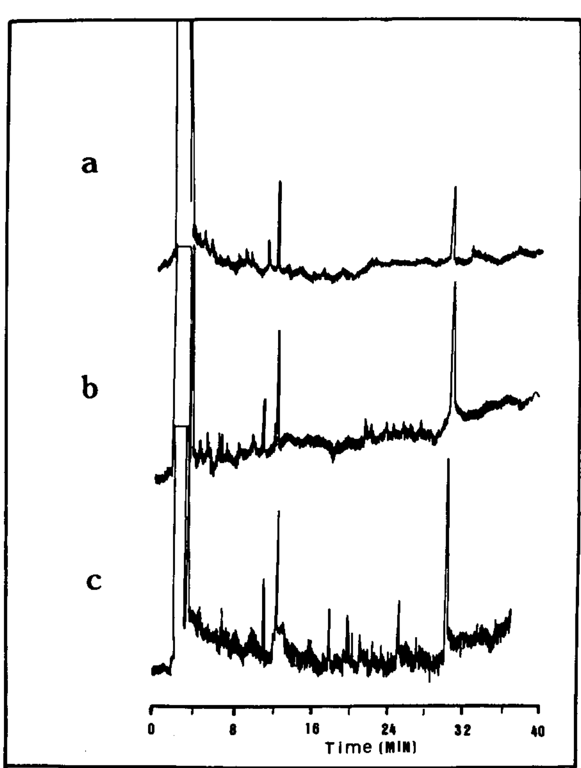

Fig. 2. Chromatograms of extracts obtained from unspiked milk with the eluents (a) n-hexane, (b) light petroleum and (c) n-hexane–light petroleum (1:1).

TABLE III

RECOVERY [MEAN ± R.S.D. (%) (n = 5)] OF ORGANOCHLORINE PESTICIDES FROM 5 ml OF MILK PLUS 10 ml OF METHANOL WITH 10 ml OF n-HEXANE AS ELUENT USING DIFFERENT AMOUNTS OF C_{18}

Compound	Amount of octadecylsilica (g)					
	0.1	0.3	0.5	0.7	1.0	1.2
Aldrin	18 ± 10	38 ± 12	53 ± 6	57 ± 6	58 ± 6	58 ± 6
Captafol	23 ± 14	28 ± 14	33 ± 6	45 ± 6	49 ± 5	48 ± 5
Captan	17 ± 10	36 ± 10	42 ± 2	43 ± 8	45 ± 9	44 ± 8
o,p'-DDD	22 ± 9	43 ± 9	65 ± 7	80 ± 9	87 ± 7	88 ± 4
p,p'-DDD	32 ± 12	45 ± 10	55 ± 8	82 ± 9	92 ± 7	93 ± 6
o,p'-DDE	33 ± 8	59 ± 9	66 ± 7	68 ± 10	84 ± 8	86 ± 5
p,p'-DDE	25 ± 12	44 ± 10	56 ± 6	74 ± 11	74 ± 7	78 ± 9
o,p'-DDT	32 ± 14	49 ± 10	65 ± 8	77 ± 8	85 ± 8	87 ± 8
p,p'-DDT	35 ± 13	56 ± 10	69 ± 7	83 ± 8	88 ± 7	90 ± 9
Dicofol	44 ± 12	62 ± 10	68 ± 10	72 ± 10	74 ± 7	75 ± 6
Dieldrin	50 ± 14	69 ± 11	82 ± 9	86 ± 9	91 ± 8	92 ± 5
α-Endosulfan	27 ± 10	55 ± 10	69 ± 9	87 ± 8	98 ± 9	99 ± 3
β-Endosulfan	36 ± 13	46 ± 13	65 ± 2	88 ± 8	90 ± 6	94 ± 8
Endosulfan sulphate	22 ± 9	52 ± 12	64 ± 7	87 ± 6	91 ± 6	92 ± 7
Endrin	33 ± 10	50 ± 12	78 ± 9	84 ± 7	93 ± 5	95 ± 9
Heptachlor	21 ± 12	35 ± 11	46 ± 9	65 ± 9	78 ± 6	72 ± 6
Heptachlor epoxide	33 ± 13	53 ± 13	74 ± 9	87 ± 9	92 ± 11	92 ± 10
α-HCH	49 ± 10	44 ± 11	64 ± 8	96 ± 11	100 ± 8	98 ± 7
β-HCH	43 ± 14	44 ± 11	80 ± 6	85 ± 9	96 ± 8	97 ± 9
δ-HCH	42 ± 12	46 ± 13	70 ± 9	82 ± 9	99 ± 7	99 ± 6
γ-HCH	45 ± 12	44 ± 11	65 ± 6	98 ± 6	106 ± 10	99 ± 8
BHC	19 ± 15	35 ± 10	66 ± 8	75 ± 13	99 ± 9	99 ± 8
Isodrin	8 ± 12	27 ± 12	68 ± 10	82 ± 10	95 ± 9	96 ± 8
Metoxichlor	22 ± 13	36 ± 12	69 ± 9	86 ± 9	90 ± 7	94 ± 8
Mirex	32 ± 14	58 ± 15	68 ± 8	69 ± 10	70 ± 9	72 ± 9
Tetradifon	45 ± 10	63 ± 10	73 ± 7	78 ± 9	82 ± 7	85 ± 10

molecular chain length is shorter because in this way the viscosity diminishes. For this reason, methanol was selected.

The effects of the volume of methanol, sample size, amount of C_{18} and eluent were tested and the results are given in Tables I, II, III and IV, respectively. The concentration of organochlorine pesticides in milk was kept constant throughout the test (see Table I). The recoveries reported are the means of five analyses.

When n-hexane, light petroleum or a mixture of the two are used as the eluent, they provide very similar recoveries. Light petroleum increases the recoveries of HCH isomers slihgtly and n-hexane increases the recoveries of the DDT isomers; mixing increases both. However, the difference is only about 5%. Fig. 2 shows chromatograms of unspiked milk obtained with each of the eluents.

The organic extract concentration was studied. The chromatograms obtained by injecting an extract of unspiked milk concentrated to different volumes are shown in Fig. 3.

The effect of the sample flow-rate though the C_{18} column (1, 5 and 20 ml min^{-1}) was also checked. No differences in the recoveries were observed.

Therefore, the optimum conditions for maximum recoveries of pesticides were established as 5 ml of milk, 5 ml of water and 10 ml of methanol mixed by sonication and passed through a microcolumn containing 1 g of C_{18}. Pesticides are eluted with 10 ml

TABLE IV

EFFECT OF THE ELUENT ON ANALYTE RECOVERIES [MEAN ± R.S.D. (%) (n = 5)] FROM C_{18} USING THE CONDITIONS INDICATED IN THE PROCEDURE

Compound	n-Hexane	Light petroleum	n-Hexane–light petroleum (1:1)
Aldrin	58 ± 6	68 ± 10	67 ± 8
Captafol	49 ± 5	38 ± 12	49 ± 9
Captan	45 ± 9	45 ± 8	45 ± 9
o,p'-DDD	87 ± 7	92 ± 8	90 ± 7
p,p'-DDD	92 ± 7	99 ± 9	101 ± 7
o,p'-DDE	84 ± 8	78 ± 5	85 ± 10
p,p'-DDE	74 ± 7	70 ± 6	76 ± 12
o,p'-DDT	85 ± 8	77 ± 8	87 ± 9
p,p'-DDT	88 ± 7	77 ± 8	89 ± 9
Dicofol	74 ± 7	98 ± 10	99 ± 8
Dieldrin	91 ± 8	89 ± 6	92 ± 7
α-Endosulfan	98 ± 9	94 ± 10	98 ± 8
β-Endosulfan	90 ± 6	86 ± 10	91 ± 5
Endosulfan sulphate	91 ± 6	89 ± 10	92 ± 11
Endrin	93 ± 5	104 ± 6	95 ± 6
Heptachlor	78 ± 6	95 ± 8	97 ± 10
Heptachlor epoxide	92 ± 11	94 ± 9	93 ± 11
α-HCH	100 ± 8	99 ± 8	102 ± 7
β-HCH	96 ± 8	101 ± 10	102 ± 9
δ-HCH	99 ± 7	101 ± 9	103 ± 10
γ-HCH	106 ± 10	102 ± 9	104 ± 10
BHC	99 ± 9	104 ± 7	102 ± 6
Isodrin	95 ± 9	96 ± 8	95 ± 9
Metoxichlor	90 ± 7	98 ± 8	99 ± 8
Mirex	70 ± 9	66 ± 8	72 ± 10
Tetradifon	82 ± 7	72 ± 7	85 ± 10

of n-hexane and concentrated to a volume of 0.5 ml.

Fig. 4 shows chromatograms of spiked milk, unspiked milk and blank analyses.

The proposed procedure was compared with a procedure using a C_{18} disc, but the small particle size (8 μm) was not appropriate for the sample, which could not pass through the pores.

To validate the method, whole milk and a certified standard of milk powder were analysed according to the present procedure and the results were compared with those obtained with the Suzuki *et al.* [1] and sulphuric acid–n-hexane [15] extraction methods. Table V gives the mean recoveries, relative standard deviations and detection limits obtained with whole milk. Although the recovery results are in general satisfactory, the sulphuric acid–hexane procedure destroys endosulfan isomers and metabolites, endrin and heptachlor epoxide, significantly diminishes the recovery of the HCH isomers and isodrin and provides the largest relative standard deviations. The Suzuki *et al.* [1] procedure provides the highest limit of detection, which may not be adequate for the low concentrations of these residues present in milk.

The results obtained with the certified standard are given in Table VI and corroborate the previous results.

To check the performance, the proposed method was applied to spiked samples of different kinds of milk. The results are presented in Table VII. The

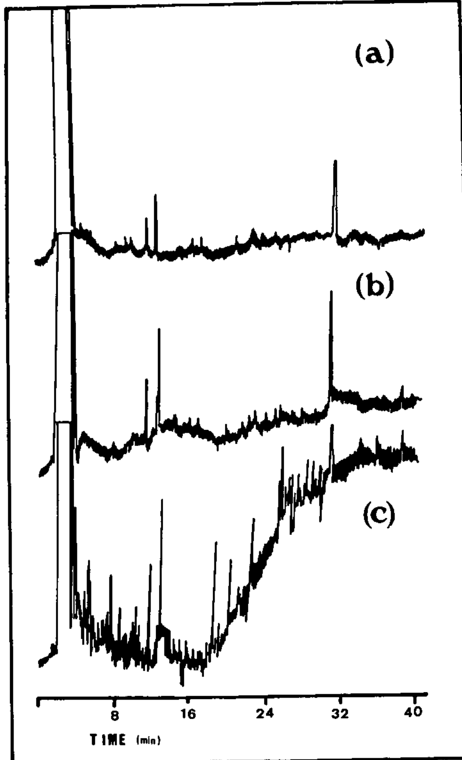

Fig. 3. Chromatograms obtained by injecting an extract of different unspiked milk concentrates: (a) 1 ml; (b) 0.5 ml; (c) 0.2 ml.

recoveries were similar for the different kinds of milk, except for condensed milk which, because of its high sugar content, yielded recoveries decreased by as much as 70%.

To verify the solid-phase extraction procedure, 45 commercial milk samples were analysed: 31 samples of sterilized milk (13 of whole milk, 9 of 2% milk and 4 of skimmed milk), 7 samples of powdered milk (3 of whole milk, 4 of skimmed milk), 5 samples of condensed milk and 2 samples of evaporated milk. Table VIII shows the contents of positive samples and Fig. 5 illustrates the chromatograms obtained from sample 4 in both chromatographic columns. The concentrations found in milk were always lower than those established by the EEC [21].

In conclusion, solid-phase extraction with octadecylsilica can be used to determine organochlorine pesticides in milk. The recoveries obtained are similar to those reported with liquid–liquid extraction methods, and the traditional advantages of solid-phase extraction, *i.e.*, simplicity, speed and low cost, are maintained.

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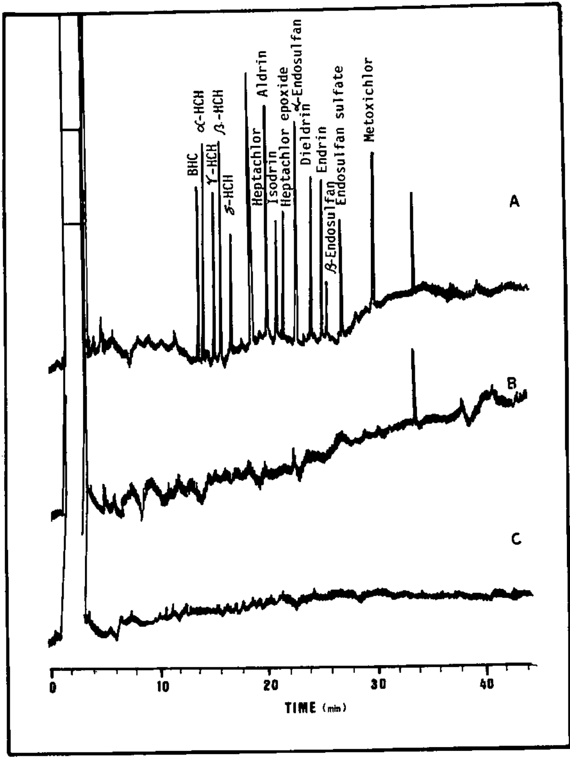

Fig. 4. Chromatograms of extracts obtained by the proposed method from (A) milk spiked with 15 OCPs, (B) unspiked milk and (C) blank.

TABLE V

RECOVERY [MEAN ± R.S.D. (%) (n = 6)] AND DETECTION LIMITS (DL) (μg/l) OF 26 ORGANOCHLORINE PESTICIDES FROM FORTIFIED WHOLE MILK USING THE PROPOSED METHOD AND LIQUID–LIQUID EXTRACTION METHODS [1,15]

Compound	C_{18}		Suzuki *et al.* [1]		H_2SO_4–*n*-hexane [15]	
	DL	Recovery	DL	Recovery	DL	Recovery
Aldrin	0.3	58 ± 6	1.0	92 ± 7	0.3	42 ± 15
Captafol	2.3	49 ± 5	9.1	52 ± 6	3.0	34 ± 12
Captan	2.3	45 ± 9	9.5	45 ± 12	5.0	36 ± 13
o,p'-DDD	0.8	87 ± 7	3.0	91 ± 8	0.8	92 ± 10
p,p'-DDD	0.5	92 ± 7	1.8	97 ± 7	0.5	94 ± 10
o,p'-DDE	0.2	84 ± 8	0.9	82 ± 6	0.2	87 ± 13
p,p'-DDE	0.4	74 ± 7	1.3	71 ± 6	0.3	83 ± 9
o,p'-DDT	0.3	85 ± 8	1.2	82 ± 9	0.3	96 ± 14
p,p'-DDT	0.4	88 ± 7	1.6	91 ± 6	0.5	90 ± 10
Dicofol	6.4	74 ± 7	25.4	82 ± 3	7.1	78 ± 15
Dieldrin	0.2	91 ± 8	1.0	94 ± 6	0.3	86 ± 9
α-Endosulfan	0.3	98 ± 9	1.1	92 ± 7	–	–
β-Endosulfan	0.2	90 ± 6	0.8	91 ± 6	–	–
Endosulfan sulphate	0.3	91 ± 6	1.3	91 ± 8	–	–
Endrin	0.3	93 ± 5	1.0	95 ± 8	–	–
Heptachlor	0.3	78 ± 6	1.0	90 ± 6	0.3	83 ± 11
Heptachlor epoxide	0.3	92 ± 11	1.0	89 ± 6	–	–
α-HCH	0.1	100 ± 8	0.5	96 ± 9	0.2	89 ± 12
β-HCH	0.2	96 ± 8	0.6	95 ± 6	0.2	75 ± 15
δ-HCH	0.3	99 ± 7	1.1	94 ± 10	0.4	73 ± 12
γ-HCH	0.1	106 ± 10	0.6	101 ± 8	0.2	84 ± 10
BHC	0.1	99 ± 9	0.5	95 ± 4	0.2	95 ± 11
Isodrin	0.3	95 ± 9	1.0	89 ± 8	0.5	59 ± 9
Metoxichlor	0.2	90 ± 7	0.7	87 ± 6	0.2	98 ± 7
Mirex	0.7	70 ± 9	2.6	60 ± 8	0.7	97 ± 9
Tetradifon	0.5	82 ± 7	2.0	90 ± 6	0.5	83 ± 13

TABLE VI

CONCENTRATIONS OF ORGANOCHLORINE PESTICIDES IN A CERTIFIED STANDARD (CMR 187)

Compound	Concentration (μg/kg)[a]			
	Certified value	C_{18}	Suzuki *et al.* [1]	H_2SO_4–*n*-hexane [15]
BHC	1.5 ± 0.2	1.4 ± 1.3	–	–
α-HCH	1.80 ± 0.14	1.8 ± 1.2	–	2.0 ± 1.6
γ-HCH	5.7 ± 0.7	6.0 ± 2.4	5.2 ± 1.6	6.5 ± 2.4
β-HEPO[b]	(1.4)[c]	–	–	–
p,p'-DDE	6.6 ± 0.6	5.6 ± 0.9	–	6.4 ± 4.9
Dieldrin	(2.3)[c]	–	–	–

[a] Contents in dry mass.
[b] β-HEPO = Heptachlor epoxide.
[c] Values are not certified.

TABLE VII

RECOVERY [MEAN ± R.S.D. (%) (n = 6)] OF 26 ORGANOCHLORINE PESTICIDES ADDED TO DIFFERENT KINDS OF MILK

Compound	Type of milk					
	Whole	2%	Skimmed	Powdered	Evaporated	Condensed
Aldrin	58 ± 6	68 ± 10	69 ± 9	79 ± 9	80 ± 6	65 ± 5
Captafol	49 ± 5	50 ± 9	54 ± 9	38 ± 5	39 ± 8	39 ± 10
Captan	45 ± 9	44 ± 10	37 ± 10	35 ± 9	37 ± 10	44 ± 12
o,p'-DDD	87 ± 7	85 ± 6	89 ± 7	85 ± 8	87 ± 9	79 ± 10
p,p'-DDD	92 ± 7	90 ± 6	92 ± 7	99 ± 5	98 ± 6	78 ± 10
o,p'-DDE	84 ± 8	88 ± 10	89 ± 8	82 ± 9	80 ± 12	92 ± 4
p,p'-DDE	74 ± 7	90 ± 10	84 ± 9	77 ± 9	82 ± 9	84 ± 5
o,p'-DDT	85 ± 8	80 ± 6	82 ± 8	101 ± 9	85 ± 10	80 ± 9
p,p'-DDT	88 ± 7	89 ± 6	90 ± 9	90 ± 9	81 ± 9	84 ± 6
Dicofol	74 ± 7	84 ± 9	80 ± 8	67 ± 6	73 ± 8	77 ± 10
Dieldrin	91 ± 8	91 ± 8	90 ± 10	95 ± 9	90 ± 10	79 ± 8
α-Endosulfan	98 ± 9	89 ± 9	92 ± 6	99 ± 10	88 ± 9	76 ± 8
β-Endosulfan	90 ± 6	87 ± 10	90 ± 9	99 ± 8	90 ± 10	74 ± 4
Endosulfan sulphate	91 ± 6	90 ± 8	94 ± 7	91 ± 7	92 ± 9	85 ± 7
Endrin	93 ± 5	93 ± 5	94 ± 5	91 ± 8	89 ± 6	76 ± 8
Heptachlor	78 ± 6	80 ± 7	85 ± 9	89 ± 8	80 ± 13	70 ± 7
Heptachlor epoxide	92 ± 11	88 ± 6	87 ± 7	102 ± 10	85 ± 5	78 ± 5
α-HCH	100 ± 8	97 ± 9	99 ± 8	94 ± 8	95 ± 9	73 ± 6
β-HCH	96 ± 8	92 ± 5	95 ± 9	114 ± 7	96 ± 8	102 ± 6
δ-HCH	99 ± 7	96 ± 6	99 ± 10	92 ± 8	93 ± 9	80 ± 9
γ-HCH	106 ± 10	100 ± 7	100 ± 6	110 ± 10	94 ± 9	85 ± 6
BHC	99 ± 9	97 ± 7	98 ± 7	95 ± 5	90 ± 9	84 ± 8
Isodrin	95 ± 9	97 ± 6	92 ± 7	85 ± 9	89 ± 12	89 ± 9
Metoxichlor	90 ± 7	90 ± 7	93 ± 9	97 ± 10	89 ± 12	86 ± 7
Mirex	70 ± 9	74 ± 8	76 ± 9	69 ± 8	69 ± 10	51 ± 9
Tetradifon	82 ± 7	80 ± 9	84 ± 6	83 ± 6	78 ± 9	80 ± 6

TABLE VIII

CONTENT OF ORGANOCHLORINE PESTICIDES IN COMMERCIAL MILK

Sample No.	Type of milk	OCP	Concentration (μg/kg)[a]
2	Sterilized whole milk	γ-HCH	2.8 ± 1.0
4	Sterilized whole milk	α-HCH	0.5 ± 0.1
		β-HCH	0.4 ± 0.2
		γ-HCH	0.4 ± 0.2
5	Sterilized whole milk	γ-HCH	1.6 ± 0.6
6	Sterilized whole milk	β-HCH	10.2 ± 2.8
11	Sterilized whole milk	γ-HCH	6.5 ± 2.6
18	Sterilized 2% milk	*p,p'*-DDE	23.3 ± 6.5
25	Sterilized skimmed milk	*p,p'*-DDE	12.2 ± 2.1
32	Powdered whole milk	Aldrin	2.0 ± 0.8
38	Powdered whole milk	Aldrin	7.3 ± 1.3

[a] Mean ± standard deviation (n = 4).

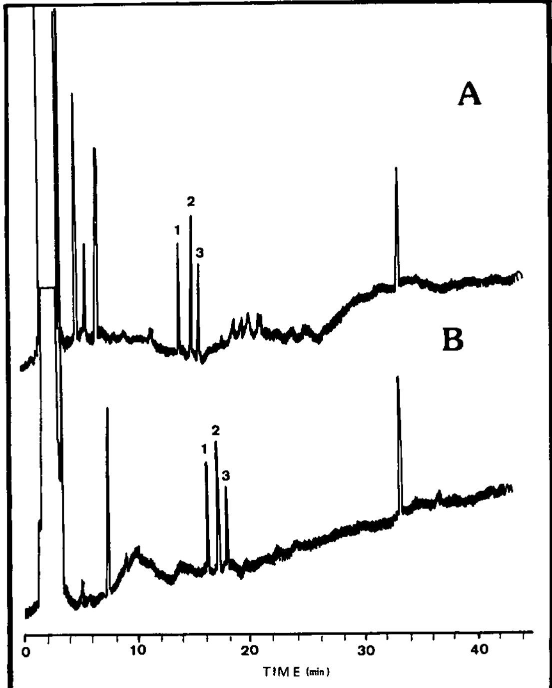

Fig. 5. Chromatograms obtained for sample 4 using two different capillary columns: (A) BP-5 and (B) DB-17. Peaks: 1 = α-HCH; 2 = γ-HCH; 3 = β-HCH.

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Size-exclusion chromatography on zeolites in the trace analysis of polyaromatic hydrocarbons and organochlorine pesticides

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ABSTRACT

In analysis of polyaromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) difficulties can arise with interference from naturally occurring compounds which behave chromatographically similarly to the target analytes. In this paper we report that much of this material (approximately 98%) can be removed simply and rapidly by passing the isolate through a short column of powdered zeolite ZSM-5. No losses of PAHs or OCPs were discernible in this procedure, which provides substantial improvements in terms of analytical performance.

INTRODUCTION

In some circumstances the analysis of polyaromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) can be confounded by the presence in isolates of long-chain oxygen-containing material which behaves chromatographically in a manner similar to the target analytes [1]. This difficulty is exacerbated in situations where the concentrations of target analytes are very low and the burden of interfering material is high; for example in biota such as oysters. To some extent the interference can be overcome by the use of size-exclusion chromatography (SEC) using gels, *i.e.* gel permeation chromatography (GPC) [2–5] and the use of selective detector systems such as in GC–MS, GC–electron-capture detection, and HPLC–ultraviolet fluorescence detection (UVF) in the final analysis stage. A disadvantage of the GPC step is that it is slow and requires the use of comparatively large volumes of solvent with the attendant risk of contamination [5,6]. In the present paper we describe a simple and rapid procedure involving SEC using a commercially available zeolite, which while not affecting the target PAH and OCP analytes which are excluded from the zeolite pores, removes most of the interfering material.

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EXPERIMENTAL

Materials

Analytical-grade *n*-pentane, dichloromethane, *n*-hexane and benzene all purchased from Ajax (Sydney, Australia), were purified by fractional distillation. Water obtained with a Millipore Milli-Q ultrapure water system fitted with an organic free kit was further purified by refluxing with potassium permanganate (approximately 0.01%, w/w) followed by fractional distillation.

Analytical-grade potassium hydroxide was finely ground, washed with dichloromethane, dried, then stored in a desiccator. Laboratory-grade anhydrous magnesium sulphate was heated to 650°C for 1 h prior to use. Both were purchased from May & Baker (Melbourne, Australia).

Ajax laboratory-reagent basic alumina (100

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mesh) was extracted with purified dichloromethane using a Soxhlet apparatus, air dried, then activated at 150°C overnight. The alumina was deactivated by the addition of purified water (1.5%, w/w) immediately before use.

ZSM-5 powder was obtained from Conteka (Delfzijl, Netherlands) as CBV 2802, a low aluminium content material (SiO_2:Al_2O_3 = 280:1) in the H^+ form. This zeolite was heated at 500°C in air for 2 h to yield organic-free material.

Standards

The PAH standard reference mixture M-610A was obtained from AccuStandard (New Haven, CT, USA). This mixture contains each of the sixteen PAHs designated as priority pollutants by the United States Environmental Protection Agency dissolved in 1 ml dichloromethane–methanol (1:1) at concentrations ranging from 0.1 to 2.0 mg ml^{-1}. This 1-ml sample was diluted to 50 ml with *n*-hexane to yield a solution ranging in concentrations of individual PAHS from 2 to 40 ng μl^{-1}.

The deuterated phenanthrene standard was purchased from the US National Institute of Standards and Technology as a solution (1.5 ml) containing 5 mg ml^{-1} [$^2H_{10}$]phenanthrene. This was diluted to 100 ml with *n*-hexane to yield a solution of 75 ng μl^{-1}

1,8-Dimethylnaphthalene (1,8-DMN) was synthesised from 1,8-naphthalic anhydride (Fluka, Buchs, Switzerland) as described by Mitchell *et al.* [7]. A standard solution of 1,8-DMN (20 mg) in 100 ml *n*-hexane (200 ng ml^{-1}) was similarly prepared.

The OCPs aldrin, dieldrin, α-chlordane, γ-chlordane, *p,p*-DDT and heptachlor were purchased from PolyScience (Evanston, IL, USA) and were similarly diluted to prepare a standard solution comprising each of the 6 OCPs from 3.2 to 18.5 ng μl^{-1}.

Isolation of PAH fractions from oysters

Approximately 100 g of oyster tissue (wet mass) was blended with purified water (60 ml) and potassium hydroxide (8 g) until a homogeneous mixture was obtained. The internal standard 1,8-DMN (400 ng) was added and the mixture refluxed under a nitrogen atmosphere for 4 h. After cooling, the digest was acidified to pH 3 with 3 *M* hydrochloric acid, then extracted with four successive portions of dichloromethane (4 × 50 ml). Where necessary, the resulting emulsion was broken by the addition of sodium chloride (*ca.* 2 g). The combined organic extract was washed with purified water (50 ml), back-extracted with a 4% potassium hydroxide solution (3 × 50 ml) then finally washed with purified water (2 × 50 ml). The organic extract was dried with anhydrous magnesium sulphate and filtered. The solvent was evaporated to leave a residue of approximately 5 ml which was further concentrated to approximately 250 μl using a Kuderna–Danish apparatus. This residue was sorbed onto deactivated basic alumina (0.5 g) and the solvent removed in a gentle stream of nitrogen. The alumina was transferred to the head of a column of basic alumina (6 g) deactivated with 1.5% (w/w) water and a fraction containing the saturated hydrocarbons and PAHs was collected by eluting the column with benzene–*n*-pentane (30:70) (50 ml). The solvent was removed as before, then the residue was dissolved in approximately 100 μl of *n*-pentane. Medium-pressure liquid chromatography (MPLC) was performed using a Merck LiChroprep Si 60 (40–63 μm) column attached to a Waters Millipore Model 510 double piston pump and a Model 440 ultraviolet absorbence detector (254 nm). The PAH fraction was collected between 6 and 20 min using a flow-rate of 4 ml min^{-1} of dichloromethane–*n*-hexane (5:95). The volume of eluate was reduced to 100 μl as described above.

ZSM-5 liquid chromatography

ZSM-5 powder (1 g) was dry packed into a Pasteur pipette which had been plugged at one end with a wad of cotton wool which had previously been washed with purified dichloromethane.

The behaviour of organochlorine pesticides was evaluated by adding a 10-μl aliquot of the mixture of pesticides containing 32–185 ng of individual OCPs to 100 μl *n*-pentane and placing this solution onto the column. This solution remained in contact with the ZSM-5 for 10 min after which the column was eluted with *n*-pentane. A fraction collected between 0 and 5 ml was concentrated to 100 μl and a 3-μl aliquot (225 ng) of the deuterated phenanthrene solution was added as a normalisation standard. The fraction was analysed by GC–MS and found to contain all of the pesticides in the mixture by comparison with an identical untreated mixture

spiked with the normalisation standard. The procedure was repeated by applying a 1-μl aliquot (2–40 ng) of the diluted M-610A mixture and spiking with a 1 μl (75 ng) aliquot of normalisation standard. Again, analysis by GC–MS showed the first 5 ml of pentane contained the PAHs.

The procedure was repeated using a PAH isolate from oyster tissue spiked with a 1 μl aliquot of the M-610A diluted solution. Analysis by GC–MS using the selected ion monitoring (SIM) mode yielded results which were identical to those obtained from analysis of the standard mixture in the absence of the oyster matrix. Another oyster tissue PAH isolate was spiked with a 2 μl aliquot (400 ng) of the 1,8-DMN as an internal standard. The isolate was divided into two with one half subjected to ZSM-5 liquid chromatography and the other remaining untreated. To each portion an aliquot (2 μl, 150 ng) of the deuterated phenanthrene solution was added as a normalisation standard prior to analysis by GC–flame ionisation detection.

Gas chromatography

Capillary GC was carried out on a 1 μl aliquot using a Varian Model 3500 gas chromatograph equipped with an on-column injector (OCI-3, SGE, Australia) and a flame ionisation detector. A 25 m × 0.22 mm I.D. cross-linked methylsilicone fused-silica column (BP 1, SGE, Australia) was used with a temperature programme of 50 to 300°C at 4°C min^{-1} then held isothermally for 15 min. Hydrogen was the carrier gas at a linear flow velocity of 40 cm s^{-1}.

Gas chromatography–mass spectrometry

GC–MS analysis was performed using a Hewlett-Packard (HP) 5970 mass-selective detector with a HP RTE/A data system and a HP 5890A gas chromatograph, fitted with a 60 m × 0.25 mm I.D. fused-silica column coated with crossed-linked 5% phenylmethylsilicone (DB-5, J&W Scientific, USA). In a typical analysis, helium was used as a carrier gas at a linear flow velocity of 30 cm s^{-1}. With the transfer line temperature held at 280°C, a 1 μl aliquot of the sample was injected into a HP on-column injection system using a HP 7673A automatic liquid sampler. The oven temperature was programmed from 70 to 300°C at 3°C min^{-1} then held isothermally at 300°C for 20 min. For the analysis of OCPs, the mass-selective detector was operated in the total ion monitoring mode with an electron ionising voltage of 70 eV. The detector was operated in the SIM mode for PAH analysis where the parent ion for each of the PAHs present in M-610A was monitored with a dwell time of 50 ms.

RESULTS AND DISCUSSION

PAH fractions were isolated from tissue of oysters collected from comparatively uncontaminated sites. Similar methods have been published previously [8], and an improved version was reported in 1988 [9]. A recent modification to our procedure has been to employ the alumina filtration step only to remove large quantities of interfering biogenic lipids and to defer the isolation of separate saturate and aromatic fractions until the silica gel MPLC stage.

Typically, GC traces similar to that shown in Fig. 1a were obtained [9]. This chromatogram is dominated by peaks with retention times between 25 and 35 min, revealing the presence of non-target analytes in amounts of approximately ten to several hundred nanograms. In view of the fact that in a typical analysis, target analytes may only be present in sub-nanogram quantities, the presence of these compounds represents a potentially serious source of interference.

After a pentane solution of this PAH isolate had been passed through a short column of ZSM-5 loosely packed in a Pasteur pipette, the gas chromatogram of the eluent, measured at both the same and at × 50 sensitivity was as shown in Fig. 1b (note the height of the internal standard 1,8-DMN). Comparing the traces in Fig. 1a and b it is clear that each of the major peaks of the trace in Fig. 1a has been removed or very substantially diminished. On the basis of total integral counts, approximately 98% of the material is sorbed by the zeolite. In fact, measured at the sensitivity used to obtain the chromatogram shown in Fig. 1a, the purified PAH sample showed an almost smooth baseline.

Recoveries of polyaromatic hydrocarbons (PAHs)

Recoveries of PAHs were estimated by spiking the PAH isolate with a portion of a commercial PAH standard mixture (M-610A) containing 2–40 ng of sixteen individual PAHs. The sample was ex-

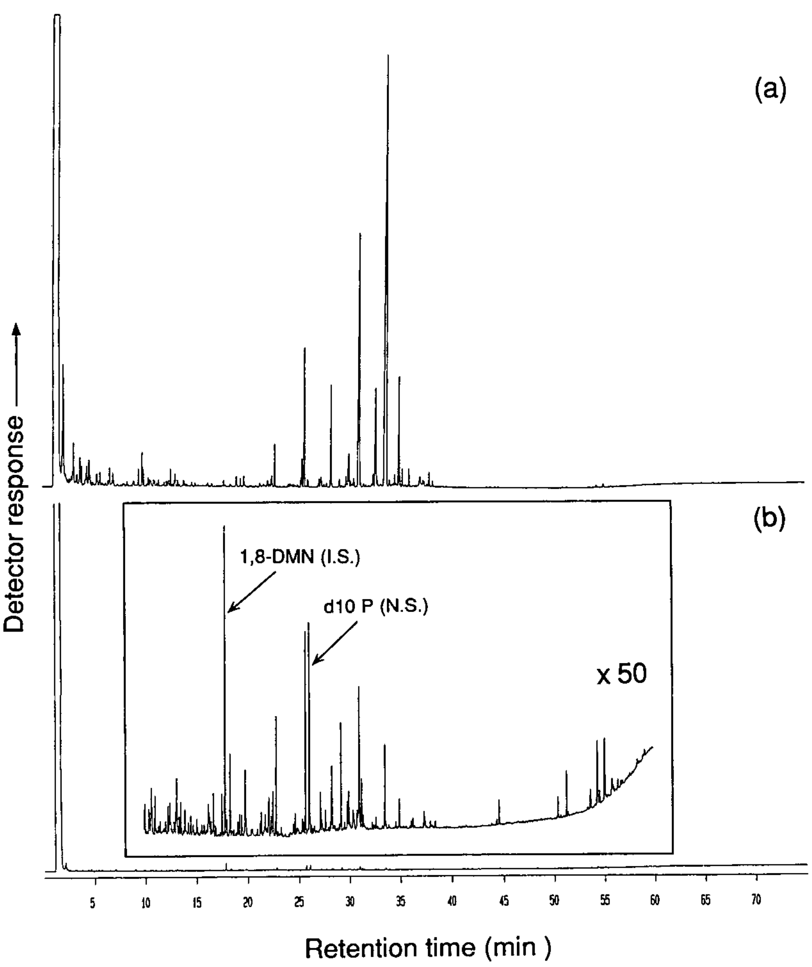

Fig. 1. Capillary gas chromatograms of a PAH isolate from oyster tissue (a) before and (b) after treatment with ZSM-5. The inset in (b) shows the chromatogram after treatment with ZSM-5 at a fifty-fold increase in sensitivity. Labelled peaks are: 1,8-DMN (I.S.) = 1,8-dimethylnaphthalene internal standard; d10 P (N.S.) = $[^2H_{10}]$phenanthrene normalisation standard.

amined by GC–MS before and after the treatment comparing peak areas relative to the normalisation standard in each case. These analyses were performed in triplicate and indicated recoveries of 98–104%.

Recoveries of organochlorine pesticides (OCPs)

Similar recovery trials to those described above were conducted by preparing a pentane solution containing 32–185 ng of the OCPs heptachlor, aldrin, γ-chlordane, α-chlordane, dieldrin and DDT and passing it through the ZSM-5 column. Analysis by GC–MS indicated that the recoveries of each of these compounds was in the range 98–104%. This is an encouraging observation, indicating that the zeolite treatment procedure may be a useful prospect for analysis of this group of compounds.

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Comparison of reversed-phase extraction sorbents for the on-line trace enrichment of polar organic compounds in environmental aqueous samples

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ABSTRACT

On-line sample handling coupling the enrichment of trace organic compounds on reversed-phase sorbents packed in small-size precolumns and their liquid chromatographic separation was investigated. Methods allowing the prediction of breakthrough volumes are reviewed and it is pointed out that the capacity factor of solutes in water, k_w', is a convenient parameter for predicting the sample volume which can be handled for the preconcentration. A comparison between the main LC-grade sorbents available for the preconcentration of organic compounds in water, alkyl-bonded silicas, non-polar styrene–divinylbenzene copolymers and porous graphitic carbons, is presented. The potential of porous graphitic carbon for trace enrichment of very polar compounds is shown.

INTRODUCTION

The use of liquid chromatography (LC) for environmental monitoring has gained in popularity in recent years owing to its suitability of the determination of polar and/or thermodegradable compounds without any derivatization step [1]. Screening for a large group of pesticides over a wide range of polarity can be easily achieved in one run by combining gradient elution reversed-phase LC and sensitive diode-array detection [2–4]. Such a screening cannot be performed by gas chromatography, which requires specific derivatization for each polar group of pesticides.

Determination of trace amounts of organic compounds in environmental aqueous samples requires a preconcentration step before LC analysis. Solid-phase extraction (SPE) techniques have grown in interest as an alternative to laborious and time-consuming liquid–liquid extraction (LLE) methods. SPE is often described as an off-line sample preparation technique. Trace organics are trapped by a suitable sorbent packed in disposable columns or cartridges or enmeshed in an inert matrix of a membrane-based extraction disc, while the water passes through, and are later recovered by elution with a small volume of organic solvent. Compared with LLE-based sample preparation methods, off-line SPE offers reduced processing time and substantial solvent savings. Percolation of samples can be performed in the field, avoiding the problem of transport and storage of voluminous samples. Automation is possible using robotics or special sample preparation units that sequentially extract samples and clean them up for automatic injections. Nevertheless, a certain amount of tedious labour remains and off-line procedures have the inherent disadvantages of a loss in sensitivity owing to the injection of an aliquot, losses in the evaporation step and some risks of contamination, so that internal standards are required.

Many of these drawbacks can be avoided by using on-line trace enrichment on a so-called precol-

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umn. After the sample percolation, the precolumn is coupled to a reversed-phase analytical column via switching valves. The concentrated analytes are then directly desorbed and transferred to the analytical column by a water–organic solvent gradient at the same time as they are separated. As there is no sample manipulation between preconcentration and analysis, no loss or contamination risk can occur and the whole of the concentrated analytes are analysed, allowing the handling of a smaller volume in comparison with off-line techniques where usually only an aliquot is analysed. If adsorption and desorption are efficient, one can except more accurate quantitative results. Automation can be easily achieved [5,6] and two automatic devices for the on-line coupling of SPE to LC are now commercially available, *i.e.*, the Prospekt (Spark Holland) and the OPS-2 (Merck). In order to avoid band broadening of analytes during their transfer from the precolumn to the analytical column, the dimensions of the precolumn have to be small compared with those of the analytical column [7] and the precolumn has to be packed with HPLC-grade sorbents. Disposable precolumns used with the Prospekt are 10 mm × 2 mm I.D. and several cartridge versions containing 30–80 mg of sorbent are available for the OPS-2. The recent commercialization of these automatic devices and of sensitive diode-array detectors will certainly help in the development of on-line trace enrichments methods in environmental analysis. It is then important to be able to select the parameters of the preconcentration step depending on the detection level required and on the solutes to be determined.

SPE can be described to a first approximation as a simple chromatographic process and retention of organics occurs provided that the organic compounds are not eluted by the water of the aqueous sample itself. A significant parameter in SPE is the sample volume that can be handled for the preconcentration without any breakthrough. There is a need to determine many organic pollutants at concentrations below the μg/l level. Monitoring drinking water in Europe requires detection limits as low as 50 ng/l, as the maximum allowed concentration limit for each pesticide is 0.1 μg/l. The sample volume that has to be handled for detection at this level should be at least 100–200 ml and depends on the detection mode. In contrast with off-line SPE, one cannot increase the breakthrough volume by increasing the amount of sorbent and therefore the retention ability of the sorbent is an important parameter in on-line trace enrichment. In this work, prediction of the breakthrough volumes was examined as a function of the nature of the sorbent and of the characteristics of the pollutant. Predictions are based on retention data. C_{18} silicas, the copolymer-based PRP1 and porous graphitic carbon, suitable for HPLC, were investigated and were compared for the trace enrichment of polar compounds.

PREDICTION OF BREAKTHROUGH VOLUMES

The breakthrough volume is the key parameter in SPE because it indicates the sample volume and therefore the amount of analyte that can be preconcentrated and that is available for detection. Two factors can be responsible for breakthrough: insufficient retention of the analytes by the sorbent and overloading of the sorbent. It is unlikely that in practical environmental analyses of organic pollutants where concentrations are typically of the order of μg/l that breakthrough will occur owing to overloading of the sorbent capacity [5,8,9].

Recording breakthrough curves

Fig. 1 presents a breakthrough curve obtained when monitoring continuously the UV signal of the effluent from an extraction precolumn. Water spiked with an organic compound at the trace level and having a UV absorbance A_0 is percolated through the precolumn. Provided that the compound is retained by the sorbent of the precolumn, the effluent does not contain it and its UV absorbance is

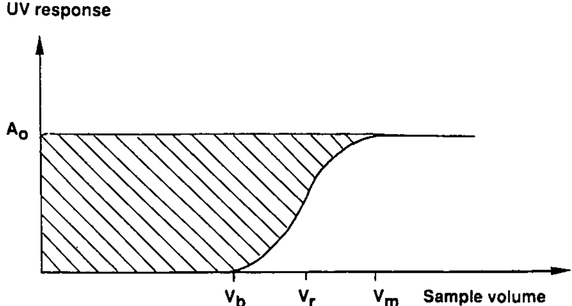

Fig. 1. Theoretical breakthrough curve obtained by percolation of a spiked sample (UV absorbance A_0) through a precolumn. See text for definition of V_b, V_r and V_m.

zero. For a volume V_b, usually defined at 1% of the initial absorbance, a frontal or breakthrough curve is recorded, and after a volume V_m, defined at 99% of the initial absorbance, the effluent has the same composition as that of the entering solution. Under ideal conditions, this curve has a biologarithmic shape and the inflection point is the retention volume, V_r, of the solute eluted by pure water (when the precolumn is not overloaded). Recovery is defined as the ratio between the amount extracted and the amount percolated. As can be seen in Fig. 1, a theoretical 100% recovery can be obtained only for sample volumes equal to or lower than V_b. The V_b values are therefore important because a simple calculation indicates if the concentration in the sample can be detected by comparing the absolute detection limit of the detection system (in nanograms injected) and the amount that can be preconcentrated in the volume V_b. The difficulty is that recording breakthrough curves is time consuming and other methods for determining or predicting V_b are necessary.

Calculations of breakthrough volumes

Werkhoven-Goewie *et al.* [10] have shown that V_b can be calculated easily from retention data. The retention volume is related to the breakthrough volume by

$$V_b = V_r - 2\,\sigma_v \quad (1)$$

where σ_v is the standard deviation depending on the axial dispersion of analyte along the bed of particles in the precolumn. V_r can be calculated easily from the knowledge of the capacity factor in pure water, k'_w, and the dead volume of the precolumn, V_0, according to

$$V_r = V_0\,(1 + k'_w) \quad (2)$$

σ_v can be calculated if the number of theoretical plates N of the precolumn is known by the equation

$$\sigma_v = \frac{V_0}{\sqrt{N}}\,(1 + k'_w) \quad (3)$$

Both V_0 and N values were determined from one experimental breakthrough curve and it was assumed that these values were the same for other solutes. V_b values can be calculated if k'_w can be predicted or easily measured.

Determination and prediction of k'_w

Several means have been proposed for determining k'_w values. Direct experimental measurement is difficult and sometimes impossible owing to high values of the order of magnitude of 100 and above, depending on the size and polarity of the solute.

With alkylsilicas as reversed-phase sorbents, quadratic relationships have been found for the variation of the capacity factor with the volume fraction of organic solvent, φ, in binary mobile phases, according to theories based on the solubility parameter concept or on interaction indices [11–13]:

$$\log k' = a - b\varphi + d\varphi^2 \quad (4)$$

It has been shown that for some solutes this equation does not provide an accurate description of the solute retention in water-rich mobile phases [14]. Nevertheless, over a limited range of binary composition, a linear relationship between $\log k'$ and φ can often be used as a good approximation, especially with methanol–water mobile phases. This linear relationship allows graphical extrapolation of k'_w by measuring $\log k'$ for at least three different mobile phase compositions. However, as pointed out by Jandera and Kubat [15], the accuracy of the extrapolated value depends strongly on the range of the experimental k' and on the range of methanol concentrations used for the determination of the experimental data points. They have shown that when using a short microcolumn (30 × 1 mm I.D.), for some solutes, it was possible to measure k'_w directly by elution methods and that for more apolar herbicides extrapolations are more rapid and more accurate because the experimental range for extrapolation is water-rich.

Jandera and Kubat [15] have also proposed a rapid sorption method; k'_w is defined as the ratio between the amount of solute in the stationary phase and in the mobile phase. A sample is pumped through a microcolumn until equilibrium and the amount of solute adsorbed on the stationary phase is measured by desorption to an analytical column via a switching valve, similarly to the on-line preconcentration device. They found that experimental values determined by the sorption method are higher than those determined by elution methods.

Another method for predicting k'_w is based on the use of the water–octanol partition coefficient, characterizing the hydrophobicity of a compound and

playing an important role in phenomena of physicochemical, biological and environmental interest [16]. These partition coefficients, P_{oct}, can be calculated by taking into account the molecular fragment and intramolecular effects [17]. Braumann [18] has clearly shown the good relationship between extrapolated log k'_w values from the linear log k'–methanol volume fraction relationship and log P_{oct}.

Experimental determination of breakthrough volumes

An experimental method for determining both breakthrough volumes and recoveries has been developed [8,19]. It is easily performed with the on-line set-up. A small volumme, V_p, spiked with a known concentration, C_p, of each analyte of interest is percolated through the precolumn. The chromatogram corresponding to the on-line elution of the precolumn is then recorded and peak areas are measured. This first volume is chosen in order that breakthrough does not occur for any solute (5 or 10 ml, for instance) and this can be easily verified by comparing the chromatogram with that obtained by loop injection of the same amount directly into the analytical column. Then, the sample volume is increased and the concentration decreased in order to have the same amount, C_pV_p, of analytes in each sample volume V_p percolated. Provided that breakthrough does not occur for any solute, the amount concentrated remains constant and the peak areas obtained on the on-line chromatogram are constant. When breakthrough occurs, the amount extracted decreases, as also do the peak areas. Breakthrough volumes can thus be estimated by three or four percolations for all the solutes of interest simultaneously. Corresponding recoveries can also be calculated by dividing peak areas obtained when percolating a volume V_p by those obtained for 10 ml. By noting the peak area A_i of one analyte in the first chromatogram without breakthrough, and the peak area A_p of the same analyte when percolating V_p, there is a relationship between A_i, A_p and V_b as follows:

$$\text{when } V_p < V_b\text{: } A_p/A_i = 1 \quad (5)$$

$$\text{when } V_p > V_m\text{: } A_p/A_i = V_r/V_p \quad (6)$$

The relationship between V_b and V_m is not simple, but it has been shown that eqn. 6 allows a good estimation of V_m values, as defined in the breakthrough curve [8]. An advantage of this method is that these determinations are performed via the whole on-line system using the same operating conditions as for the quantitative analyses.

EXPERIMENTAL

Apparatus

On-line percolation of water was performed with a Chromatem (Touzart et Matignon, Paris, France) or a Milton Roy (LDC, Riviera Beach, FL, USA) pump. Precolumn elutions and analyses were carried out with a Model 5060 liquid chromatography equipped with a UV 200 variable-wavelength spectrophotometer (Varian, Palo Alto, CA, USA) and a Coulochem Model 5100 electrochemical detector (ESA, Bedford, MA, USA). Precolumns and analytical column switching were connected with two Rheodyne (Berkeley, CA, USA) valves. Quantitative measurements of peak areas were provided by a CR3A integrator–computer from Shimadzu (Kyoto, Japan). The on-line experimental set-up is described, *e.g.*, in ref. 5, the stainless-steel precolumn being placed in the sample-loop position of the six-port liquid switching valve. The recording of breakthrough curves is described in ref. 8.

Stationary phases and columns

A commercially available column packed with Hypercarb porous graphitic carbon (100 × 4.6 mm I.D., 7-μm particle size) (Shandon, Runcorn, UK), a laboratory-packed column with the PRP-1 copolymer (100 × 4.6 mm I.D., 10-μm particle size) (Hamilton, Reno, NV, USA) and a laboratory-packed column with LiChrosorb RP-8 or RP-18 (100 × 4.6 mm I.D., 5-μm particle size) (Merck, Darmstadt, Germany) or a 250 × 4.6 mm I.D. column prepacked with spherical 5-μm Zorbax octylsilica (Interchim, Paris, France) were used for retention measurements. Stainless-steel precolumns (21 mm × 10 mm I.D.) available from Chrompack (Middelburg, Netherlands) and laboratory-made stainless-steel precolumns (22 mm × 4.6 mm I.D. or 27 mm × 4.6 mm I.D.) were used for high retention measurements in water-rich mobile phases and for on-line preconcentration. They were laboratory-packed using a thick slurry and a microspatula. The void volume was determined by injection of a 2 *M* solution of sodium nitrate.

Chemicals

HPLC-grade acetonitrile was obtained from Rathburn (Walkerburn, UK) and methanol from Prolabo (Paris, France). LC-grade water was prepared by purifying demineralized water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). Other chemicals were obtained from Prolabo, Merck or Fluka (Buchs, Switzerland). Stock solutions of selected solutes were prepared by weighing and dissolving them in methanol. LC-grade water samples were spiked with these solutions at the ppb or ppt level. The final standard solutions did not contain more than 0.5% of methanol.

RESULTS AND DISCUSSION

Alkyl-bonded silica sorbents

Experimental measurements of V_b. The different methods for predicting breakthrough values were first investigated with *n*-alkylsilica stationary phases, as many data have been published using these sorbents. Fig. 2a shows experimental breakthrough curves obtained for three herbicides with a 10 mm × 2.1 mm I.D. precolumn packed with C_{18} silica. These curves have a different shape and are spread over a larger volume when the compounds are more strongly retained, owing to the low plate number in the precolumn. The front corresponding to linuron is spread over nearly 100 ml from a V_b value of 70 ml to a V_m value of 165 ml. First, the determination of V_b at 1% of the initial absorbance on the front curves cannot be accurate when the front is not sharp. Also, if one wants a 100% recovery, the percolated volume has to be lower than 70 ml. Nevertheless, it can be seen that increasing the percolated volume to 165 ml increases considerably the amount preconcentrated, up to nearly 50%. The corresponding recovery at that point is below 100%, but overcoming the breakthrough volume is often of interest when traces of organic compounds have to be determined in water samples with relatively low organic contamination. When many solutes are to be determined together, this situation occurs, of course, for some analytes. The volume V_r is then a good indication of the sample volume that can be preconcentrated with good recoveries. It is important to note that with on-line methodologies, once the experimental conditions have been selected,

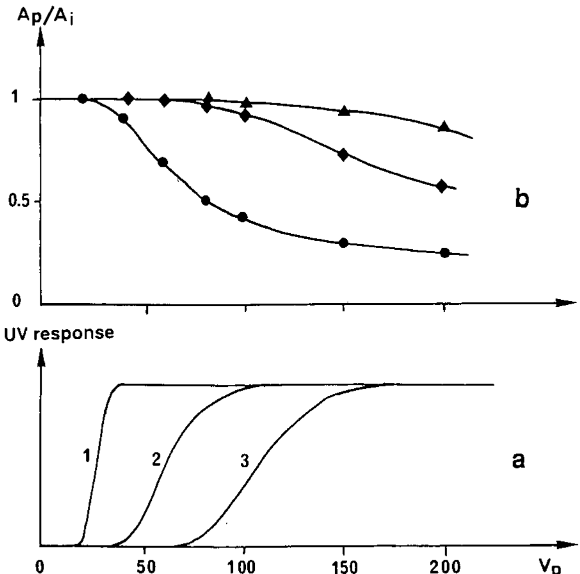

Fig. 2. (a) Experimental breakthrough curve recorded with a 1 cm × 0.21 cm I.D. precolumn packed with RP-18 silica. Sample: solution spiked with 100 μg/l of (1) simazine, (2) atrazine and (3) linuron. (b) Experimental variations of the ratio between peak area obtained for the on-line preconcentration and elution of a sample volume V_p spiked at a concentration C_p and peak area obtained for a 10-ml percolation volume spiked with 50 μg/l of each of the three herbicides (amount preconcentrated = 0.5 μg). The product C_pV_p is constant and equal to 0.5 μg. Solutes: ● = simazine; ◆ = atrazine; ▲ = linuron.

quantitative analyses are carried out with spiked samples that are preconcentrated and analysed on-line via the whole system. Knowing accurately the recoveries of analytes is therefore useless.

The experimental method derived from the percolation of spiked samples with increasing volumes and decreasing concentration is represented in Fig. 2b for the three herbicides together. For simazine similar V_b values are found, whereas for atrazine and linuron higher V_b values are estimated on the curves representing the variations of A_p/A_i with the percolated volume than those measured on breakthrough curves recorded for each compound separately. This can be explained by the fact that V_b values can be slightly different when analytes are percolated together on the precolumn. This is why we consider it is better to estimate both V_b and recovery values by spiking samples and using the whole on-line system under similar conditions to those used for the analyses of real samples.

TABLE I

V_r VALUES MEASURED ON BREAKTHROUGH CURVES AND CALCULATED USING EXPERIMENTAL LOG k'_w VALUES OR EXTRAPOLATED LOG k'_w VALUES

See text for calculations.

Solutes	Breakthrough curves			Experimental values		Extrapolated values	
	V_b (ml)	V_r (ml)	V_m (ml)	Log k'_w	V_r (ml)	Log k'_w	V_r (ml)
2-Nitrophenol	1.5	2.5	7	1.9	1.8	1.9	1.8
Toluene	2.5	5	9	2.45	7 ± 1	2.75	13 ± 1
Simazine	19	26	36	3.1[a]	30 ± 3	2.7[a]	12 ± 2
Atrazine	37	60	110	3.55[a]	80 ± 7	3.2[a]	33 ± 3

[a] Values taken from ref. 15.

Calculations of V_r from log k'_w. From a practical point of view, the curves above indicated that V_r is a good indication of the sample volume that can be handled. As many values of k'_w have been published, the practical problem is to relate this value to V_r, which can be easily done by the equation $V_r = (1 + k'_w)V_0$. The void volume is the product of the geometric volume of the precolumn by the porosity ε of the sorbent. An average value between 0.65 and 0.70 was taken for the porosity value of *n*-alkylsilicas. Table I reports the experimental values measured on breakthrough curves and the calculated values from experimental measurements of k'_w. The agreement is correct if one takes into account the fact that for simazine and atrazine breakthrough curves have been recorded with RP-18 silica and k'_w values are taken from ref. 15 and have been measured with Silasorb SPH C_{18} silica. It is well known that retention varies with the characteristics of the C_{18} silica, *i.e.*, the number of octadecyl groups bonded at the surface [20]. Nevertheless, experimental values of k'_w are seldom available and, as seen earlier, only extrapolated values from the log k' *vs.* methanol content relationship or from the log k'_w–log P_{oct} relationship are found. For the four compounds reported in Table I, it is evident that agreement is obtained from experimental values and that calculations with extrapolated values can be different depending on the difference between the experimental and predicted values. One example of the difference in these two values is reported in Fig. 3, showing the variation of the experimental

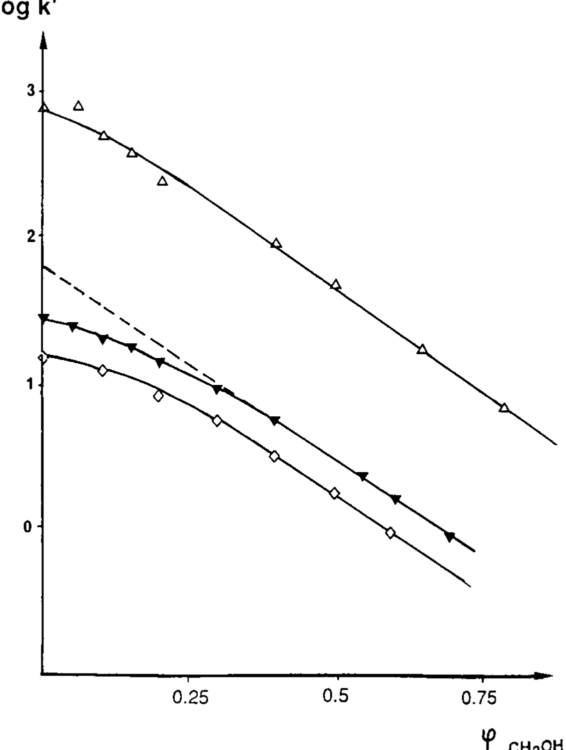

Fig. 3. Variation of the capacity factor of benzene with the volume fraction of methanol contained in the water–methanol mobile phase. ▼ = Measured on a 10 cm × 0.46 cm column laboratory-packed with RP-8 silica; △ = measured on a 5 cm × 0.46 cm column laboratory-packed with PRP-1 copolymer; ◇ = measured on a 10 cm × 0.46 cm column packed with Hypercarb.

TABLE II

EXPERIMENTAL AND EXTRAPOLATED LOG k'_w VALUES AND THE DIFFERENCE (Δ) BETWEEN THEM

Extrapolations were made in the linear range of methanol volume fraction indicated. Log P_{oct} values are mainly from ref. 18.

Solute	Log k'_w		Linear range	Δ (log k'_w) (exp. − extrap.)	Log P_{oct}
	Experimental	Extrapolated			
Chlorobenzene	2.1	2.4	0.3–0.7	−0.3	2.84
Fluorobenzene	1.6	1.8	0.2–0.7	−0.26	2.27
Benzene	1.45	1.75	0.3–0.7	−0.3	2.14
4-Nitrophenol	1.65	1.60	0.05–0.7	0.05	1.91
Nitrobenzene	1.77	1.68	0.1–0.7	0.09	1.84
4-Chloroaniline	1.63	1.60	0.05–0.7	0.03	1.83
Acetophenone	2.08	1.70	0.3–0.7	0.38	1.70
Benzonitrile	1.72	1.65	0.05–0.7	0.07	1.56
Phenol	1.14	1.09	0.05–0.7	0.03	1.48
Benzaldehyde	1.71	1.53	0.05–0.7	0.25	1.45
4-Nitroaniline	1.49	1.33	0.1–0.7	0.06	1.39
Benzyl alcohol	1.27	1.20	0.1–0.7	0.07	1.10
Aniline	0.85	0.85	0–0.7	0	0.91

capacity factor of benzene with the methanol volume fraction of the mobile phase. The extrapolated log k'_w is higher than the experimental value. The extrapolated value was obtained from the linear range for methanol contents above 30%, which corresponds to the way that data are usually extrapolated in the literature. In order to investigate the differences between real and extrapolated values, experimental retention volumes were measured in water using short precolumns packed with C_8 silica instead of C_{18} silica in order to obtain shorter and measurable values. Table II reports the experimental log k'_w values, the extrapolated values log k'_w values from the log k'–φ relationship and the φ range used for the graphical extrapolation, the difference between experimental and extrapolated log k'_w and the water–octanol partition coefficient. As pointed out by Schoenmakers *et al.* [14], the difference between experimental and predicted log k'_w values can be positive or negative, depending on the solutes. For benzenes monosubstituted by halogen or alkyl groups, the experimental values are found to be lower that the predicted values and there is a factor of two between the two values, as indicated by the average difference of 0.3 in logarithmic units. For other compounds, the predicted values are lower than or similar to the experimental values. Jandera and Kubat [15] found lower values for the nine herbicides they examined. From our results and other published data [14,15], the difference between predicted and experimental values are small and lower than 0.10–0.15 logarithmic units for solutes having experimental log k'_w values below 1.5–1.6. The more polar and water-soluble are the compounds, the less retained they are by *n*-alkylsilicas and the closer are the extrapolated and experimental log k'_w values.

The water–octanol partition coefficients are widely used in the biological field and efforts should be made to collect these data for many environmental pollutants. Table III reports some results for a few moderately polar (log P_{oct} between 1 and 3) and weakly apolar (log P_{oct} between 3 and 4) compounds. The variation of extrapolated log k'_w from the log k'_w–methanol content relationship are reported as a function of the log P_{oct} of all the compounds in Table III in Fig. 4. The good correlation obtained between these two values confirms that log k'_w can be estimated for any compounds having a log P_{oct} value between 1 and 4. It has been observed that the relationship is not verified for log P_{oct} values lower than 1. Therefore, extrapolated

TABLE III

OCTANOL–WATER PARTITION COEFFICIENTS, LOG k'_w VALUES EXTRAPOLATED FROM THE LOG k'–METHANOL VOLUME FRACTON RELATIONSHIP AND CORRESPONDING CALCULATED RETENTION VOLUME IN WATER, V_r, USING 1 cm × 0.2 cm I.D. PRECOLUMNS PACKED WITH C_{18} SILICA AND PRP-1

The difference between extrapolated log k'_w values measured on PRP-1 and on C_{18} silica is given in the last column. V_r was calculated assuming a porosity of 0.7 for C_{18} and 0.65 for PRP-1.

Solute	Log P_{oct}	C_{18} silica		PRP-1		Δ (log k'_w) (PRP-1 − C_{18})
		Extrapolated log k'_w	V_r (ml)	Extrapolated log k'_w	V_r (ml)	
Monosubstituted						
Ethylbenzene	3.15	3.4	55	4.8	1390	1.4
Toluene	2.76	2.75	12	4.14	304	1.39
Benzene	2.14	2.2	3.5	3.52	73	1.32
Phenol	1.48	1.55	0.8	2.45	6.2	0.9
Benzyl alcohol	1.10	1.40	0.6	2.45	6.2	1.05
Aniline	0.91	1.08	0.3	2.48	6.6	1.40
Benzoic acid	1.77	1.90	1.4	3.3	44	1.40
Benzaldehyde	1.45	1.73[a]	1.2	2.94	19	1.2
Benzonitrile	1.56	1.80[a]	1.4			
Nitrobenzene	1.84	2.05	2.5	3.62	92	1.57
Acetophenone	1.70	1.8	1.4	3.1	28	1.3
Di- and trisubstituted						
4-Nitroaniline	1.39	1.5	0.7	2.8	14	1.3
4-Chloroaniline	1.83	1.84[a]	1.5	2.8	14	0.96
2-Chlorophenol	2.16	2.11[a]	3	3.2[b]	35	1.09
2,6-Dichlorophenol	2.84	2.76[a]	12	3.92[b]	180	1.16
3,5-Dichlorophenol	3.56	3.49[a]	68			
2,4,5-Trichlorophenol	4.1	3.96[a]	200			
2-Methylphenol	1.93	1.8	1.5	3.3	44	1.5
4-Nitrophenol	1.91	1.84	1.5	2.8	11	0.96
1,3-Dinitrobenzene	1.49	1.6	0.9	3.26	40	1.66

[a] Value from ref. 18.
[b] Value estimated from curves in ref. 19.

values from the general log k'_w–log P_{oct} relationship are very useful for V_r calculations which indicates rapidly the order of magnitude of the sample volume that can handled without any breakthrough. No k' measurement is then required for these estimations. The calculations of V_r indicate that for moderately polar compounds breakthrough occurs rapidly. V_r is lower than 5 ml for compounds such as phenol and methyl- and chlorophenol, about 20 ml for dichlorophenols and 200 ml for the more apolar trichlorophenols. If the minimum sample volume required for detection is about 50 ml, then an on-line preconcentration using small cartridges packed with C_{18} silica can only be applied to organic compounds with log P_{oct} values above 3. Nevertheless, it is always possible to use larger precolumns than that used for calculations in Table III. This size was selected because it is the size of disposable cartridges that have been selected by manufacturers for automatic on-line enrichment-LC apparatus. However, for a classical 15 cm × 0.46 cm I.D. analytical column, a precolumn up to 15 mm × 3.2 mm I.D. can be employed without introducing band broadening in the on-line enrichment chromatogram. With such a precolumn, retention volumes V_r are more than three times higher than those calculated in Table III. It can be seen that by increasing the size of the analytical column to 25 cm, the length of the precolumn can be up to 2 cm.

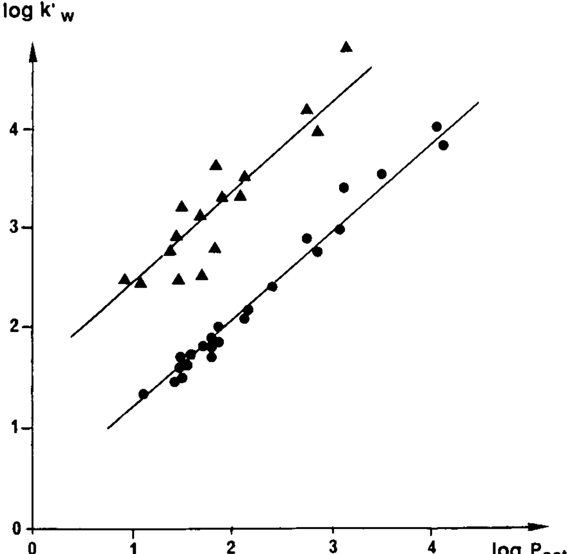

Fig. 4. Variations of extrapolated log k'_w values from the log k'–methanol volume fraction relationship with the water–octanol partition coefficients of solutes. Values obtained for (●) RP-18 silica and (▲) PRP-1.

Styrene–divinylbenzene non-polar copolymers

XAD-type styrene–divinylbenzene copolymers have been widely used for the off-line extraction of analytes from environmental samples, as pointed out in some reviews [21–23]. The ability of these types of sorbents to concentrate polar organics compounds has been demonstrated. These sorbents cannot be used in on-line methodologies because they are not pressure resistant. Porous, rigid and pressure-resistant styrene–divinylbenzene copolymers are commercially available for LC under trade names PRP-1 (Hamilton) and PLRP-S (Polymer Laboratories). They also have the advantage of being stable over the pH range 1–14, in contrast to silica-based materials which are stable only in the pH range 3–8. Frei and co-workers, who were the pioneers and promoters of on-line methodologies, have shown that precolumns packed with PRP-1 retained moderately polar compounds such as chlorophenols much more than C_{18} silicas [5,8,19,24]. Precolumns packed with PLRP-S have also been employed for the on-line trace enrichment of polar pesticides with suitable breakthrough volumes [2,25]. The retention behaviour of analytes is governed by hydrophobic interactions similarly to C_{18} silicas but, owing to the aromatic rings in the network of the polymer matrix, one can expect strong electron-donor interactions (π–π) with aromatic rings of the solutes. It should be also sensitive to changes in the solute electron density caused by the electron-withdrawing ability of solute substituents. The retention behaviour of analytes on PRP-1 has not been studied in depth and we have tried to compare log k'_w values obtained with PRP-1 with those obtained with C_{18} silicas. In Fig. 3, we also reported the variations of the capacity factor of benzene with the methanol content of the mobile phase. First, whatever the mobile phase composition, benzene is about 25–30 times higher more retained by PRP-1 than it is by RP-8. It can be also seen that the variations of log k' with the mobile phase are parallel, indicating that the change in retention observed is mainly due to the mobile phase effects, specially to changes in the solubility of benzene in the methanol–water mixture, as was demonstrated for *n*-alkylsilicas [21]. A similar linear relationship is obtained in the same φ range. Similar observations have been made for many other compounds and similar differences to those obtained with C_8 silica have been measured, positive or negative. A consequence is that similar differences exist between extrapolated and predicted values and that log k'_w can also be extrapolated from the log k'_w–methanol content relationship. However, we can expect the log k'_w–log P_{oct} relationship to be less linear owing to the π–π interactions being different from one solute to another. In Table III are also reported the extrapolated log k'_w values obtained with PRP-1 sorbent. All these solutes are much more retained by PRP-1 than they are by RP-18 and the corresponding V_r values are consequently higher. The difference between the logarithm of the retention on the two sorbents is reported in the last column of Table III and varies between 0.9 and 1.66 with an average value of 1.27, indicating that V_r values obtained with PRP-1 are about twenty times higher than those obtained with C_{18} silica. The difference is the highest for benzene derivatives substituted by nitro groups having a strong electron-withdrawing effect, and the smallest for derivatives substituted by hydroxy groups with an electron-donating effect. The extrapolated log k'_w–log P_{oct} relationship is, of course, less linear than that with C_{18} silica, as shown in Fig. 4, and cannot

be used for accurate enough values of log k'_w. Nevertheless, the average values of 25 ± 12 times higher verified for many compounds are important and the prediction of the retention volume can be derived to a first approximation from predictions carried out with C_{18} silicas.

Moderately polar compounds can be determined at trace levels using this sorbent in on-line methodologies, but early breakthrough is still observed for polar compounds such as phenol or aniline. For polar compounds, we tried to increase as far as possible the size of the precolumn. A longer analytical column (25 cm × 0.46 cm I.D.) was selected for the chromatographic separation and the size of the precolumn was increased to 2.2 cm × 0.46 cm I.D. These dimensions are high for optimization of the coupling. An application to the on-line preconcentration of some triazines and degradation products was carried out using this precolumn [26]. This size was necessary for preconcentration of deisopropyl-

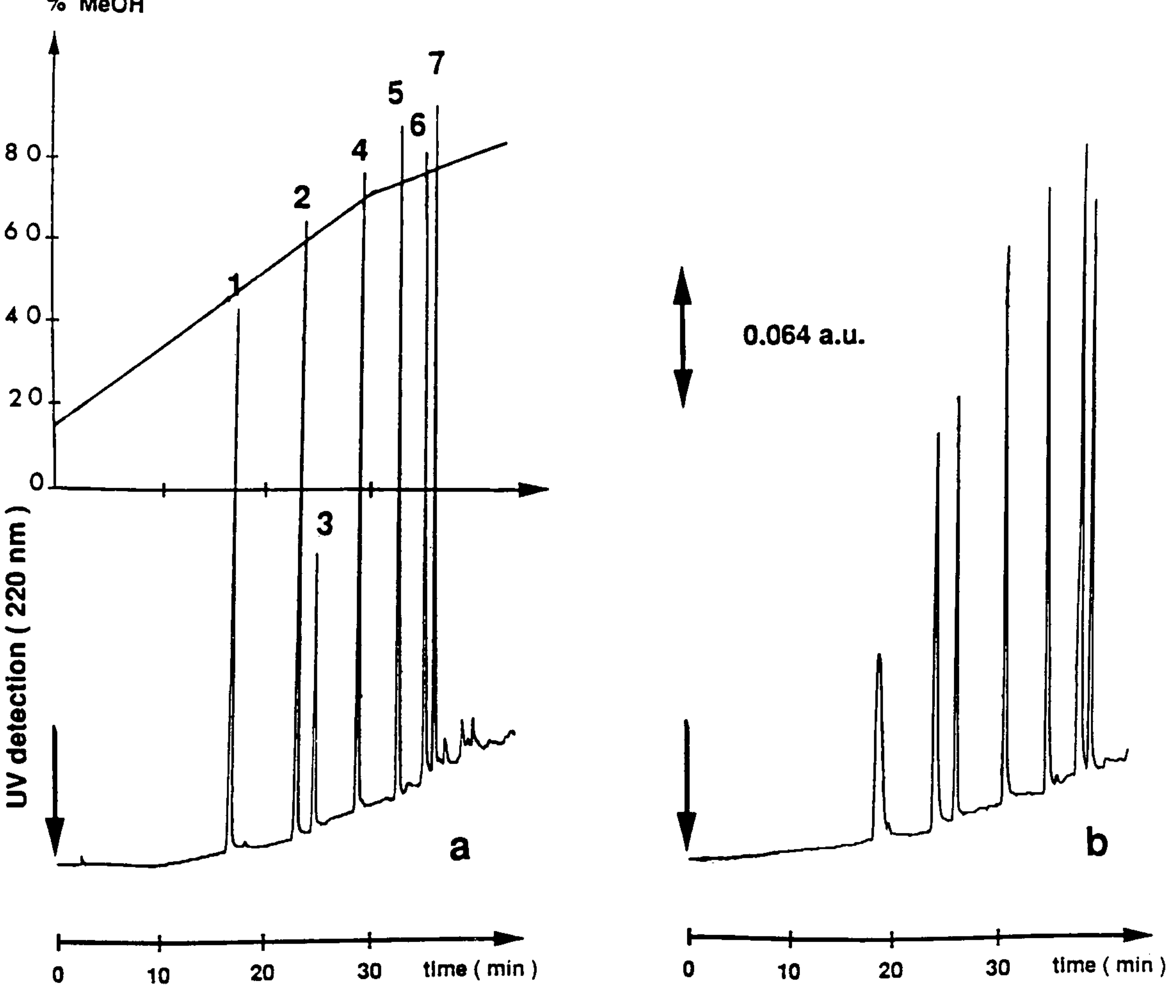

Fig. 5. Efficiency of the on-line coupling of a large-size precolumn packed with PRP-1 with a 25-cm long analytical column packed with C_{18} silica. Comparison between (a) direct 20-μl loop injection and (b) on-line preconcentration of 50 ml of LC-grade water spiked with 5 μg/l of each compound. Solutes: 1 = deisopropylatrazine; 2 = deethylatrazine; 3 = hydroxyatrazine; 4 = simazine; 5 = atrazine; 6 = propazine; 7 = terbutylazine. Analytical column: 25 cm × 0.46 cm I.D. prepacked with 5-μm C_8 Zorbax silica. Precolumn: 2.2 cm × 0.46 cm I.D packed with PRP-1 copolymer. Mobile phase: methanol gradient with potassium phosphate buffer (pH 7) as shown. Flow-rate: 1 ml/min. UV detection at 220 nm.

atrazine, deethylatrazine and hydroxyatrazine, which are more polar than atrazine with breakthrough volumes of 80, 100 and 150 ml, respectively, measured with this 2.2-cm long precolumn. The chromatogram shown in Fig. 5b corresponds to the preconcentration of 50 ml of LC-grade water spiked with 5 μg/l of each analyte. On comparison with the chromatogram obtained with direct loop-injection of 20 μl into the analytical column in Fig. 5a, slight band broadening can be observed for the two first peaks and none for the other products. The large dimensions of the precolumn and the fact that compounds are more retained by the sorbent in the precolumn than by the sorbent in the analytical column should cause band broadening, which was visible when using isocratic elution. In that event, isocratic conditions should be avoided and, when applying a rapid gradient, band broadening does not occur, as can be seen in Fig. 5. Increasing the size of the precolumn to these dimensions allows the V_r values in Table III to be more than ten times higher.

Another advantage of the PRP-1 and PLRP-S sorbents is their high stability in the pH range 1–14. These sorbents retained analytes in their neutral form but not in their ionic form. As an example, the breakthrough volume of aniline measured with 9 cm × 0.46 cm I.D. PRP-1 column was 180 ml at pH 6 whereas it was 6 ml at pH 2. Polar and ionizable compounds have been determined at trace levels using on-line methodologies with two precolumns. The first, packed with PRP-1, traps the compounds in their neutral form. They are later desorbed and reconcentrated on a second precolumn packed with an ion exchanger in their ionic form by eluting the two precolumns in series with a small volume of deionized water adjusted to a convenient pH. The first PRP-1 precolumn is never analysed on-line and this is why its dimensions can be increased considerably for retention of very polar compounds. This methodology allows the use of the selective ion-exchange sorbents which cannot be used directly with natural water samples because these water samples contains many inorganic ions at the mg/l level and the ion exchanger is rapidly overloaded. With the combination of the two precolumns, the water sample is never percolated through the precolumn containing the ion exchanger. This method has been applied to trace levels of aniline derivatives, chlorotriazines, phenols and phenoxy acids [27–30].

Porous graphitic carbon

Carbonaceous sorbents were certainly the first materials used for off-line extraction of medium- to low-polarity organic compounds from water. Graphitized carbon black has been successfully employed for the preconcentration of chlorophenols and chloroanilines and other moderately polar pesticides [31–33], but its practical use in LC is prevented by its poor mechanical properties. Pyrocarbon-modified silicas and pyromodified carbon black [34,35] were synthesized by Colin *et al.* as LC stationary phases. Although these materials were not commercialized, their suitability for on-line trace enrichment of chlorophenols [10] and other medium-polarity compounds such as nitrophenol and nitrobenzene [36] has been reported. Recently a porous graphitic carbon became available as an LC stationary phase [37] under the trade name Hypercarb. This sorbent shows a reversed-phase behaviour, as can be seen in Fig. 3, where the variation of the capacity factor of benzene with the methanol content of the mobile phase is also reported. It is a reversed-phase behaviour in the sense that retention decreases when the methanol content of the mobile phase increases.

The curve corresponding to the porous graphitic carbon (PGB) is also parallel to those obtained with the RP-8 and the PRP-1 sorbents. This indicates that log k'_w can be extrapolated from the linear log k'–φ relationship as for the other reversed-phase sorbents. Owing to its crystalline structure, made of large graphitic sheets held together by weak Van der Waals forces, it is often presented as a more retentive reversed-phase sorbent than C_{18} silica [38], requiring mobile phases with higher organic/aqueous ratios to achieve elution comparable to C_{18} silica-based sorbents. Our results indicates that its depends greatly on the solute polarity and, for instance, Fig. 3 shows clearly than benzene is less retained by PGB than it is by RP-8 silica. It has been pointed out that one basic difference between these reversed-phase sorbents is that solute–stationary phase interactions play an important role with carbon-based sorbents whereas they can be neglected to a first approximation with C_{18} silicas. The affinity of PGC towards very polar and water-soluble polyhydroxybenzenes has been shown [39]. The capacity factor in water of the very polar 1,3,5-trihydroxybenzene (phloroglucinol) is about 1000 with PGC whereas it

TABLE IV

COMPARISON OF EXTRAPOLATED LOG k'_w VALUES OBTAINED WITH RP-18 SILICA, PRP-1 AND PGC

Solute	k'_w		
	RP-18	PRP-1	PGC
Monosubstituted			
Benzene	2.2	3.5	1.45
Aniline	1.08	2.5	1.35
Phenol	1.55	2.4	1.8
Benzoic acid	1.9	3.2	2.4
Nitrobenzene	2.05	3.6	2.45
Polysubstituted			
4-Aminophenol		1.1	2.05
1,4-Diaminobenzene		1.2	2.4
4-Aminobenzoic acid		2	2.85
4-Hydroxybenzoic acid		2.3	2.7
3,5-Dihydroxybenzoic acid		1.35	3
1,3-Dihydroxybenzene		1.35	2.35
1,4-Dihydroxybenzene		0.83	2.15
1,3,5-Trihydroxybenzene		0.5	2.7

was found to be 3 with PRP-1. This compound is not retained by C_{18} silica and it was even proposed as an experimental probe for determining the void volume of C_{18} columns [40].

Other extrapolated or real log k'_w values have been measured for mono- and polysubstituted benzene derivatives with RP-18, PRP-1 and PGC. Results are reported in Table IV. First, when comparing the values for monosubstituted benzenes, compounds are more retained by PRP-1 than they are by PGC. It is interesting to compare the retention behaviours of these two sorbents because they both can involve π–π interactions. Nevertheless, the difference in specific area should be taken into account as there is a factor of three between the two areas. The comparison between RP-18 and PGC indicates that benzene and nitrobenzene are less retained by PGC than they are by RP-18 and that aniline, phenol and benzoic acid are more retained. In contrast to results on PRP-1, indicating that the retentions of all the solutes were higher with PRP-1 than that with C_{18} silicas, no correlation was found between the retention of monosubstituted benzenes on PGC and that on C_{18} silicas. The disubstituted benzenes in Table IV are polar compounds and are not, or only slightly, retained by C_{18} silicas, explaining why log k'_w values have not been reported. The comparison between the retentions obtained on PRP-1 and on PGC are interesting. With PRP-1, the values of log k'_w obtained with two polar substituents are always lower than those measured for each corresponding monosubstituted benzene, whereas the opposite is observed with PGC. For instance, log k'_w of aminophenol is 1.1 with PRP-1 and is lower than those of both phenol (2.4) and aniline (2.5). With PGC, log k'_w of aminophenol is 2.05 and is higher than those of both phenol (1.8) and aniline (1.35). The retention mechanism is therefore very different for the two sorbents.

The capacity factor is defined as the ratio between the sum of solute–stationary phase interactions and the sum of solute–mobile phase interactions. The addition of a second substituent to the benzene ring of the solute increases the polarity of the molecule and therefore increases the solubility of the molecule in water and the overall solute–mobile phase interactions. As the retention time decreases on adding the second substituent, the increase in polarity is higher than the modification of the solute–stationary phase (π–π) interactions induced by this second substituent. The same effect is observed with C_{18} silicas and the retention mechanism with PRP-1 sorbent is mainly governed by hydrophobic interactions. In contrast with PGC, the retention increases on adding a second polar substituent and this indicates that the increase in the solute–stationary phase interactions involved is higher than the increase in the mobile phase interactions which occurs. The separation mechanism involved is obviously different from a hydrophobic mechanism and is more similar to an adsorption process, as already pointed out [36]. The understanding of this process and the possibility of predicting log k'_w are under study [41].

The important practical point is the high retention in water for some polar compounds that cannot be analysed in aqueous samples at present because they are too water soluble to be extracted by liquid–liquid extraction and not retained by classical sorbents. Using PGC as a reversed-phase sorbent is therefore useful for trace analysis in on-line methodology but the coupling with analytical C_{18} columns is impossible when the analytes of interest are very polar. The difference in retention is too strong and it is impossible to separate the compounds by C_{18} silica

owing to their high polarity. Their separation requires water-rich mobile phases unable to desorb and transfer analytes from the PGC precolumn to the C_{18} analytical column. We have reported previously an on-line determination of pyrocatecol, resorcinol and phloroglucinol in a 50-ml drinking water sample with detection limits as low as 50 ng/l using a PGC precolumn and a commercial 10-cm long analytical column prepacked also with PGC [39].

CONCLUSIONS

A significant parameter of solid-phase extraction coupled on-line to an analytical separation is the sample volume that can be handled without breakthrough of analytes. This volume is related to the knowledge of the retention volume of analyte in waters, and predictions can be made if the retention mechanism involved is known. Prediction of retention volume can be easily carried out with C_{18} silicas from the water–octanol partition coefficients. A collection of these values for priority pollutants and pesticides would be useful, because calculation of these constants rapidly becomes difficult for non-simple molecules. The advantage of using styrene–divinylbenzene copolymers for the preconcentration of moderately polar compounds is obvious, but the prediction of the retention of solutes depending on their characteristics is less accurate. The potential of porous graphitic carbon for both trace enrichment and on-line elution of very polar compounds has been demonstrated even though the prediction of the sample volume to handled has not yet been achieved.

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Review

Matrix solid-phase dispersion extraction and the analysis of drugs and environmental pollutants in aquatic species

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ABSTRACT

The classical methods of analyte isolation using homogenization and liquid–liquid partitioning have served for several decades as the standard for the analysis of drugs and environmental pollutants in aquatic species. However, these methods often are costly in terms of analyst time and solvent use and often cannot be accomplished before the materials in question have gone to market. While there have been improvements in screening and determinative techniques, these classical extraction methods are now a limiting factor in residue monitoring. We present here an overview of tissue residue methods for the analysis of drugs and chlorinated pesticides in aquatic resources and offer a comparison to newer extraction technologies, such as solid-phase extraction, supercritical fluid extraction, and matrix solid-phase dispersion (MSPD), as alternatives. MSPD, in particular, shows a great potential to reduce labor and solvent costs and improve sample throughput for residue monitoring programs directed toward aquatic species.

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1. INTRODUCTION

Aquatic resources are monitored for the presence of tissue residues of chemical agents for two main reasons: (1) for food safety —to identify and remove from commercial markets any edible tissues that contain potentially hazardous levels of drug or other chemical residues and (2) for environmental monitoring —to help identify geographical areas where environmental quality may have been significantly compromised.

With increasing reliance on aquatic species as a

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source of dietary protein there is a strong public interest in the safety of edible aquatic resources. This interest is based on concerns about potential unacceptable health risks associated with eating fish containing residues of drugs and environmental pollutants [1]. Such residues may exist in both fish bought by consumers in commercial markets and in fish caught for recreational purposes from rivers, lakes, and oceans. Further, seafood sold in the markets of one country may often have been imported from another with different regulatory policies concerning drug and pesticide use in aquatic environments. For example, imports accounted for over 60% of the fish and shellfish consumed by the United States in 1990 [2]. Therefore, methods are needed for compounds that may be present in either domestic or international products. There is also a need for an international consensus regarding residue levels and concerns.

In this regard, the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) serves as a scientific advisory body to FAO, WHO, the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), and the Codex Committee on Food Additives and Contaminants, concerning the safety of residues of food additives, contaminants, and veterinary drugs. Recommended acceptable daily intake and maximum residue level (MRL) for these substances have been proposed by JECFA and are used by many countries to formulate regulations regarding chemical residues in foods —including aquatic food resources. The analytical needs of an effective residue monitoring program are in part determined by the MRLs as set by a nation's regulatory authorities. Appropriate analytical methods for these programs are recommended by the CCRVDF. A listing of reports and other documents published by the JECFA is available [3].

Although aquatic species are sporadically monitored for various environmental contaminants, existing environmental monitoring efforts are not designed to be of direct use in evaluating many aspects of seafood safety concerns. This is, in part, due to the fact that many environmental programs lack sufficient geographical scope and sufficient focus on the edible portions of many aquatic species. Because many studies are conducted by various university researchers and by state and federal agencies, the programs also tend to lack a common methodological approach to analysis.

As a result of public concern, the failure of environmental and drug monitoring programs to contribute valuable residue data for human food analysis, and the fact that present seafood monitoring and inspection programs lack both the frequency and direction sufficient to ensure effective implementation of current regulatory limits for seafood safety, several governmental bodies, including the US government, have declared their intention to develop a new seafood inspection system [4]. There is early recognition that the key to the success of this new system will be development and application of more efficient and cost-effective analytical methods.

Two of the major classes of chemicals that will need to be included in any aquatic food safety program and in existing and future environmental monitoring programs are drugs and chlorinated pesticides. This review offers a summary of existing methods for the analysis of many of the drugs and for chlorinated pesticides in aquatic species. Several major drawbacks of the methods are discussed and three relatively new methods that offer solutions to these problems are described.

2. METHODS FOR RESIDUE ANALYSIS OF DRUGS AND POLLUTANTS IN AQUATIC SPECIES

Analytical methods are needed for screening, quantitation, and confirmation of chemical residues in aquatic species for research and regulatory purposes. A review of the literature for methods used to extract, isolate, and quantify chemical (drugs and chlorinated pesticides) residues in aquatic species (Tables 1 [5–42] and 2 [43–77]) reflects the confusion currently felt in the field concerning which protocols are most efficient, accurate, reliable, and cost-effective. For example, most methods currently being used by monitoring agencies for pesticide analysis are based on five "classical" multi-residue methods, some developed over thirty years ago. These methods are commonly called the non-fatty (MOG), fatty (Mills), Luke, Storherr, and Krause methods. Together they detect approximately 321 pesticides or pesticide-related compounds [78]. Most of these methods have undergone rigorous multi-laboratory calibration studies, such as those

TABLE 1

REPRESENTATIVE EXTRACTION AND LIQUID CHROMATOGRAPHIC METHODS FOR DRUGS USED IN AQUACULTURE[a]

Compound(s)	Matrix	Sample preparation	Organic solvent (ml)	Analytical column	Mobile phase	Detection method and analysis time	Limit of detection	Reference
Tetracycline, oxytetracycline, chlortetracycline	Fish	Homogenization, filtration, SPE using Amberlite XAD2 resin	100	(A) Wako Gel (dimethylsilica) 10 μm or (B) Shimadzu Gel (polystyrene gel) 10 μm	(A) 0.05 *M* phosphate buffer–ACN (9:1) (B) 0.04 *M* KH_2PO_4–0.08 *M* EDTA–MeOH (1:1:8)	UV at 370 nm 15 min	20 ng TC, OTC 60 ng CTC on column	5
Tetracycline, oxytetracycline, chlortetracycline	Salmon muscle	Homogenization, SPE using Bond Elut C_{18}	29	Merck Hibar LiChroCART RP-18 7 μm	0.01 *M* oxalic acid–ACN–MeOH (73:17:10)	Photodiode array at 355 nm 15 min	90 ng/g TC 80 ng/g OTC 500 ng/g CTC	6
Oxytetracycline	RBT muscle	Homogenization, SPE using Amberlite XAD2 resin	825	Hypersil SAS 5 μm	0.1 *M* citric acid–0.1 *M* trisodium citrate–0.1 *M* Na_2EDTA–ACN (340:5:5:150)	UV at 370 nm 8 min	5 ng/g	7
Oxytetracycline	RBT muscle and liver	Homogenization, SPE using Sep-Pak C_{18}	10	Shandon ODS Hypersil 5 μm	[(5 g DAHP + 5 ml DEA)/810 ml water]–ACN–DMF (81:19:6)	UV at 365 nm 8 min	5 ng/g	8
Oxytetracycline	RBT serum, liver, muscle	Homogenization, liq–liq, SPE using Bond Elut C_{18}	5	Spheri ODS 5 μm	ACN–DMF–0.01 oxalic acid (27:6:67)	UV at 355 nm 8 min	50 ng/ml serum 50 ng/g muscle 100 ng/g liver	9
Oxytetracycline	Fish liver, muscle, slime, hide, vertebrae	Homogenization, SPE using Bondesil C_8 or C_{18}	11	Supelcosil LC-18 DB 5 μm	0.005 *M* phosphate buffer–ACN–THF (81:10:9)	UV at 357 nm 10 min	5 ng/g muscle 10 ng/g liver	10
Oxytetracycline	RBT plasma	Protein precipitation using trifluoracetic acid	0	Cyano Spheri-5 MPLC 5 μm	0.02 *M* oxalic acid–MeOH–DMF (95:5:5)	UV at 350 nm 6 min	4 ng on column	11
Oxytetracycline	Channel catfish muscle	MSPD	16	MicroPak C_{18} MCH-10	0.02 *M* oxalic acid–ACN–MeOH (70:27.5:2.5)	Photodiode array at 365 nm 6 min	50 ng/g (1.25 ng on column)	12
Sulfadimethoxine, ormetoprim	Catfish muscle, liver, kidney	Homogenization liq–liq	10	μ-Porasil	$CHCl_3$–MeOH–H_2O–conc. NH_4OH (1000:28:2:0.6)	UV at 288 nm 20 min	50 ng/g each	13
Sulfadimethoxine, ormetoprim	Chinook salmon muscle	Homogenization, SPE using Sep-Pak C_{18}	25	Ultrasphere ion-pair 5 μm	ACN–MeOH–0.1 *M* H_3PO_4 (17:10:73)	UV at 280 nm 30 min	200 ng/g each	14
Sulfadimethoxine	Channel catfish muscle	MSPD	16	MicroPak C_{18} MCH-10	0.017 *M* H_3PO_4–ACN (65:35)	Photodiode array at 270 nm 10 min	50 ng/g (1.25 ng on column)	15

(Continued on p. 228)

TABLE 1 (continued)

Compound(s)	Matrix	Sample preparation	Organic solvent (ml)	Analytical column	Mobile phase	Detection method and analysis time	Limit of detection	Reference
Sulfadiazine, sulfamerazine, sulfamethazine, sulfadimethoxine, sulfapyridine	Coho salmon muscle	MSPD	16	Supelcosil LC 18-DB	ACN–0.01 *M* ammonium acetate gradient composition	Photodiode array at 270 nm 57 min	*ca.* 100 ng/g SDZ 66 ng/g SMR 228 ng/g SMT 150 ng/g SDM 48 ng/g SP	16
Furazolidone	Atlantic salmon muscle and liver	Homogenization, liq–liq, SPE using Bond Elut NH_2	57	ODS Hypersil 3 μm	ACN–water (16:84) containing 0.001 *M* Na_2EDTA and 0.1 *M* KNO_3	UV at 400 nm 8 min	5 ng/g	17
OA, NA, PA, FZD, DFZ, NPN, FMZ, SDM, SMM, SSZ, SMR	Eel, yellowtail, RBT meat	Homogenization, liq–liq, SPE using alumina	367	Nucleosil C_{18}	THF–ACN–H_3PO_4–water (29:1:0.06:69.94)	UV at 260 nm 40 min	20 ng/g OA 40 ng/g SMR, FZD, NA 60 ng/g SMM, SSZ, SDM, DFZ 80 ng/g PA, NPN, FMZ	18
Oxolinic acid, nalidixic acid, piromidic acid	Eel, RBT, sweetfish, red sea bream, yellowtail tissue	Homogenization, SPE using Baker 10 Amino Cartridge	65	Nucleosil 3 C_{18} 3 μm	ACN–MeOH–0.01 *M* oxalic acid (3:1:6)	UV at 295 nm 8 min	50 ng/g each	19
Flumequine, oxolinic acid	Salmon plasma	SPE using Bond Elut C_2 or on-line polystyrene–divinylbenzene	10	Polystyrene–divinylbenzene PLRP-S 5 μm	ACN–THF–0.02 *M* orthophosphoric acid (20:15:65)	Fluorescence 262 nm excitation 380 nm emission 13 min	5 ng/ml OA 10 ng/ml FEQ	20
Flumequine, oxolinic acid	Atlantic salmon liver	Homogenization, liq–liq, on-line dialysis, on-line SPE using polystyrene–divinylbenzene	5	Polystyrene–divinylbenzene PLRP-S 5 μm	ACN–THF–0.02 *M* orthophosphoric acid (20:14:66)	Fluorescence 325 nm excitation 365 nm emission 15 min	4 ng/g OA 7 ng/g FEQ	21
Flumequine, oxolinic acid	Atlantic salmon muscle	Homogenization, liq–liq, on line dialysis, on-line SPE using polystyrene–divinylbenzene	10	Polystyrene–divinylbenzene 5 μm	ACN–THF–0.02 *M* orthophosphoric acid (20:15:65)	UV at 260 nm or fluorescence 325 nm excitation 365 nm emission 13 min	2 ng/g OA 3 ng/g FEQ using fluorescence	22
Flumequine, oxolinic acid	Salmon and RBT muscle and liver	Homogenization, liq–liq	10	PLRP-S polymer 5 μm	0.002 *M* H_3PO_4–ACN–THF (64:21:15)	Fluorescence 260 nm excitation 380 nm emission 12 min	5 ng/g OA 10 ng/g FEQ	23
Flumequine	Atlantic salmon muscle	Homogenization, liq–liq	43	ODS Hypersil 3 μm	0.1 *M* citric acid–MeOH–ACN–THF (60:30:5:5)	Fluorescence 324 nm excitation 363 nm emission 8 min	5 ng/g	24

Flumequine	Eel plasma, aquaria water	Liquid–liquid	4	Nucleosil	ACN–DMF–[(3 g H_3PO_4 + 1 g TMAC)/675 ml water] (125:200:675)	Fluorescence 245 nm excitation 350 nm emission	2 ng/ml	25
Oxolinic acid	RBT serum	SPE using Sep-Pak Accell or liq–liq	4	Nova-Pak C_{18} 4 μm	MeOH–(7.5 g/l $KH_2PO_4 \cdot 2H_2O$ + 2.5 g/l $Na_2HPO_4 \cdot H_2O$ (4:6)	UV at 258 nm 15 min	1 ng/ml	26
Oxolinic acid	RBT serum, muscle, liver	Serum - direct inj. Muscle, liver, homogenization, SPE using Bond Elut C_{18}	0 10	Regis Pinkerton GFF ISRP 5 μm	ACN–0.1 M KH_2PO_4 (1:9)	UV at 254 nm 12 min	10 ng/ml serum (direct inj.) 10 ng/g liver, muscle	27
Oxolinic acid	Salmon muscle	Homogenization, liq–liq	26	Partisil ODS-3 5 μm	ACN–MeOH–0.01 M oxalic acid (3:1:6)	Fluorescence 327 nm excitation 369 nm emission 4 min	1–2 ng/g	28
Oxolinic acid	Channel catfish muscle and bile	MSPD	16	Versapak C_{18} 10 μm	MeOH–0.05 M GAA gradient composition	UV at 260 nm 65 min	50 ng/g	29
Nalidixic acid	Channel catfish muscle and liver	MSPD	16	Verspak C_{18} 10 μm	MeOH–0.05 M GAA gradient composition	UV at 257 nm radio-label monitoring 65 min	20 ng/g muscle 60 ng/g liver using radioactivity	30
Nalidixic acid	RBT and amago salmon serum, muscle, liver, kidney, bile	Homogenization, liq–liq	260	ToyoGel DEAE-2SW	0.015 M phosphate buffer–ACN (65:35)	UV at 278 nm	Muscle 50 ng/g serum 50 ng/ml liver, kidney 100 ng/g bile 100 ng/ml	31
Miloxacin, M-1 metabolite	Eel, yellowtail, red sea bream and RBT muscle	Homogenization, SPE using Bond Elut C_{18}	110	L-column ODS	0.05 M NaH_2PO_4–ACN (65:35)	UV at 260 nm or fluorescence 325 nm excitation 365 nm emission 10 min	10 ng/g each	32
Enrofloxacin	Atlantic salmon and RBT serum, muscle, liver	Serum - SPE using C_2 muscle and liver - homogenization, liq–liq. SPE using C_{18}	2 61	PLRP-S polymer 5 μm	0.002 M H_3PO_4–ACN (8:2)	UV at 289 nm or fluorescence 278 nm excitation 440 nm emission 12 min	1 ng/ml serum 1 ng/g tissue using fluorescence	33
Sarafloxacin	Fish serum	SPE using Bond Elut C_2	2	PLRP-S polymer 5 μm	0.002 M H_3PO_4–ACN–MeOH (72:20:8)	Fluorescence 278 nm excitation 440 emission 7 min	5 ng/g	34
Enrofloxacin, sarafloxacin	Atlantic salmon muscle, liver	Homogenization, liq–liq	11	RLRP-S polymer 5 μm	0.002 M H_3PO_4–ACN–MeOH (73:19:8)	Fluorescence 278 nm excitation 440 nm emission 8 min	5 ng/g enrofloxacin 10 ng/g sarafloxacin	35

(Continued on p. 230)

TABLE 1 *(continued)*

Compound(s)	Matrix	Sample preparation	Organic solvent (ml)	Analytical column	Mobile phase	Detection method and analysis time	Limit of detection	Reference
Ciprofloxacin	RBT and african catfish plasma	liq–liq	3	Spherisorb-5 ODS	ACN–DMF–[(1.13 g H_3PO_4 + 0.38 g TMAC)/700 ml water] (1.5:1.5:7)	UV at 278 nm	–	36
Ampicillin	Yellowtail tissue	Homogenization, SPE using Sep-Pak Florisil	134	Nucleosil C_{18}	MeOH–0.02 *M* Na_2PO_4–0.01 *M* citric acid (15:42.5:42.5)	UV at 222 nm 40 min	30 ng/g (3 ng on column)	37
Thiamphenicol	Yellowtail tissue	Homogenization, liq–liq SPE using Florisil	–	TSK gel ODS-120T	MeOH–water (15:18)	UV at 225 nm 20 min	1.25 ng	38
Thiamphenicol, florfenicol, chloramphenicol	Yellowtail muscle	Homogenization, liq–liq, SPE using Sep-Pak Florisil	245	Chromatorex ODS 5 μm	MeOH–water (15:85)	UV at 225 and 270 nm 30 min	10 ng/g each	39
Febendazole	Channel catfish plasma, kidney, fat, muscle, bowel contents, urine	Homogenization, SPE using diatomaceous earth, liq–liq	49	MicroPak C_{18} MCH-10	Water–0.05 *N* H_3PO_4–ACN (6:6:88) gradient flow-rate	UV at 290 nm 30 min	low ng/g range	40
Malachite green	RBT muscle and liver	Homogenization, liq–liq	16	PLRP-S polymer 5 μm	0.02 *M* H_3PO_4–ACN–THF (49:40:1)	UV at 615 nm 6 min	1 ng/g muscle 10 ng/g liver	41
Malachite green	Pond and tap water	SPE using Baker 10 diol	2	μBondapak C_{18} 10 μm and PbO_2 postcolumn reactor	MeOH–(0.05 *M* Na acetate + 0.1 *M* GAA) (85:15)	UV at 618 nm 17 min	2.83 ng/l chromatic form 2.01 ng/l leuco form	42

[a] Abbreviations: ACN = acetonitrile; CTC = chlortetracycline; DAHP = diammoniumhydrophosphate; DEA = diethanolamine; DFZ = difurazone; DMF = dimethylformamide; FEQ = flumequine; FMZ = furamizole; FZD = furazolidone; GAA = glacial acetic acid; MSPD = matrix solid-phase dispersion; NA = nalidixic acid; NPN = nifurpirinol; OA = oxolinic acid; OTC = oxytetracycline; PA = piromidic acid; RBT = rainbow trout; SDZ = sulfadiazine; SDM = sulfadimethoxine; SMR = sulfamerazine; SMT = sulfamethazine; SMM = sulfamonomethoxine; SP = sulfapyridine; SPE = solid-phase extraction; SSZ = sulfisozole; TC = tetracycline; THF = tetrahydrofuran; TMAC = tetraethylammonium-chloride.

TABLE 2

METHODS FOR DETERMINATION OF CHLORINATED PESTICIDE RESIDUES IN AQUATIC RESOURCES[a]

Compound(s)	Matrix	Sample preparation	Detection	Reference
EPA "16" except for endosulfan I and II	Whole oyster homogenate	MSPD [8 ml acetonitrile–methanol (9:1)]	GLC–ECD	43
EPA "16" except for endosulfan I and II	Crayfish and lobster hepatopancreas	MSPD (8 ml acetonitrile)	GLC–ECD	44
DDT, DDE and DDD (*para* isomers); lindane; heptachlor; hept. epox.; aldrin; dieldrin; endrin	Fish muscle	MSPD (8 ml acetonitrile)	GLC–ECD	45
ΣDDT (also PCBs, PAHs)	Mussel, oyster	NOAA methods	GLC–ECD	46
ΣDDT, lindane, heptachlor, hept. epox., aldrin, dieldrin, α-chlordane, *trans*-nonachlor, mirex	Fish liver, whole mollusk	NOAA methods	GLC–ECD	47
ΣDDT, lindane, heptachlor, hept. epox., aldrin, dieldrin, α-chlordane, *trans*-nonachlor, mirex (also HCB, PCB, PAHs)	Oyster	NOAA methods	GLC–ECD	48
Endosulfan I and II, endosulfan sulfate	Whole crayfish	Modified EPA methods	GLC–ECD (another column for confirmation)	49
ΣDDT, lindane, heptachlor, aldrin, dieldrin, chlordane, mirex, methoxychlor, endosulfan, toxaphene (also PCBs, trifluralin)	Whole fish	Modified EPA methods	GLC–ECD (3 different columns for confirmation)	50
ΣDDT, lindane, heptachlor, aldrin, dieldrin, chlordane, mirex, methoxychlor, endosulfan, toxaphene (also PCBs, trifluralin)	Clam, oyster, mussel, and quahog	Modified EPA methods	GLC–ECD	51
DDT, DDE and DDD (*para* isomers); α-HCH; dieldrin; *trans*- and *cis*-chlordane; *trans*-nonchlor; octachlor epoxide (also HCB, PCBs)	Fish muscle (with and without skin)	FDA	GLC–ECD	52
DDE and DDD (*ortho* and *para* isomers)	Crayfish abdominal muscle and hepatopancreas	FDA (modified Florisil procedures)	GLC–ECD (another column for confirmation)	53
DDT, DDE and DDD (*para* isomers); *o,p'*-DDT, α- and β-HCH; lindane; heptachlor; hept. epox.; aldrin; dieldrin; oxychlordane; *trans*-nonachlor (also HCB, Aroclors 1254 and 1260)	Fish "edible portions"–all flesh and skin	FDA for high moisture, non-fatty food	GLC–ECD (another column and TLC for confirmation)	54

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TABLE 2 *(continued)*

Compound(s)	Matrix	Sample preparation	Detection	Reference
ΣDDT, ΣHCH, dieldrin, Σchlordane, *trans*-nonachlor, mirex, toxaphene (also Σchlorobenzenes, PCBs)	Fish liver	Solvent extraction, GPC for cleanup, Florisil column chromatography for fractionation	GLC–ECD (GLC–MS for confirmation)	55
EPA "16" except for endrin aldehyde	Oyster and clam	Solvent extraction, reversed GPC for cleanup, Florisil column chromatography for further cleanup	GLC–MS	56
ΣDDT, α-HCH, lindane, heptachlor, hept. epox., aldrin, dieldrin, endrin, *trans*- and *cis*-chlordane, oxychlordane, *trans*- and *cis*-nonachlor, mirex, methoxychlor, toxaphene (also HCB; Aroclors 1242, 1248, 1254 and 1260; dacthal, pentachloroanisole)	Whole fish composites	Solvent extraction, automated GPC for cleanup, Florisil column chromatography for cleanup and initial fractionation, silica gel chromatography for further fractionation	GLC–ECD (GLC–MS for confirmation)	57
Organochlorine pesticides	Fish	SFE, silica gel or alumina chromatography for cleanup	GLC	58
α- and β-Endosulfan; endosulfan sulfate, diol, ether and lactone	Fish	Semipreparative liquid chromatography–homogenization of tissue with trisodium citrate, disodium hydrogen orthophosphate, and Na_2SO_4; silicic acid/alumina chromatography for cleanup	GLC–ECD	59
DDT, DDE and DDD (*para* isomers); DDT and DDD (*ortho* isomers); dieldrin	Whole fish	Semipreparative liquid chromatography–homogenization of tissue with trisodium citrate, disodium hydrogen orthophosphate, and Na_2SO_4; silicic acid/alumina chromatography for cleanup	GLC–ECD	60
DDT, DDE and DDD (*para* isomers); lindane; hept. epox.; dieldrin; endrin; *trans*-chlordane; *cis*-nonachlor	Fish, crabmeat, shrimp, scallop	Solvent extraction, SPE columns (C_{18} and Florisil) for cleanup	GLC–ECD	61
p,p'-DDT-d8	Clam without gut contents	Solvent extraction, SPE columns for cleanup, Florisil column chromatography for further cleanup	GLC–MS	62
Endosulfan	Fish, oyster, and clam	Soxhlet extraction, AC for cleanup	GLC–ECD	63
DDT, DDE and DDD (*para* isomers); α-BHC; lindane; γ-chlordane; mirex (also chlorobenzenes, PCBs)	Whole mussel	Soxhlet extraction, AC for cleanup	Dual capillary column GLC	64

DDT, DDE, DDD, α-HCH, lindane, heptachlor, hept. epox., aldrin, dieldrin (also PCBs)	Fish muscle	Soxhlet extraction, AC for cleanup and fractionation	GLC–ECD	65
EPA "16" except for dieldrin, endrin aldehyde, and endosulfan sulfate	Fish muscle	Soxhlet extraction, AC for cleanup and fractionation	GLC–ECD	66
ΣDDT, HCH isomers, heptachlor, hept. epox., aldrin, dieldrin (also PCBs)	Whole fish and shellfish	Soxhlet extraction, AC for cleanup, saponification (alcoholic KOH) or one fraction	GLC–ECD	67
DDT, DDE and DDD (*pra* isomers); lindane; heptachlor; hept. epox.; aldrin; dieldrin; endrin; mirex; methoxychlor; toxaphene	Oyster, mussel, clam	Soxhlet extraction, liquid–liquid partitioning, AC for cleanup	GLC–ECD (3 different columns and TLC used for confirmation)	68
p,p'-DDT, toxaphene, parathion	Oyster	Soxhlet extraction, liquid–liquid partitioning, AC for cleanup	GLC–ECD	69
DDT, DDE and DDD (*para* isomers); *o,p'*-DDT (also PCBs)	Mussel	Soxhlet extraction, H_2SO_4 cleanup	GLC–ECD	70
DDT, DDE, β-HCH, dieldrin, hexachloroepoxide	Fish fat	Soxhlet extraction, on-line SEC for cleanup	GLC–ECD	71
DDT, DDE and DDD (*para* isomers); dieldrin	Fish	Solvent extraction, AC for cleanup	GLC–ECD	72
DDT, DDE and DDD; α- and β-HCH; heptachlor; dieldrin; endrin; α- and γ-chlordane; *trans*- and *cis*-nonachlor; toxaphene; compound E (also PCBs)	Fish	Solvent extraction, AC for cleanup	GLC–ECD	73
ΣDDT (also Aroclors 1242 and 1254, B[α]P)	Fish muscle	Solvent extraction, AC for cleanup	GLC–ECD (GLC–MS for confirmation)	74
Heptachlor, photodieldrin, *cis*-chlordane; photo-*cis*-chlordane (all [^{14}C]-labeled)	Whole fish	Solvent extraction, AC for cleanup	GLC–ECD and TLC Radioactivity also measured	75
ΣDDT (also PCBs)	Fish liver, whole mollusk	Solvent extraction, AC for cleanup and fractionation	GLC–ECD (GLC–MS for confirmation)	76
Lindane	Whole fish and fish liver, kidney and spleen	Solvent extraction, H_2SO_4 for cleanup	GLC–ECD	77

[a] Abbreviations: AC = adsorption chromatography; B[α]P = benzo[α]pyrene; BHC = benzene hexachloride; ECD = electron capture detector; EPA = United States Environmental Protection Agency; FDA = United States Food and Drug Administration; GLC = gas–liquid chromatography; GPC = gel permeation chromatography; HCB = hexachlorobenzene; HCH = hexachlorohexane; hept. epox. = heptachlor epoxide; MS = mass spectrometry; MSPD = matrix solid-phase dispersion; NOAA = United States National Oceanic and Atmospheric Administration; PAH = polyaromatic hydrocarbons; PCB = polychlorinated biphenyl; SEC = size-exclusion chromatography; SFE = supercritical fluid extraction; SPE = solid-phase extraction; TLC = thin layer chromatography.

needed to obtain official acceptance by the Association of Official Analytical Chemists (AOAC). These methods are the backbone of residue analysis protocols for governmental agencies such as the US Food and Drug Administration (FDA) [79], US Environmental Protection Agency (EPA) [80], and the US National Oceanic and Atmospheric Administration (NOAA) [81] (Table 2). These methods work well under certain conditions and for certain purposes. However, perhaps the greatest drawback to their continued use is their inefficiency as screening methods. These methods are sufficiently complex as to not allow the generation of relevant data in time to prevent contaminated foods from entering the marketplace. For example, the FDA is responsible for prohibiting interstate marketing of food containing illegal pesticide residues. In many cases, food is sold before the FDA completes the analyses needed to confirm the presence of the illegal residues [82]. Results are obtained too late to prevent enforceable removal of the contaminated product. A similar problem exists in screening for drugs that are commonly used in aquaculture.

A variety of methods for the analysis of drugs in aquatic species have been reported. Historically, microbial inhibition tests have been used to detect and quantitate antibiotic residues in fish tissues [83–89] but these tests often lack the specificity and sensitivity required for residue detection at MRLs, may be affected by non-specific inhibitors, do not detect microbiologically inactive metabolites [90], and often have a 20–24 h incubation time.

Colorimetric methods published for drug residue testing in fish generally lack the sensitivity and specificity required for residue analysis, but may be of use in experimental studies [91–93]. Experimental methods using radiolabelled drug and liquid scintillation counting [90,94–97] or whole body autoradiographic studies [98,99] have been used to provide information on pharmacokinetic behavior, metabolite formation, disposition, depletion rate, and extraction efficiencies for compounds used in aquaculture. These methods are, however, unsuited for use in routine drug residue analysis.

The development of rapid screening tests that are practical and rugged would allow for routine monitoring of larger numbers of samples in a shorter time period with greater sensitivity and selectivity than is often currently available using conventional methods. The development of these tests may soon be required by many governments as a part of the methods package needed for drug approval of new animal drugs [100]. Tests that show promise as screening methods for aquatic species include thin-layer chromatography (TLC) [101], high-performance liquid chromatography (LC) (Table 1), and ligand receptor techniques such as immunoassay [102–104], bacterial cell receptor assay [105], and radioimmunoassay [106,107]. These techniques have the greatest potential applicability to regulatory monitoring programs, but must be combined with newer extraction methods or used with a reference biological fluid, not requiring extraction, to indicate the residue level in a target tissue. However, the ability of a screening assay to accurately detect positive and negative samples must be evaluated based on performance parameters such as sensitivity, specificity, cross-reactivity, predictive values (positive and negative) and efficiency before the test can be included in a residue monitoring program.

Suspects identified with screening tests require quantitation and confirmation of the presence of residues exceeding the MRL in the target tissue; hence rapid tissue extraction, quantitation, and confirmatory methods must be available for regulatory purposes. For many such analyses, chromatographic methods provide the necessary specificity and sensitivity required for both qualitative and quantitative drug analyses. Liquid chromatography is the most commonly reported method for quantitation of drugs in aquatic species, but extraction procedures and/or sample pretreatment are often needed before injection on conventional reversed-phase analytical columns. Newer analytical columns employing internal surface reversed-phase or immunoaffinity packing permit direct injection of plasma and other liquid matrices that cannot ordinarily be used with conventional LC columns [27]. With these improvements LC procedures may approach screening tests in speed and simplicity [108]. As illustrated in Table 1, a variety of analytical columns and mobile phases are reported for a number of drugs. Reversed-phase C_{18} packing is most commonly used but other packings reported are dimethyl silica, polystyrene gel, ion-pair, polystyrene divinylbenzene, and Regis Pinkerton internal surface reversed-phase. Detection by UV using fixed- or variable-wavelength detectors or diode ar-

ray is most frequently reported, but fluorescence detection gives greater sensitivity for the quinolone antibiotics. Reported limits of detection for some of the LC methods listed in Table 1 are above the current MRLs of many countries and few of the methods have undergone interlaboratory validation studies, however. Therefore, methods validated for detection of drugs and pollutants about the MRL are needed for many compounds used in aquaculture.

Several multi-residue gas–liquid chromatographic methods (GLC) have also been reported for aquaculture drugs [109,110] but GLC is not as frequently utilized for analysis as LC (Table 1). Nevertheless, absolute confirmation of the presence of a compound required for regulatory action may often be secured using gas–liquid chromatography–mass spectrometric detection and confirmation (GLC–MS). LC–MS is becoming more available and will someday exceed GLC for the purpose of confirming many of the polar drugs used in aquaculture. However, even these technologies require the use of tissue isolation methods that are simple, fast, and efficient.

3. PROBLEMS DUE TO REQUIREMENTS FOR SAMPLE PREPARATION, ISOLATION, AND CLEANUP

Sample preparation, isolation, and cleanup are becoming the major rate-limiting factors in sample analysis as improvements in analytical methods proceed [111]. This fact is especially important in light of efforts to introduce rapid screening tests such as immunoassays. Several approaches have been used over the years for the preparation, isolation, and cleanup of drug and environmental residues from aquatic matrices. The classical approach to isolation of the drugs is homogenization followed by liquid–liquid partitioning. Liquid–liquid partitioning may also be used with biological fluids. Homogenization and liquid–liquid partitioning methods provide adequate separation of the drug from matrix but are often expensive in terms of time, labor, material use, and organic solvent disposal. Such approaches also tend to be highly non-specific in their isolation of the target drug(s).

Furthermore, these methods may be generating more pollution than they are satisfactorily resolving. Table 1 gives an indication of the problem by showing a list of the required volumes of solvent for extraction of various drugs from aquatic tissues. Many of these solvents are of greater environmental concern than the compounds they are used to isolate. During extraction and isolation procedures much of this solvent volume is evaporated into the atmosphere, and solvents are often not disposed of properly. This leads to further contamination of the atmosphere, aquifers, aquatic habits, and resources.

As previously mentioned, a further problem with excessive use of solvents is that they make these methods very expensive to perform. The purchase price and subsequent disposal costs of organic solvents and wastes can be limiting factors in analyses performed by government agencies operating on a restricted budget. Therefore, costs for materials can limit the number of samples which can be consistently analyzed to provide a statistically sound evaluation of the magnitude of contamination.

Employee costs can also be a limiting factor in residue analysis. Official methods generally require extensive training of laboratory technicians in order to guarantee consistent, reliable results and most such methods are not amenable to automation.

All of the above problems indicate that the use of classical methods for screening purposes should be severely curtailed and phased out as new, more appropriate methods are developed and validated for sample screening.

4. TISSUE RESIDUE PROBLEMS CAUSED BY DRUGS

Diseases are the single most important cause of economic loss in intensive aquaculture [2] and necessitate the use of antibacterial and other therapeutic compounds to maintain the health and production of cultured species. Although there is a degree of variability there are numerous therapeutants which are consistently used worldwide in aquaculture [112–114]. These agents belong to a wide range of chemical and therapeutic classes such as antibacterials (*e.g.*, sulfonamides and potentiated sulfonamides, aminoglycocides, β-lactams, tetracyclines, quinolones, macrolides, etc.), parasiticides (*e.g.*, mebendazole and dichlorvos), disinfectants, piscicides, herbicides, algacides, anesthetics, water treatments, and dyes. This large range of chemical classes presents a problem for residue monitoring, and requires the use of screening tests to adequately detect the presence of illegal residues.

A residue can be defined as "any compound present in edible tissues of the target animal that results from the use of the sponsored compound, including the sponsored compound, its metabolites and any other substances formed in or on food because of the sponsored compounds use" [100]. Metabolites are considered to be as toxic as the parent compound unless shown otherwise [100]. Clearly, extraction and analytical methods used in monitoring programs must be capable of extracting and analyzing both parent drug and metabolites present in the tissues of interest at or less than MRLs. However, MRLs are not always static. Toxicological data are always being updated and the JECFA periodically issues recommendations for MRLs based on such available toxicology information for selected veterinary drugs including those used in aquaculture. Many countries have established MRLs based on JECFA recommendations for a number of these compounds. A listing of WHO publications containing JECFA recommended MRLs is available [3].

The use of therapeutants in aquaculture not only may result in unacceptable residues in edible tissues but also in the environment. Drugs used in aquaculture may be directly introduced into the environment, as with ectoparasiticides, or indirectly introduced in medicated feeds (via non-consumption of the feed, poor bioavailability, and limited biotransformation). The environmental degradation, accumulation, and persistence of these agents is affected by water temperature, sediment microenvironment, and factors affecting dispersion [115]. It has been estimated that 70 to 80% of orally administered oxytetracycline remains in the environment [116]. Furthermore, there are large variations in the persistence of antibiotics in sediments from fish farms. Furazolidone exhibits a very short half-life (18 h) [117] and oxytetracycline a half-life of 32 to 64 days depending on sediment conditions [116]. The environmental fate [115–122] and effects [117,122–127] of several compounds commonly used in aquaculture has been the subject of recent studies. However, the environmental metabolism, fate, and effects of most drugs introduced into the aquatic environment is poorly understood and relatively few methods are available for the multitude of compounds, environmental matrices, and environmental conditions that are of import in assessing the environmental impact of these compounds. Environmental impact assessment is now required for aquaculture drug approval in the United States [128]. Additionally, periodic monitoring of fish farm effluents for drug residues may be required. Therefore, methods of analysis of therapeutic agents in environmental samples are now part of the drug approval process and should be part of our continuing environmental concern.

Development of new methods of analysis of therapeutants was identified at a recent joint FDA-US Department of Agriculture (USDA) sponsored Interregional Research Project Number 4 (IR-4) meeting as a priority need in aquaculture [129]. In general, new methods for drug residue analysis in aquatic species must be suitable for screening, quantitating, and confirming tissue residues of drugs used domestically and drugs present in imported aquacultural products. The methods must also be suitable for analyzing environmental samples for drug and contaminant residues.

5. TISSUE RESIDUE PROBLEMS CAUSED BY PESTICIDES

Pesticides have been and continue to be applied extensively in the United States for agricultural purposes on animals, farmland, and forests and for mosquito control in urban areas. Many of these chemicals ultimately find their way into aquifers, rivers, lakes, and oceans mostly through transfer in the water itself, through adsorption to sediments and other organic layers in the water, or through air. In addition, because many aquaculture ponds are built on land that was formerly used for agriculture, the sediments and organic materials of these ponds could also contain high levels of pesticides.

Some of the pesticides that may be found in the environment are the chlorinated hydrocarbon, organophosphorus, carbamate, and pyrethrin/pyrethroid pesticides; chlorophenoxy acid, triazine, trichlorobenzoic acid, chlorophenol, and glyphosate herbicides; viticulture fungicides; and grain fumigants. The EPA has established tolerance levels for over 300 pesticides in various foods or food groups [130], and the FDA has determined action levels for many pesticides and their metabolites and degradation products in seafood [131]. Some of these pesticides are included in current seafood monitoring

programs conducted by groups such as the FDA and US National Marine Fisheries Service and also included in environmental monitoring programs conducted by groups such as the NOAA.

Most of the pesticides are readily degraded in the environment and therefore, are normally not a problem as tissue residues. However, some of the pesticides, especially the chlorinated hydrocarbons, such as DDT, are persistent in the environment and can be commonly found as residues in mammalian and aquatic species. Most of the chlorinated pesticides have been banned from wide-ranging use in this country for over twenty years, but they continue to be of concern to regulatory agencies because of their occurrence in food and their unknown health effects. Many are classified as suspected carcinogens [132].

In general, the persistence of the chlorinated pesticides and potential to undergo biomagnification, their continued use in some countries, and the concerns for their known and unknown toxicity make them a very important class for regulatory agency attention. State, federal, and international monitoring programs will need to continue to include this class of compounds in their testing for decades to come. Because efficient, cost-effective, universal methods for the extraction, detection, quantitation, and confirmation of these residues in aquatic matrices do not exist, a need for a better approach to analysis has recently been acknowledged. The research and development plans of regulatory agencies, such as the FDA [78,133] and the USDA Food Safety Inspection Service [134], currently include commitments to increase and improve capabilities for testing for pesticide residues.

6. FUTURE METHODS OF RESIDUE ANALYSIS

There have been recent advances in the field that offer several promising techniques as possible solutions to the problems caused by outmoded and complex analytical methods. Three techniques, supercritical fluid extraction (SFE), solid-phase extraction (SPE), and matrix solid-phase dispersion (MSPD), are receiving attention because they have the potential to greatly reduce analysis costs and reduce analyst-generated waste and pollution.

6.1. Solid-phase extraction

In the SPE process, a compound is isolated from a liquid sample based on its relative solubility in the liquid mobile phase compared to its solubility in a solid support-bound liquid stationary phase of differing polarity or to a solid support stationary phase of differing polarity. Isolation is accomplished by passing the analyte dissolved in solvent (organic or aqueous) through a column containing the stationary phase with subsequent elution using an appropriate solvent. Several solid-phase extraction methods have been developed to facilitate the extraction and cleanup of biological liquid and tissue samples.

For liquid matrices, acceptable residue recovery may be obtained using protein precipitation and direct injection of plasma without cleanup with SPE [11], but the many impurities present can affect the chromatogram and accumulate on the analytic column, thus resulting in increasing back pressure. SPE cleanup helps avoid these problems and works well with biological fluids such as plasma, urine, and cerebral spinal fluid. In addition, SPE extraction and analysis can be automated and done on-line [20–22] and/or with on-line dialysis and column switching.

Before SPE can be used with solid tissue (*e.g.*, muscle and liver), a separate homogenization step and often multiple filtration, sonication, centrifugation, and liquid–liquid cleanup steps are required. While SPE may improve cleanup of these solid tissue samples, the additional labor and materials costs make SPE less suitable, in some cases.

Solid-phase extraction methods published for fish tissues are often combinations of SPE with other methods such as homogenization, liquid–liquid partition, filtration, sonication, and centrifugation (Table 1). Because choice of SPE column depends on the matrix and on the particular compound of interest, a wide range of solid-phase columns of differing polarities have been used for drug extraction in fish and include C_2, C_8, C_{18}, NH_2, amberlite resins, and PLRP(polystyrene–divinylbenzene) polymers (Table 1).

6.2. Supercritical fluid extraction

With the SFE process, supercritical fluids [usu-

ally supercritical carbon dioxide ($SC\text{-}CO_2$)] are used in place of organic solvents to extract residues [135]. Carbon dioxide becomes a supercritical fluid if handled above its critical temperature and pressure. Because various chemicals and associated tissue lipids are soluble in the $SC\text{-}CO_2$, they are extracted and then collected once the pressurized CO_2 is brought back to atmospheric pressure. No large volumes of organic solvents are needed. One drawback to the procedure is that because the extracts contain contaminating lipids, a cleanup step is usually needed before samples can be injected onto instruments such as gas chromatography apparatuses. Cleanup is usually performed with gel permeation chromatography or adsorption chromatography with Florisil. In-line cleanup could be conducted by using disposable or reusable SPE cartridges or newer disc SPE technologies and changing the pressures of the supercritical fluid. Coupling this system directly to an LC–MS type interface or a GLC–MS interface could provide a complete analytical process for the desired analysis.

More work will be necessary to further develop this process. Its application to fish tissues [58] is quite limited. However, the process has the potential to provide a near solventless, in-line, automated process for the rapid analysis of the lipophilic chemical species from edible aquatic resources.

6.3. Matrix solid-phase dispersion

Of the three techniques being considered, matrix solid-phase dispersion, in particular, has the strongest potential to meet the demands of future residue monitoring of aquatic resources for drugs and pollutants.

In general terms, the process involves blending a tissue sample (0.1–1.0 g) with lipophilic polymer-derivatized silica particles [*e.g.*, octadecylsilyl (ODS)-derivatized silica (C_{18})], which simultaneously disrupts and disperses the sample. This mixture of C_{18} and tissue becomes part of a potentially multiphasic column that possesses unique chromatographic character. Elution of the MSPD column with a solvent or solvent sequence can provide a high-resolution fractionation of target analytes that can be further purified by simultaneous use of co-columns of Florisil, silica, or alumina. The final eluate can, in most cases, be directly analyzed or further concentrated or manipulated to meet the demands of the individual analysis. The extracts obtained from these methods are most often detected by LC (in the case with drugs) or GLC with electron capture detection or mass spectrometry (in the case with pesticides). However, they can also be used in immuno- [103] or receptor assays.

Additionally, the MSPD process is generic and can be modified for a particular application by (1) a change in the eluting solvent or solvent sequence, (2) use of a different polarity polymer or solid support, and (3) blending of the C_{18}/tissue in the presence of modifiers such as chelators, acids, bases, etc.

MSPD could also be used in conjunction with SFE. The water in biological matrices often interferes with the SFE process [135] and analysts have used samples blended with diatomaceous earth to remove water from the sample. However, blending samples first with polymer-coated silicas, as is done in the MSPD process, would remove water and provide an initial stage of fractionation at the point of elution of the analytes with supercritical fluid and modifiers.

In general, the three main advantages of MSPD are (1) it allows for rapid turnover of samples and hence, access to timely data on residue levels present in samples, (2) because of its required small sample size, it considerably decreases solvent use compared to the classical methods, which in turn decreases environmental contamination and increases worker safety, and (3) it is suitable to robotics automation. Therefore, MSPD has the potential to meet the future demands for conducting drug and pesticide analysis for large numbers and varieties of samples.

6.4. MSPD applied to aquatic resources

As seen in both Tables 1 and 2, MSPD has been used to provide for single or multi-residue analysis of various drugs and environmental contaminants in several aquatic matrices. Drugs isolated from aquatic animal tissues by this method include oxytetracycline [12] and sulfadimethoxine [15.103] from fortified channel catfish muscle and oxolinic acid as an incurred residue from channel catfish muscle and bile [29]. Reimer and Suarez [16] reported a multi-residue method for MSPD isolation and LC analysis of five sulfonamides in fortified salmon

muscle. Jarboe [30] has demonstrated its applicability to the isolation of incurred residues of nalidixic acid from channel catfish muscle and liver. Walker and Barker [103] evaluated the performance of several enzyme immunoassays for the detection of sulfadimethoxine residues using MSPD extracts of fortified channel catfish muscle as well. Other compounds used in aquaculture or related compounds have been extracted from various non-aquatic matrices using MSPD methods [136] and these methods could potentially be applied to aquatic matrices.

Pesticides extracted and isolated by this method include 14 chlorinated hydrocarbon pesticides from fortified whole oyster homogenate and crayfish hepatopancreas [43,44] and 9 chlorinated pesticides from fortified catfish muscle [45]. These methods are a significant advance in the ability to screen more samples due to their simplicity and efficiency.

7. DISCUSSION

Methods development for residue determination should focus on rapid screening tests, multi-residue capabilities, metabolite detection, and improved sensitivity [137]. Further, the use of determinative methods generally requires a method of isolating the compound(s) of interest from edible or marker tissues that is rapid, inexpensive, and does not generate large volumes of solvents for disposal. Classical isolation methods using homogenization and/or liquid–liquid partitioning of biological tissues and fluids may be sufficient for some applications but are poor for screening purposes because they are often lengthy, involving multiple steps and use large volumes of solvents (Table 1). Solvent disposal is becoming increasingly expensive and environmentally unsound. Therefore, methods using low solvent volumes are desirable. A main purpose of this review was to present a case for phasing out existing official methods in favor of newer technologies that require less sample, less solvent, less employee time, and less cost per sample. Newer techniques such as supercritical fluid extraction [135], solid-phase extraction (Tables 1 and 2), and MSPD (Tables 1 and 2) [136,138] offer alternative isolation strategies. When compared to the classical methods, these new methods greatly reduce labor and solvents costs and improve throughput. There are a few drawbacks to the new methods and more work is needed to further develop SPE, SFE, and MSPD for use with the many different types of matrices that may contain residues of chemical contaminants. However, of the three new methods, MSPD shows tremendous potential.

MSPD methods have been published for the isolation of a wide range of compounds in a variety of matrices indicating this approach may provide a generic technique for single and multi-residue extraction of drugs, environmental pollutants, and their metabolites. In particular, MSPD has already been used to provide a two-step process for the single- or multi-residue analysis of various drugs [12,15,16,29, 30,103] and chlorinated pesticides [43–45] in several aquatic matrices. This process, when compared to classical methods, has been estimated to reduce solvent use by approximately 98% and analysis time by 97%. Furthermore, once the MSPD column is prepared, the process of solvent elution, collection, and analysis can be automated by the use of robotics. Cost of analysis is decreased because less solvent is needed and fewer laboratory technicians need to undergo training. Safety and environmental protection are increased because less solvent is needed. Finally, data is generated more quickly because of the ease of the process and its potential to be automated. These features of MSPD make it a general and perhaps significantly useful method in designing future residue analysis screening programs for aquatic as well as other food animal resources.

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Review

Liquid–gel partitioning and enrichment in the analysis of organochlorine contaminants

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ABSTRACT

A review is given of methods for analysis of organochlorine compounds in which the lipophilic gel Lipidex is used for extraction and purification. Some of the compounds analysed are DDT, DDE, hexachlorobenzene (HCB), hexachlorocyclohexane (α-, β-, γ-HCH), dieldrin, oxychlordane, *trans*-nonachlor, pentachlorophenol, polychlorinated biphenyls (PCBs), including the non-*ortho* planar congeners, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and metabolites of chlorinated paraffins. Applications to analyses of human milk, cod liver oil, bile, urine, water and indoor air are discussed.

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1. INTRODUCTION

Organochlorine compounds are widely spread in the environment, and many of them constitute a danger to the environment and risks for our health. Such well-known contaminants include pesticides (*e.g.*, DDT, hexachlorobenzene, dieldrin, chlordane), industrially used products [*e.g.*, polychlorinated biphenyls (PCBs), naphthalenes (PCNs) and paraffins (CPs)] and unintentionally produced contaminants such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). These compounds possess several common properties; they are persistent, lipophilic and accumulate in mammals. The most toxic of the compounds, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been extensively studied, and a structure–toxicity relationship with this compound has been confirmed for many of the organochlorine aromatics [1,2]. Analysis of the organochlorine compounds in different compartments of the environment and in humans is of great interest in order to establish the present condition and time-related trends and to evaluate the effects of the contaminants. The multiplicity of compounds and the low levels to be analysed from complex matrices make the analytical work complicated. Generally, the analyses include the following steps: extraction of lipids and lipid-soluble compounds, isolation of organochlorine compounds from the bulk of the co-extracted material, purification and separation into groups for identification and determination of concentrations by electron-capture gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

A variety of methods have been used for the extraction of biological materials, and some reviews of the common methods have been published [3–5]. The classical partitioning between solvents of different polarity [6,7] has been modified and applied in many cases [8–10]. The drawback with this method is that several extractions must be made and emulsions may be troublesome. These problems can in some cases be eliminated by addition of sodium sulphate. The sample is mixed with sodium sulphate to a dry matter which is extracted by maceration [11] or shaking with a non-polar solvent [12,13], or it can be filtered or eluted [14,15]. The dry sample can also be transferred to a Soxhlet apparatus and refluxed with a non-polar solvent. This method is reported to give lower levels of PCBs than the method of saponification and subsequent liquid–liquid extraction [16]. In recent years, successful attempts to use supercritical extraction of biological [17–19] and environmental samples [20] have been described. However, problems with interfering contaminants from carbon dioxide have been reported.

After the preliminary isolation of the compounds, co-extracted material must be removed. Usually several purification steps are required to get the sample clean enough for GC or GC–MS analysis. In the case of biological samples, the first step is to remove lipids and other co-extracted components without losing the lipid-soluble organochlorine compounds. For this purpose saponification and treatment with sulphuric acid have been used. However, strong alkali degrades PCDDs and PCDFs [21,22] and concentrated sulphuric acid destroys certain pesticides, *e.g.*, dieldrin [23]. Column chromatography on aluminium oxide, silica gel, Florisil and activated charcoal is most frequently used for further purification and separation of the compounds. The activities of the adsorbents have been varied and the silica gel is sometimes impregnated with silver nitrate or sulphuric acid or modified to strong basic silicates [3–5,24]. Several techniques have been applied in order to improve the efficiency of the chromatographic systems. In this respect, favourable fractionation of organochlorine compounds from butterfat has been demonstrated using high-performance liquid chromatography (HPLC) with silica columns [5]. Gel-permeation chromatography with cross-linked polystyrene resins (Bio-Beads) has also been used for effective removal of lipids [26–28] and for separation of chlorinated paraffins from other halogenated compounds [29].

The identification of analytes and the determination of their levels are difficult even with clean extracts. There are a large number of chlorinated compounds with similar chemical and physical properties, and not all the compounds are separated by any available single GC column. Furthermore, the concentrations of the compounds to be analysed may differ in the range of ppm to ppt (10^{-6} to 10^{-12}, w/w), making the quantitation of the lower concentrations in the mixture impossible. Therefore, the compounds are separated into groups suitable for further analysis. Such group separations are achiev-

ed by column chromatography using the abovementioned adsorbents in different combinations. Activated charcoal has generally been used for separation of planar (PCDDs, PCDFs and certain PCBs) from non-planar compounds [24,29–32]. Recently, Haglund *et al.* [33] managed to separate PCBs and PCNs into groups, characterized by the planarity of the substances, using HPLC on 2-(1-pyrenyl)ethyldimethylsilylated silica.

Usually pesticides and PCBs are analysed in the same samples and separate analyses are performed for *e.g.* PCDDs and PCDFs. With the increasing number of pollutants and the possibility of interaction between compounds in their toxic activity, there is a need for multicomponent methods for analysis of a range of analytes in the same sample. For this purpose methods have been developed for simultaneous analysis of a large number of halogenated compounds [29,31,34].

Simple and effective methods are needed for extraction and purification of biological samples for multicomponent trace analysis by GC and GC–MS. In order to replace the laborious liquid–liquid extractions in separatory funnels, other techniques for partitioning have been developed. A liquid–liquid partition method in which one phase is supported on macroporous diatomaceous earth has been used for extraction of milk with light petroleum saturated with acetonitrile [35] and for extraction of oil and fat samples with hexane–acetonitrile mixtures [36]. Solid phase extraction using octadecylsilane-bonded (ODS) silica has been used in the analysis of, for example, water [37] and serum [38].

In our laboratory liquid–gel partition using the lipophilic gels Lipidex 1000 and 5000 is employed for extraction and preliminary purification of biological samples. These gels have been successfully used in the analysis of steroids and bile acids and have also been found to be efficient for other lipid-soluble compounds. Depending on the sample matrix, different procedures have been developed for the transfer of analytes into the gel. The present paper reviews procedures for enrichment on Lipidex in the analyses of organochlorine contaminants.

2. LIPIDEX GELS

2.1. Structure

Lipophilic gels were originally synthesized by methylation [39] or hydroxypropylation of the hydroxyl groups of the cross-linked dextran matrix of Sephadex [40,41]. The hydroxypropylated product, Sephadex LH-20 (Pharmacia, Uppsala, Sweden), has been additionally substituted in order to obtain gels with different functional groups and properties. The Lipidex gels are derivatives of Sephadex LH-20 in which the hydroxypropyl groups have been reacted with long-chain epoxides [41,42] to introduce non-polar alkyl substituents. Two neutral lipophilic gels, Lipidex 1000 and Lipidex 5000, are commercially available from Packard Instruments, Downers Grove, IL, USA. Similar gels are also available from Sigma, St. Louis, MO, USA, as hydroxyalkoxypropyl dextrans. Lipidex 1000 contains 10% and Lipidex 5000 contains 50% (w/w) hydroxyalkyl groups. The higher degree of substitution makes Lipidex 5000 hydrophobic and less polar than Lipidex 1000. The gels are delivered in methanol. Before use they are washed with aqueous ethanol at 70°C and ethanol to remove contaminants from the synthesis [43]. The gels can also be re-used after appropriate washing.

2.2. Properties and mechanisms

The properties of Lipidex and the mechanisms for distribution of compounds in liquid–gel chromatography have been discussed and reviewed [39,41,44]. The Lipidex gel consists of a three-dimensional network. The hydrophobic substituents make the gel repel water and contract in polar solvents while swelling in less polar organic solvents. The bed volume varies with the polarity of the solvent and can be modified by mixing different solvents. The distribution of analytes between the mobile (solvent) and gel phases depends on the polarities of the analytes, the solvent and the gel. Extraction of aqueous solutions with Lipidex gels closely resembles extraction with a solvent of medium polarity and differs markedly from extractions with ODS silica and Amberlite XAD-2, in which adsorption occurs to both polar and non-polar sites. In aqueous media, Lipidex 1000 and 5000 have a higher selectiv-

ity for non-polar compounds than, for example, ODS silica and other solid sorbents [44]. This is an advantage, since cleaner extracts are obtained when analysing non-polar compounds. This is also seen from the yield of organic compounds from urine: the two solid sorbents extracted 2–3% of the total dry weight, while Lipidex 1000 extracted less than 0.1% [45]. The size-exclusion properties of the gel matrix are important for exclusion of macromolecules from the extract but are of little discriminatory importance for the extraction of compounds of medium molecular size.

3. APPLICATIONS

Owing to their selectivity and high capacity, Lipidex gels have been applied in several areas of analysis. In our laboratory, Lipidex 1000 has been successfully used for extraction and purification of steroids of low and medium polarity from different biological samples, *e.g.*, urine [45], tissues [46] and human milk [47]. Bile acids have been extracted from aqueous media as ion pairs [48]. The more hydrophobic Lipidex 5000 has been used for extraction of tetrahydrocannabinol from human fat [49]. An automated method using lipophilic ion-exchanging gels has been developed for enrichment of bile acids and metabolites of mono(2-ethylhexyl) phthalate from urine [50]. This indicates future possibilities for development of simpler procedures for sample treatment using these gels for extraction and purification.

The Lipidex gels have also found several applications in the analysis of organochlorine contaminants. Lipidex 1000 has been used for separation of the more polar conjugated metabolites from the non-polar unconjugated products of chlorinated paraffins [51]. In the early studies, Lipidex 1000 was shown to be an efficient sorbent for fat and *p,p'*-DDT from cow's milk [52]. In this experiment *p,p'*-[^{14}C]DDT was added to the milk and the pesticide could be eluted and separated from 90% of the lipids. In subsequent studies, the more hydrophobic Lipidex 5000 has been used for extraction and purification of organochlorine pesticides, PCBs, PCDDs and PCDFs [31,32,34,53–55].

The gels can be used for chromatography both with normal- and reversed-phase systems [41]. The extraction of compounds with Lipidex is performed in a reversed-phase mode, while the purification and separation can be performed in a normal-phase system. The distribution of organochlorine compounds in a normal-phase system from Lipidex 5000 is shown in Table 1. A mixture of compounds was applied on top of a column of 5 g of Lipidex 5000 prepared in hexane. The gel was eluted with hexane and then with dichloromethane–hexane (1:1). The

TABLE 1

ELUTION OF ORGANOCHLORINE COMPOUNDS FROM LIPIDEX 5000

Recoveries in 5-ml fractions of hexane (fractions 1–20) and dichloromethane–hexane (1:1) (fractions 21–24). + = Compound is present but was not quantified.

Compound	Recovery (%) in fractions											
	1	2	3	4	5	6	7	8–20	21	22	23	24
Hexachlorobenzene		16	84									
α-HCH					86							
β-HCH											56	44
γ-HCH						54	46					
trans-Nonachlor				90								
Heptachloroepoxide				97								
Dieldrin				100								
p,p'-DDT			+	71								
p,p'-DDE			90									
p,p'-DDD						84						
PCBs (Clophen A50)			+	+								
Camphechlor			+	+	+							

separation of dieldrin and β-HCH from other compounds has been used as a purification step in the GC analysis of breast milk [34].

The partitioning of compounds from a sample solution into the Lipidex gel is dependent on the combined effect of the nature of the gel, the solvent associated with the network of the gel and the analyte. Thus, the efficiency of transfer can be modified by rinsing the gel before use with an appropriate solvent. In the analysis of organochlorine contaminants, the Lipidex was washed with methanol [34,53] or 2-propanol [32] and the solvent was removed by suction. The "dry" gel still having some enclosed polar solvent in the matrix was used in the analysis. The need for 2-propanol in Lipidex for quantitative yields of non-polar lipids was first noted in studies on the extraction of sterols and sterol esters in plasma [56].

4. EXTRACTION PROCEDURES

Because of the widespread occurrence of organochlorine contaminants, the nature of the samples to be analysed varies greatly. It is an advantage if analytical strategies can be used which are independent of the nature of the sample. One way to achieve this is to bring all samples into a common form before the start of the purification and separation steps. The aim of our studies is to design methods to transfer the contaminants from different types of samples into Lipidex gels from which column beds can be prepared for subsequent washing and elution.

4.1. Filtration of fluids

4.1.1. Bile

The simplest method for enrichment and purification is to filter a solution through a column bed of the gel [44,45,48,52]. This method was applied in a study of metabolites of chlorinated paraffins in bile [51]. A column bed of Lipidex 1000 in methanol was washed with water. The bile was diluted with 0.6 *M* acetic acid and filtered through the column. The metabolites conjugated with N-acetylcysteine and glutathione were retained and could be eluted with methanol. The unconjugated material was mainly eluted with methanol–chloroform. The more polar conjugated bile acids, which are present in high concentrations in bile, are not retained. Thus, they are removed and will not interfere in subsequent analytical steps.

4.1.2. Water

The somewhat less polar Lipidex 5000 was used in the analysis of pentachlorophenol (PCP) in water and urine [53]. The gel was washed with methanol and the solvent was removed by suction. The gel (1 g) was transferred to a chromatographic column and washed with 20% methanol in water. The sample of water (100 ml) was mixed with 2 ml of formic acid and passed through the gel. The column was then eluted with 10 ml of 20% and 5 ml of 50% methanol in water. This gives a gradual change of the gel character with subsequent swelling of the gel and enables elution with a non-polar solvent. The aqueous solvent in the void volume was expelled with nitrogen and the retained DDT and TCDD were eluted with 35 ml of hexane. When 1 ml of hexane had passed through the column, the flow was stopped for 10 min to permit maximum swelling of the gel in this solvent. Finally, PCP was eluted with 10 ml of acetone. The PCP was derivatized and analysed by GC.

4.1.3. Urine

Non-hydrolysed and hydrolysed urine samples (2 ml) have been analysed for PCP [53]. The hydrolysis was performed with hydrochloric acid or enzymatically with *Helix pomatia* digestive juice. The samples were then treated in the same way. Formic acid (0.5 ml) was added to the hydrolysate and the mixture was passed through a bed of 2 g of Lipidex 5000 followed by 50 ml of water, 10 ml of 50% and 25 ml of 70% methanol in water. Nitrogen was blown through the column to expel the remaining polar solvent and the elution was then performed with 35 ml of hexane and 15 ml of acetone. The elution with 70% methanol and hexane efficiently removed the bulk of different endogenous compounds and interfering substances to give an extract which was sufficiently clean for GC analysis of PCP after derivatization. This method exemplifies the combination of a reversed-phase mode for partitioning of the compounds into the gel and normal-phase elution for purification of the sample on the same Lipidex bed.

4.2. Filtration of gases

4.2.1. Indoor air

Pentachlorophenol is very strongly sorbed to Lipidex and was shown to be directly transferred from laboratory air to Lipidex [53]. A flow of air through a column (2 cm I.D.) containing 5 g of Lipidex 5000 was obtained by suction. The outlet of the column was connected to a flask containing isooctane and ended in the solvent. At different time intervals the sampling was stopped and the column was eluted with 50 ml of hexane to eliminate most of the non-polar compounds and then PCP was eluted with 50 ml of methanol. However, this fraction was not clean enough for GC analysis and purification was achieved by treatment with sulphuric acid. The linear increase of PCP with time of sampling clearly indicated a constant level of PCP in the laboratory air [53].

4.3. Batch extraction

4.3.1. Human milk

Milk has a high fat content and the non-polar lipids are present in chylomicrons surrounded by a layer of phospholipids and proteins. This layer has to be disrupted for the lipids to be extracted, and conditions for quantitative extraction by a fast filtration method have not yet been established. Instead, the fat and fat-soluble compounds can be partitioned into the gel by shaking a mixture of milk and Lipidex under suitable conditions [34]. The extraction was performed in a flask (Erlenmeyer shape) with a PTFE-lined screw cap. A 10-ml aliquot of milk in the flask was mixed with 10 ml of formic acid and 5.0 g of Lipidex 5000 (washed with methanol and dried by suction) was added. The flask was placed at 35°C and shaken for 2.5 h. The mixture was then poured into a glass column (2 cm I.D.) and the solvent was drained. The resulting gel bed was washed with 40 ml of 30% methanol followed by 50 ml of 50% methanol in water. The organochlorine compounds and part of the lipids were eluted with 75 ml of acetonitrile. Remaining lipids were eluted with 60 ml of chloroform–methanol–hexane (1:1:1, v/v/v). The acetonitrile fraction was submitted to further purification and separation steps (chromatography on aluminium oxide, silica gel, activated charcoal) before analysis by GC and GC–MS. Details of the method are described in refs. 31 and 34. The addition of formic acid to the milk was essential for complete transfer of organochlorine compounds and lipids into the gel. Formic acid decreases the binding of chlorinated compounds to proteins [57]. Probably it disrupts the chylomicrons and facilitates the extraction of organochlorine compounds in the lipid core. The slightly elevated temperature keeps the lipids dissolved and the sample mixture uniform. The method has been used in trend studies of organochlorine contaminants, including DDT, DDE, hexachlorobenzene (HCB), isomers of hexachlorocyclohexane (HCH), dieldrin, oxychlordane, *trans*-nonachlor, pentachlorophenol and congeners of PCBs, PCDDs and PCDFs, in milk from individual and pooled samples from Swedish mothers, collected from 1972 to 1989 [31,54,55], and in studies of the correlation of levels with the fat content of milk [54]. Milk from the early sampling periods, 1972–1980, was previously analysed by a method using liquid–liquid partitioning [9]. The agreement between the two methods was established by analysing the same milk by both methods [34]. By the retrospective analysis of stored milk it was possible to calculate the time-related changes in the levels of compounds (PCDDs, PCDFs, planar PCBs) for which there were no techniques available at the time of sample collection [54,55] (Tables 2–4).

4.3.2. Cod liver oil

An oil or solution of oil cannot be filtered through Lipidex or treated in the same way as milk. Because of the non-polar character of both the sample and the gel, there will be no transfer of non-polar compounds to the gel phase. However, the polarity of an oil solution in hexane can be changed by addition of 2-propanol and water, which causes a partitioning of the organochlorine compounds into the Lipidex. This is demonstrated in the analysis of PCBs in cod liver oil [32]. The oil (10 g) was dissolved in hexane (100 ml) and a 2-ml aliquot was subjected to analysis. The sample was mixed with 15 ml of 2-propanol in an Erlenmeyer flask and 5.0 g of Lipidex 5000 (washed with 2-propanol and dried by suction) were added. During the extraction procedure, water (40 ml) was added from an attached dropping funnel equipped with a pressure equalizer at a rate of 0.5 ml/min. The extraction was per-

TABLE 2

MEAN LEVELS OF PCDDs AND PCDFs IN HUMAN MILK FROM SWEDISH MOTHERS

All values are pg per g of fat.

Year	1972	1976	1980	1985	1989
Number of mothers	227	245	340	102	100
2,3,7,8-Tetra-CDD	5	5	3	1	3
1,2,3,7,8-Penta-CDD	9	7	6	5	7
1,2,3,6,7,8-Hexa-CDD	45	40	31	30	38
1,2,3,4,6,7,8-Hepta-CDD	119	96	70	69	57
Octa-CDD	458	371	338	244	268
2,3,7,8-Tetra-CDF	4	3	3	2	2
2,3,4,7,8-Penta-CDF	32	29	17	14	17
1,2,3,6,7,8-Hexa-CDF	14	14	8	8	7
1,2,3,4,6,7,8-Hepta-CDF	24	21	7	8	8
Octa-CDF	6	4	5	5	2

formed in a water bath at 35°C for 2.5 h. The mixture was then transferred to a glass column and subsequently eluted as described for milk. This procedure separated about 60% of the lipids from the PCB-containing fraction (eluted with acetonitrile). Further purification and separations were made prior to analysis by GC and GC–MS [32]. The method was successfully used in an international intercalibration of methods for analysis of planar PCBs.

TABLE 3

MEAN LEVELS OF TOXIC NON-*ORTHO* PCBs IN HUMAN MILK FROM SWEDISH MOTHERS

All values are pg per g of fat.

Year	1972	1976	1980	1985	1989
Number of mothers	195	204	431	102	140
3,3′,4,4′-Tetra-CB (PCB 77)	76	41	29	35	27
3,3′,4,4′,5-Penta-CB (PCB 126)	298	253	166	102	98
3,3′,4,4′,5,5′-Hexa-CB (PCB 169)	67	74	65	43	47

TABLE 4

MEAN LEVELS OF TOXIC MONO-*ORTHO* PCBs IN HUMAN MILK FROM SWEDISH MOTHERS

All values are pg per g of fat.

Year	1972	1976	1980	1985	1989
Number of mothers	135	153	431	102	140
2,3,3′,4,4′-Penta-CB (PCB 105)	15	16	8	6	7
2,3′,4,4′,5-Penta-CB (PCB 118)	60	46	31	20	25
2,3,3′,4,4′,5,-Hexa-CB (PCB 156)	20	19	13	11	13

5. CONCLUSIONS

The liquid–gel partitioning technique is advantageous compared with liquid–liquid partitioning as no emulsions are formed and the passage of sample through a column bed replaces repeated extractions and centrifugations. By suitable modifications of the liquid–gel partitioning procedure organochlorine contaminants from different sample matrices can be transferred into the gels. In this way a common starting point is obtained for subsequent purification, and analytical strategies can be similar for many types of samples. The structure and polarity of the gels permit sequential use of solvents in the reversed- and normal-phase modes, so that the extraction–transfer step can be combined with separation and purification. Finally, the methods have been developed for small sample sizes permitting the use of small column systems and solvent volumes.

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Sandwich-type extraction column with on-line sulphuric acid treatment for the determination of organochlorine compounds in vegetable oil or oil seeds by gas chromatography with electron-capture detection

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ABSTRACT

An effective clean-up technique for the determination of hexachlorobenzene (HCB), α-hexachlorocyclohexane (HCH), β-HCH, γ-HCH (lindane), heptachlor, heptachlor epoxide, aldrin, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT and endosulfan in vegetable oils and oil seeds by gas chromatography with electron-capture detection is described. The separation of these organochlorine pesticides from normally co-extracted fatty material of plant origin was achieved by the use of a new "sandwich"-type extraction column, allowing adsorption of polar matrix interferences on Florisil, Extrelut and sodium sulphate and "on-line" degradation of usually at least partially co-eluting triglycerides with sulphuric acid in a single step. Quantification down to the 1–5 ppb level was performed by external or internal standard calibration using pentachlorobenzene and Mirex as internal standards.

INTRODUCTION

The contamination of vegetable oils with organochlorine pesticides (OCPs) is caused by the uptake of traces of pesticides during the growth period of plants from the surrounding biocompartments such as soil, air and water. Owing to the poor solubility of OCPs in water [1], there is particular concern over the long-lasting adsorption of traces of pesticides onto colloids in the soil and over specific transportation phenomena of highly volatile and sublimable organochlorine compounds in the air [2,3].

Contamination levels can be reduced by refinement of the oil [4], hence also partly decomposing the content of valuable highly unsaturated fatty acids and vitamins, which is provided by cold pressing of the oil seeds such as olives and many other seeds. However, this may have to be paid for by a higher content of OCPs.

The method presented in this paper was specially designed and evaluated for the determination of OCPs in Styrian pumpkin seeds or pumpkin seed oil, but proved also to be a general sample pretreatment technique for various oil seed extracts and vegetable oils.

The Styrian pumpkin, *Cucurbita pepo* var. *styriaca*, is cultivated in some agricultural regions in Styria (Austria), Slovenia and Hungary only and is used for the production (by a special cold pressing procedure) of characteristic dark-coloured, aromatic tasting salad oil distinguished by its high content of unsaturated fatty acids (about 50% linoleic acid [5,6]). Pumpkin seeds have been used in Europe for a long time as a remedy for prostate gland problems due to the content of particular phytostertols [7,8] and have recently becoming popular as snacks (comparable to peanut snacks).

Traces of hexachlorobenzene (HCB) and many other OCPs have been detected in such products and the extracted oil. In most countries the legislative authorities set limits and regulations for OCPs

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in oil seeds (*e.g.*, for Austria 250 ppb (w/w) for HCB, 20 ppb for lindane and 10 ppb for other OCPs in oil seeds [9]), which have to be very low considering the high potential for accumulation of these compounds in all human fatty tissues with particular concern over OCP accumulation in human milk as recently reported in many European countries [10–15].

For monitoring traces of HCB and OCPs in various lipids and oils of plant origin, highly sensitive chromatographic methods have to be employed. For providing reliable results the lipid-soluble contaminants have to be accurately separated from the co-extracted fatty plant material (*e.g.*, long-chain alcohols, fatty acids and esters), which is usually the most painstaking part of the analytical procedure. If not carried out properly, sensitive GC capillaries and detectors can deteriorate and/or false-positive results are obtained owing to various chromatographic interferences.

An effective technique for the separation of analytes from lipids is chemical degradation of the main matrix components by saponification with strong bases or acids as with a potassium hydroxide–alcohol mixture [16–18] or concentrated sulphuric acid [19,20], forming more polar products that can be easily washed out of the organic sample extract with water. The applicability of this method is limited owing to the resistance of the analytes to strong bases and acids, which is, however, fulfilled by many organochlorine compounds.

Clean-up by adsorption chromatography, also termed solid-phase extraction (SPE), is the most common method and has official status in many countries (AOAC [21]). Lipids are retained in an extraction column filled with polar sorbents and the analytes are eluted with organic solvents. Suitable adsorbents are alumina [22], magnesia [17,23,24], Florisil [18,23,25] and plain silica [20].

Gel permeation chromatography (GPC) on Bio-Beads SX-3 (a polystyrene gel) with ethyl acetate–cyclohexane [26–30] utilizes the difference in the molecular size of pesticides (M_r 200–400) and lipids (M_r 600–1500) for the accurate and moderate separation of analytes and matrix interferences [31,32].

For routine monitoring of OCPs in vegetable oil extracted or pressed from pumpkin seeds, an accurate, simple and rugged sample purification method is particularly needed owing to the exceptionally high content of various matrix compounds of plant origin such as phytosterols, chlorophylls (unique among all other oil seeds [33]) and many others besides the usual triglycerides. For reliable OCP determination we therefore had to combine different clean-up techniques previously reported for other vegetable oils and directed our attention especially to problems concerning the determination of OCPs using external and internal standard calibration.

EXPERIMENTAL

Instrumentation and chromatography

All analyses were performed using a Hewlett-Packard HP 5890 A gas chromatograph equipped with a ^{63}Ni electron-capture detector and a fused-silica column (30 m × 0.25 mm I.D.) coated with 0.25-μm cross-bonded 65% dimethyl–35% diphenylpolysiloxane (RTX-35; Restec) or alternatively for peak identification a fused-silica column (25 m × 0.25 mm I.D.) coated with 0.25-μm cross-linked 95% dimethyl–5% diphenylpolysiloxane (RTX-5; Restec). The carrier and make-up gases were nitrogen (5.0 quality) (TEGA) equipped with a moisture and oxygen trap at 18 p.s.i. (125 kPa) column head pressure (corresponding to a flow-rate of 55 ml/min at split vent) and a 55 ml/min make-up flow-rate. The septum purge flow-rate was 0.3 ml/min. A 1-μl volume of the sample was injected using an autosampler (HP 7673 A) equipped with a 10-μl Hamilton syringe into a capillary inlet with glass liner and silanized glass-wool in the splitless mode with a split delay of 60 s. The temperatures of the injector and detector were 290 and 350°C, respectively. The oven temperature was held at 60°C for 1 min followed by temperature programming to 220°C at 20°C/min, then to 230°C at 3°C/min and to 290°C at 6°C/min and finally held at 290°C for 2 min. The GC conditions for peak identification on the RTX-5 capillary were 60°C for 1 min, programmed to 290°C at 10°C/min and finally held at 290°C for 5 min. For data storage and integration an HP ChemStation 5895 A was employed.

Standards and reagents

Solvents (acetonitrile, hexane) were of Pestanal® quality from Merck (Darmstadt, Germany). Light petroleum (b.p. 40–60°C) was of Pestanal quality from Riedel-de Haën (Seelze, Germany). Extrelut

(a polar macroporous magnesia–silicate material) was purchased from Merck. Florisil of research grade (0.15–0.25 mm, 60–100 mesh, specific surface area 298 m^2/g, 15% MgO, 84% SiO_2, <1% Na_2SO_4) was obtained from Serva (Heidelberg, Germany). H_2SO_4 (95–97%, 36 *M*), anhydrous Na_2SO_4 and ethanol (96%) were of analytical-reagent grade. Pesticide standards, mix IV [α-HCH, β-HCH, γ-HCH (lindane), HCB, heptachlor, heptachlor epoxide, endosulfan I] and mix V (2,4′-DDD, 2,4′-DDE, 2,4′-DDT, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, dieldrin, endrin), 1 ng/μl each in cyclohexane, and all single standard compounds (10 ng/ μl each in cyclohexane) were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Methods

All solvents were monitored for possible OCP traces prior to use by GC with electron-capture detection (ECD). After concentration by a factor of 50 the OCP contamination was found to be below 0.01 ppb. The Florisil purity was tested by extracting a 7-g amount of Florisil with 20 ml of light petroleum and, after concentration to 1 ml, injecting a 1-μl volume into the GC–ECD system. The resulting chromatogram demonstrated the lack of any ECD-sensitive compounds. The same results were obtained for anhydrous sodium sulphate. Similar extraction of Extrelut revealed interferences in the retention window from β-HCH to heptachlor epoxide, which were completely removed by loading the material with concentrated sulphuric acid. Any contact of samples or sample extracts with materials made of paper or plastic was avoided, as these materials contained light petroleum-extractable ECD-sensitive compounds (EOCls, phthalates and others) interfering especially with β-HCH and 4,4′-DDE determinations, often leading to false-positive results. Therefore, only PTFE-lined stoppers (Soxhlet apparatus) and crimp caps (autosampler vials) were used and all Soxhlet extraction cartridges were pre-extracted by Soxhlet extraction for 4 h with 96% ethanol prior to use. Standard solutions could be stored in silylated glassware (preventing adsorption of traces of OCPs on the large glass surface) at −18°C in a freezer for at least 3 months.

Elution of OCPs from the "sandwich"-type extraction columns described in this paper was performed with a special eluent of constant polarity. We used the "upper phase" of a two-layer system obtained by simply mixing light petroleum, acetonitrile and ethanol (100:25:5), which represents a saturated solution of acetonitrile in light petroleum with ethanol as an emulsifier [25]. The exact composition of the "upper phase" was determined by GC with flame ionization detection (FID) and is given in Table I. It should be emphasized that the composition of the "upper phase" was always guaranteed by simply mixing the components in the given proportions (assuming constant room temperature and an appropiate equilibrium time of 12 h). The benefits of this eluent are discussed later.

Sampling

Harvested seeds as sunflower, rape, poppy, soya, thistle, linseed, zulunut (*Cyperus esculentus*) and Styrian pumpkin seeds were dried after harvest at ambient temperature in special rooms on each farm to a humidity of about 10%. For pumpkin seed analysis the entire field crop (500–900 kg/ha) was mixed and after removing of the hulling materials by special machines stored at ambient temperature in metallic containers in a large store. A representative mixed sample of at least 500 g was transferred to the analytical laboratory in paper bags after sampling ten aliquots from different positions in each container.

TABLE I

COMPOSITION OF ELUENT FOR SPE–LPE EXTRACTION OF ORGANOCHLORINE PESTICIDES FROM "SANDWICH"-TYPE EXTRACTION COLUMN

Component	Retention time (min)[a]	Composition (%, v/v)	
		"Upper phase"[b]	"Lower phase"
Light petroleum	5.4–5.8	96.0	23.8
Ethanol	9.7	0.9	10.5
Acetonitrile	12.5	3.1	65.7

[a] GC–FID on a 50 m × 0.32 mm, 0.52μm film thickness, FFAP capillary. Temperature programmed from 50 to 60°C at 3.5°C/min, held for 1 min, then increased to 80°C at 3.0°/min.

[b] Eluent for SPE extraction of organochlorine pesticides (mainly light petroleum saturated with acetonitrile and ethanol).

A 50-g aliquot of each pumkin seed sample was finely ground with a grain mill. The degree of fineness was adapted with care to avoid any heating of the mill, otherwise large OCP losses could occur owing to their volatility.

Extraction

In an appropriate Soxhlet extraction cartridge a 10-g aliquot of ground pumpkin seed granulate was mixed with 10 g of anhydrous sodium sulphate and extracted with 180 ml of light petroleum by a 4-h Soxhlet extraction. The extractable content was between 45 and 55% (w/w). After cooling, the extract was concentrated to about 8 ml by rotary evaporation at ambient temperature and reduced pressure. The oleaginous extract was transferred into a calibrated vial, diluted to 10 ml with light petroleum and can be stored at 5°C in a refrigerator for several days.

This procedure worked equally well for all other oil seeds.

Clean-up

A 1-ml aliquot of the above-described (pumpkin) seed extract, which represents a 1:1 dilution of the oil extract with light petroleum) or a 1-ml aliquot of a 1:1 dilution of pumpkin seed oil or any vegetable oil was transferred to the top of a prerinsed (with 15 ml of "upper phase"; for composition see *Methods*) "sandwich-type" extraction column (see Fig. 1) followed by penetration into the column. After a delay time of 10 min the column was rinsed with 2 × 10 ml of "upper phase" (method I) or 3 × 10 ml of "upper phase" (method II), whereby only the remaining OCPs were eluted. The total eluate was collected in a conical vial with a 1-ml calibration mark, concentrated by a gentle stream of nitrogen at room temperature to about 0.6–0.8 ml and finally diluted to 1 ml with light petroleum. The final sample extract was transferred to an autosampler vial with PTFE-lined crimp cap and could be stored in a refrigerator at 5°C for at least 2 weeks prior to GC analysis.

RESULTS AND DISCUSSION

Sample pretreatment and clean-up

The off-line treatment of fatty samples and oil seeds with concentrated sulphuric acid [19,20] and the use of SPE clean-up with Florisil as an adsorptive matrix for the determination of OCPs has been reported by several workers [18,23,25]. In this work, a method was developed utilizing the advantages of Florisil and Extrelut as adsorptive materials for sample clean up and the effective removal of triglycerides with concentrated sulphuric acid by an online technique using a new "sandwich"-type extraction column.

The preseparation of non-volatile oleagenous compounds from OCPs is essential for reliable and

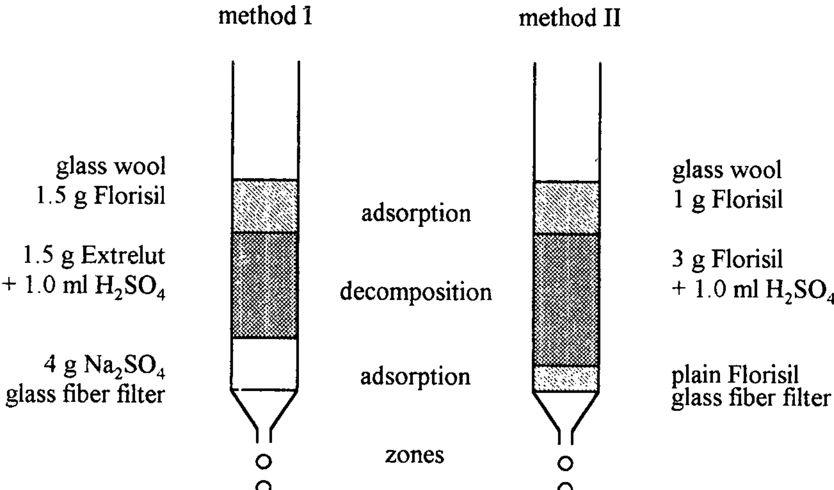


Fig. 1. "Sandwich"-type clean-up columns performing solid-phase extraction (SPE), on-line treatment with concentrated sulphuric acid and liquid-phase extraction (LPE) for the determination of OCPs in vegetable oils. Dimensions of glass column, 200 mm × 9 mm I.D.

rugged capillary GC–ECD of OCPs to prevent uncontrolled adsorption phenomena in the injector inlet or deterioration of the film coating of the GC capillary. However, preloading of the adsorption material in the centre of the specific clean-up column with concentrated sulphuric acid allows complete saponification and decomposition of triglycerides and oxidation of mucilaginous compounds without deterioration of the analytes of interest (with the exception of dieldrin and endrin). The resulting more polar reaction products are essentially not extractable with light petroleum and remain in the sulphuric acid layer and/or are adsorbed on the lower sodium sulphate (method I) and Florisil (method II) layers. The described "sandwich"-type clean-up column offers and combines two different separation techniques: (a) solid-phase extraction (SPE), more correctly termed liquid–solid-phase extraction (LSPE) between the eluent and the Florisil packing material and (b) liquid-phase extraction (LPE) via liquid–liquid partitioning between the non-polar eluent and the polar sulphuric acid layer adsorbed (loaded) on the Extrelut or Florisil surface.

The effectiveness of purification from other interfering matrix components, the recoveries and elution behaviours of several OCPs using this combined SPE–LPE clean-up system using magnesia–silicate materials such as Florisil (method II) and Extrelut (method I) have been studied.

The two "sandwich-type" extraction columns depicted in Fig. 1 revealed similar "clean" extracts with a minimum of ECD-sensitive compounds but not interfering with the OCP analytes. Nevertheless, the higher adsorption strength of Florisil (method II) resulted in high elution volumes and therefore a higher solvent consumption and an increase in sample clean-up time, which makes the column in method I more favourable.

In both instances a top layer of 1.5 g of Florisil was successful in adsorbing first the chlorophyll, revealed by the dark green colour of this zone and further preventing the column bed from plugging, which was particularly convenient when using Extrelut columns.

Elution of OCPs from Florisil or silica gel columns is usually performed with light petroleum, hexane or 6% diethyl ether in hexane [18]. However, the polarity of the adsorbents has to be preadjusted and standardized for reliable results and constant recoveries for pesticides; this could be achieved by using a polar prerinsing eluent as the "upper phase" of a two-layer system of light petroleum–acetonitrile–ethanol (100:25:5). By this preadjustment of the polarity of the adsorbents, which is otherwise usually carried out by laborious and time-consuming procedures (high temperature treatment of the adsorbents and re-equilibration with polar solvents), the sample work-up procedure is essentially simplified.

Table II presents the elution profiles and total recoveries of OCPs (100 ppb each in pumpkin seed granules) by elution of the adsorbed OCPs from Extrelut (method I) or Florisil (method II) "sandwich"-type extraction columns with light petroleum or "upper phase", revealing better recoveries and less solvent consumption for the Extrelut "upper phase" technique (method I) for most of the investigated OCPs except γ-HCH and endosulfan I.

Fig. 2A shows the profile of OCPs in pumpkin seeds after clean-up according to method I and final GC–ECD analysis. MS detection with electron impact (EI) ionization specific for each pesticide or co-chromatography (Fig. 2B) on a second capillary (RTX-5) was used for peak identification, showing inversion of retention for HCB and α-HCH and for 4,4′-DDT and 4,4′-DDD.

The described SPE–LPE technique with on-line sulphuric acid treatment failed only in the determination of dieldrin and endrin, owing to irreversible deterioration of these compounds by oxidation to undefined products, which could not be detected by GC–ECD. However, the incidence of these pesticides in Austrian oil seeds is rare. Nevertheless, one way of separating dieldrin and endrin from fatty products was to use SPE on Florisil (Fig. 1, method II) with "upper phase" elution but with omission of sulphuric acid treatment. The extracts obtained were relatively "clean" and ready for injection into the GC column, but it turned out that they were not completely free from triglycerides and therefore this method cannot be recommended for long-term routine analysis, owing to the decrease in detector sensitivity and deterioration of the separation efficiency of the GC capillary.

Nevertheless, the combination of decompositional (SPE–LPE) and non-decompositional (SPE) methods can be used as "chemical proof" for the

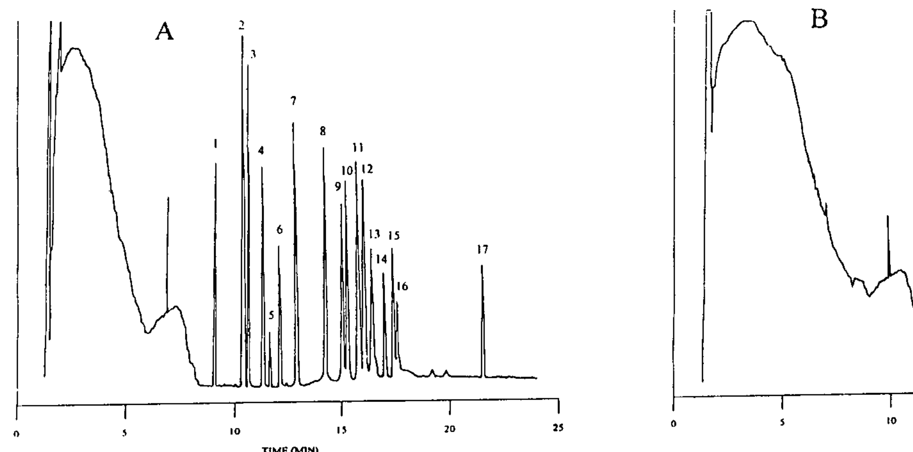
TABLE II

RECOVERIES AND ELUTION PROFILES OF ORGANOCHLORINE PESTICIDES FROM "SANDWICH"-TYPE SPE–LPE COLUMNS WITH DIFFERENT ELUENTS

A = Method I (Extrelut) with light petroleum; B = method I with "upper phase"; C = method II (Florisil) with light petroleum; D = method II with "upper phase". "Upper phase" = supernatant phase of light petroleum–acetonitrile–ethanol (100:25:5).

Compound	Pesticide recovery (%)																							
	A						B						C						D					
	Total recovery	Fractions[a]					Total recovery	Fractions[a]					Total recovery	Fractions[a]					Total recovery	Fractions[a]				
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
HCB	65	91	5	3	1	–	92	83	16	1	–	–	63	39	46	10	3	2	98	91	9	–	–	–
α-HCH	60	94	3	2	1	–	83	82	15	3	–	–	71	46	52	2	–	–	83	87	13	–	–	–
γ-HCH	63	96	4	–	–	–	82	82	18	–	–	–	72	15	49	26	10	–	88	37	54	9	–	–
β-HCH	97	69	13	8	6	4	97	52	42	6	–	–	58	36	40	17	7	–	66	23	37	32	8	–
Heptachlor	73	98	2	–	–	–	86	88	12	–	–	–	75	84	16	–	–	–	51	100	–	–	–	–
Aldrin	47	100	–	–	–	–	83	93	7	–	–	–	68	85	15	–	–	–	78	96	4	–	–	–
Heptachlor epoxide	44	93	7	–	–	–	81	90	10	–	–	–	51	15	42	21	14	8	81	83	17		–	–
Endosulfan I	11	93	7	–	–	–	69	69	31	–	–	–	40	9	22	28	22	19	70	86	14	–	–	–
2,4′-DDE	75	90	8	2	–	–	88	91	7	2	–	–	51	36	51	9	4	–	56	64	36	–	–	–
4,4′-DDE	94	95	5	–	–	–	86	92	7	1	–	–	59	81	19	–	–	–	77	95	5	–	–	–
Dieldrin	–	–	–	–	–	–	7	–	–	–	–	–	30	–	15	24	30	31	30	77	23	–	–	–
2,4′-DDT	85	92	7	1	–	–	96	86	9	5	–	–	55	70	30	–	–	–	53	55	45	–	–	–
Endrin	–	–	–	–	–	–	6	18	82	–	–	–	32	–	10	28	27	35	73	69	28	2	1	–
4,4′-DDT	85	93	6	1	–	–	97	85	11	4	–	–	53	70	30	–	–	–	19	71	29	–	–	–
4,4′-DDD	87	90	7	2	1	–	96	83	12	3	2	–	52	57	40	2	1	–	71	83	16	1	–	–

[a] Elution with 5 × 10 ml of light petroleum or "upper phase"



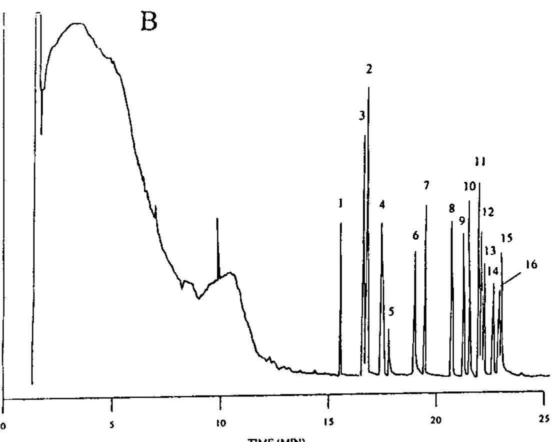
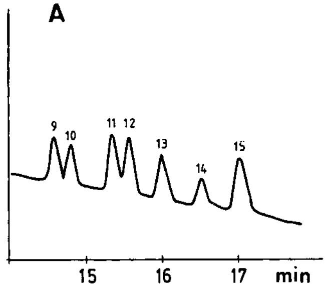


Fig. 2. GC–ECD of OCPs (100 ppb each) in spiked pumpkin seeds after clean-up according to method I, (A) on RTX-35 and (B) on RTX-5 capillary columns. For experimental details, see text. 1 = PCB (pentachlorobenzene); 2 = HCB (hexachlorobenzene); 3 = α-HCH (α-hexachlorocyclohexane); 4 = γ-HCH (lindane); 5 = β-HCH; 6 = heptachlor; 7 = aldrin; 8 = heptachlor epoxide; 9 = 2,4′-DDE; 10 = endosulfan; 11 = 4,4′-DDE; 12 = dieldrin; 13 = 2,4′-DDT; 14 = eldrin; 15 = 4,4′-DDT; 16 = 4,4′-DDD; 17 = Mirex. Pentachlorobenzene and Mirex were added as internal standards.



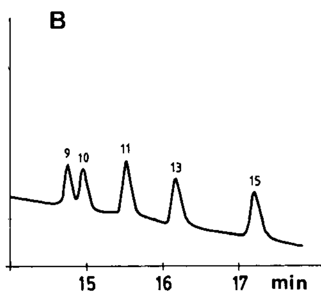


Fig. 3. GC–ECD of OCPs (5 ppb each) in spiked pumpkin seeds showing the retention window of 9 = 2,4'-DDE, 10 = endosulfan, 11 = 4,4'-DDE, 12 = dieldrin, 13 = 2,4'-DDT, 14 = endrin and 15 = 4,4'-DDT after SPE clean-up according to (A) method II without sulphuric acid treatment and (B) method I with sulphuric acid treatment. Note that in (B) peaks 12 and 14 are missing but the other peaks reveal similar peak areas.

presence of dieldrin and endrin in oil samples (Fig. 3).

Very low recoveries of OCPs, especially HCB, were observed after accidentally concentrating the SPE or SPE–LPE extracts to dryness. Addition of high-boiling solvents such as 1-phenyl-2-propanol, 1-phenylethanol and 1-decanol to the light petroleum extract before concentration can prevent evaporation completely to dryness and undesirable volatilization of OCPs. However, this method was not convenient. To avoid additional sources of contamination with organochlorine compounds, we decided to ensure complete OCP recoveries only by accurate concentration to 1 ml by a gentle stream of nitrogen at ambient temperature.

To prevent the formation of emulsions and irreproducible SPE–LPE performance, all extracts have to be completely dry. This could be achieved very simply by mixing the oil seed granules with anhydrous sodium sulphate prior to extraction with light petroleum.

Quantification

Quantification of OCPs in oil seeds was performed using external standard or internal standard calibration methods.

External standard calibration suffers from erratic GC-injections and irregularities in sample pretreatment. As a potential risk we observed an undesirable change in detection sensitivity and retention behaviour with a sequence of multiple injections of oily extracts containing 100 ppb of HCB, although special care was taken with sample pretreatment. However, after injection of at least six "dummies" the accuracy becomes sufficient for external calibration. These effects could also be avoided by prolonging the final heating and equilibration time of the GC column, but this has to be paid for by a longer analysis time. For compensation of deviations during sample pretreatment, calibration standards in the range 1–250 ppb, prepared by spiking oil seeds of very low contamination (< 1 ppb), were treated in exactly the same way as all other samples. All these precautions allowed external standard calibration for accurate determination of the major OCPs.

Day-to-day and within-day reproducibility tests on two "real" samples contaminated with 120 and 2 ppb of HCB, revealed acceptable relative standard deviations for trace analysis (Table III). The calibration points for OCPs in the range 1–250 ppb were sufficiently well correlated (correlation factors between 0.997 and 0.998 were achieved).

Most of the inconveniences in quantification with external standard calibration could be overcome by using calibration with internal standards. An ideal internal standard should have similar chemical and physical properties to the analytes, allowing identical extraction, sample pretreatment, chromatography and detection. In this work, pentachlorobenzene (PCB) and Mirex (MIR) were used as internal standards for the determination of OCPs in pumpkin seeds and vegetable oils, as they best fitted all the requirements listed above. Evaluation of more than 1000 pumpkin seed samples showed that the extracts are essentially free from these compounds, and free from any interferences in the respective retention windows. The use of two internal standards

TABLE III

DAY-TO-DAY AND WITHIN-DAY REPRODUCIBILITY OF DETERMINATIONS OF HCB IN PUMPKIN SEEDS BY EXTERNAL STANDARD AND INTERNAL STANDARD CALIBRATION

For experimental details, see text. All samples were pretreated and purified according to method I.

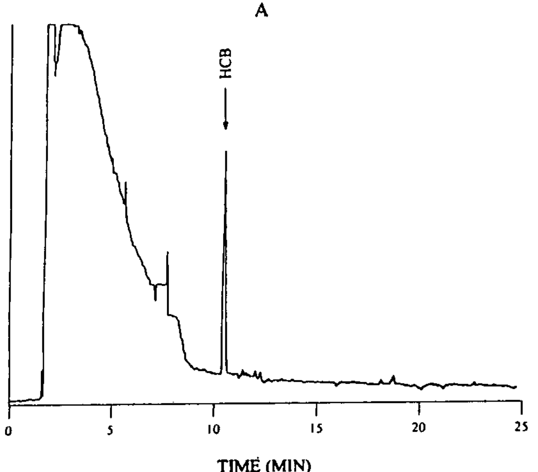
Pumpkin seed spiked with HCB (ppb)	External standard calibration (%)		Internal standard calibration[a] (%)	
	Within-day[b]	Day-to-day[c]	Within-day[b]	Day-to-day[c]
120	8.5	10.6	5.5	7.6
2	10.6	23.1	9.9	19.3

[a] Internal standard = pentachlorobenzene (PCB).
[b] Relative standard deviation of six determinations.
[c] Relative standard deviation of five determinations.

with different volatilities (appearing in the early and late part of the gas chromatogram) allowed better problem solving in cases of observed irregularities. The loss of highly volatile PCB gives a hint of undesirable losses of analytes by evaporation in the course of sample pretreatment, whereas the loss of Mirex indicates erratic aliquoting and dilution procedures or problems in the GC injector line. The loss of both compounds points to irregular SPE–LPE clean-up or aberrant GC injection.

By this procedure, the time-consuming co-determinations of external standard samples could be omitted and all systematic errors and instrumental deviations could be minimized. The reproducibility of HCB determinations was improved to a relative standard deviation of 3.5% and linearity of calibration was extended to 400 ppb with good correlation factors of 0.998–0.999. The limited range of linearity with electron-capture detection was compensated for by internal standard calibration, leading to an increase in the linearity of the overall method by a factor of 1.5.

Table IV gives the detection limits and limits of determination for all the investigated OCPs in pumpkin seeds achieved by the present clean-up technique (Fig. 1, method I). The described method was especially designed for the highly sensitive determination of HCB, allowing a detection limit of



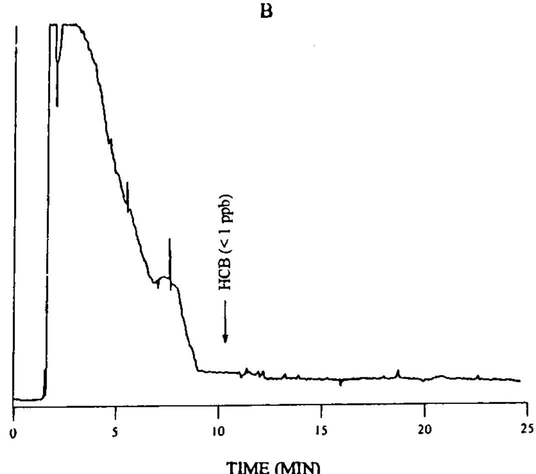


Fig. 4. GC–ECD chromatogram of (A) 70 ppb of HCB in natural contaminated pumpkin seeds and (B) of a non-contaminated "blank" pumpkin seed sample, pretreated according to method I. GC capillary: RTX-35 (for detailed conditions, see Experimental).

TABLE IV

SENSITIVITY OF GC–ECD AND LIMITS OF DETERMINATION OF SEVERAL OCP COMPOUNDS IN VEGETABLE OILS AND OIL SEEDS AFTER CLEAN-UP WITH "SANDWICH"-TYPE SPE–LPE EXTRACTION COLUMNS

OCP	Detection limit (ppb)[a]	Limit of determination in pumpkin seeds (ppb)[b]	
		Method I	Method II
HCB	0.5	1	2
α-HCH	1	2	5
β-HCH	2	5	5
γ-HCH (lindane)	1	2	5
Heptachlor	2	5	5
Aldrin	1	2	5
Heptachlor epoxide	1	5	5
2,4'-DDE	1	2	5
Endosulfan I	4	5	10
4,4'-DDE	1	2	2
2,4'-DDT	3	5	10
4,4'-DDT	3	5	10

[a] Signal-to-noise ratio = 3:1.
[b] Samples are rated as positive for peak areas six times higher than the standard deviation of peak areas from a blank "sample".

500 ppt for standard solutions and a limit of determination of 1 ppb in pumpkin seeds (the ratio of the signal to the standard deviation of the background peaks is 6:1).

The overall recovery with the described method was 80–100% (see Table II) for all major OCP compounds (except dieldrin and endrin), combined with sufficient reproducibility for reliable monitoring of OCP contamination in vegetable oils, particularly pumpkin seed oil.

It was found that 99% of all pumpkin seed samples investigated from the 1990 crop showed HCB contamination in the range 1–400 ppb (median = 22 ppb). The Austrian limit of 250 ppb was exceeded by only 1.6% of the samples, but nearly 75% exceeded the limit of 10 ppb set by some countries in the European Community (EC). Fig. 4 shows a GC–ECD trace of HCB in naturally contaminated pumpkin seeds.

Table V gives a survey of OCP contamination of Styrian pumpkin seeds harvested in 1990 including all the investigated compounds.

Other oil seeds such as soya, sunflower, rape, poppy, linseed, thistle and zulunut, also grown on HCB-contaminated sites, revealed no HCB contamination. However, HCB was occasionally detected in commercially avaiable oils of these seeds in the low ppb range.

TABLE V

OCP CONTAMINATION OF STYRIAN PUMKIN SEEDS OF THE 1990 CROP

A collection of 110 samples harvested in 1990 were investigated for determination of OCPs after sample purification according to method I including OCP elution with the special "upper phase". For additional experimental details, see text.

Contaminant	Positives (%)	Range (ppb)	Mean (ppb)	Median (ppb)	Limit (ppb)[a]
HCB	99	0–400	51.1	25	250
α-HCH	1	0–35	–[b]	–[b]	100[c]
β-HCH	15	0–98	20.8	<5 100[c]	
γ-HCH (lindane)	2	0–24	–[b]	–[b]	150
Aldrin	30	0–16	7.4	<5 10	
ΣDDT[d]	18	0–30	13.2	<2 100	
Endosulfan I	4	0–30	–[b]	–[b]	200

[a] From ref. 9.
[b] Number of positives too low.
[c] ΣHCH = 100 ppb.
[d] ΣDDT (all isomers of DDE, DDD and DDT).

In an interlaboratory test programme, this method using the "sandwich"-type SPE–LPE clean up column (method I) proved to be similar in accuracy to the commonly used SPE and gel permeation chromatographic (GPC) methods but was faster. Compared with these other clean-up techniques, the lifetime of the analytical GC capillary was exceptionally high and even after 3000 sample injections the GC column showed good performance without the need for washing, which was essential for GC columns after GPC clean-up [26].

CONCLUSIONS

A simple and highly reproducible sample pretreatment method for the GC–ECD determination of various OCPs in vegetable oils and oil seeds such as pumpkin, soya, sunflower, rape, poppy, linseed, thistle and zulunut has been developed.

The described method (preferably method I) is reasonably rugged, allowing the routine determination of several OCPs in different vegetable oils and oil seed extracts, and will be applied in further studies of the environmental fate of HCB and the parameters that influence the uptake of HCB in oil seeds, which might help in elucidating some of the human health-relevant aspects of OCP contamination.

We see also a particular advantage of this rugged sample preparation technique for laboratories that occasionally have only a small series of oil samples to analyse for HCB and other OCPs; its installation is simple and inexpensive.

ACKNOWLEDGEMENTS

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Review

Analytical-scale supercritical fluid extraction: a promising technique for the determination of pollutants in environmental matrices

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ABSTRACT

Analytical-scale supercritical fluid extraction (SFE) has become of increasing interest in the last few years because of its efficiency and rapidity concerning the extraction of organic compounds prior to separation and detection by chromatographic techniques. SFE has several advantages over classical solvent extractions such as faster analysis, better selectivity, higher efficiency and the absence of toxic solvent waste, which reduces safety hazards. This paper describes the principles of SFE and its coupling with chromatographic techniques; numerous environmental applications are also reported.

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1. INTRODUCTION

Sample preparation methods generally used by analytical chemists are both time and solvent consuming. According to a recent survey, two thirds of the analysis time are devoted to sample preparation and this step accounts for at least one third of the error generated during the performance of an analytical method [1]. The improvement and automation of sample preparation will therefore lead to a reduction in analysis time and to a greater precision of results.

Recent concern about the hazards associated with most of the solvents used, and the costs and environmental dangers of waste solvent disposal, have led to the development of alternative sample extraction methods such as solid-phase extraction (SPE) and supercritical fluid extraction (SFE). Until recently, SFE has been used mainly for large-scale processing applications (*e.g.*, decaffeinization of coffee) [2]; but for several years the use of SFE methods for analytical applications has attracted considerable interest. The potential advantages of this technique come from the unique properties of supercritical fluids.

2. SUPERCRITICAL FLUID PROPERTIES

A fluid is said to be in its supercritical state when both its temperature and pressure are above their critical values. If only one of these two parameters fulfils this condition, it is said to be in its subcritical state.

Supercritical fluids possess unique physico-chemical properties which make them attractive as extraction solvents (Table 1 [3]). Concerning kinetics,

TABLE 1

APPROXIMATE VALUES OF DENSITIES, VISCOSITIES AND DIFFUSION COEFFICIENTS OF GASES, SUPERCRITICAL FLUIDS AND LIQUIDS

From ref. 3.

Fluid	Density ($g\ cm^{-3}$)	Viscosity (Pa s)	Diffusion coefficient ($cm^2\ s^{-1}$)
Gas	$(0.6–2) \cdot 10^{-3}$	$(1–3) \cdot 10^{-5}$	0.1–1.0
Supercritical fluid	0.2–0.9	$(1–3) \cdot 10^{-4}$	$(0.1–5) \cdot 10^{-4}$
Liquid	0.6–1.6	$(0.2–3) \cdot 10^{-3}$	$(0.2–3)) \cdot 10^{-5}$

their viscosity is 5–20 times lower than that of ordinary liquids: the diffusion coefficients of solutes are consequently greater. This clearly provides a means for faster and more efficient extractions compared with classical liquid phases, owing to a more rapid and more complete penetration of solid matrices.

From a thermodynamic point of view, supercritical fluids have densities 100–1000 times greater than those of gases, which gives them a solvating power closer to that of liquids. In additon, their density is closely related to the pressure and, to a lesser extent, the temperature (Fig. 1) [4]. As the solvent strength of a supercritical fluid is directly related to its density (it can be described by the empirical correlation $\delta = 1.25P_c^{1/2}(\rho_g/\rho_l)$, where δ is the Hildebrand solubility parameter, P_c is the critical pressure of the fluid, ρ_g is the density of the supercritical fluid, and ρ_l is the density of the fluid in its liquid state), the solvating ability of a supercritical fluid toward a species can easily be modified by changing the extraction pressure and/or the temperature. That allows the adjustment of a large scale of density values and the selective extraction of compounds by varying the extraction pressure. For example, supercritical carbon dioxide can selectively extract alkanes and polyaromatic compounds from diesel exhaust particulates [5]: at 45°C and 75 atm, 85% of the alkanes were removed from the particulates after 5 min (with these operating conditions polyaromatic compounds were not extracted), whereas 300 atm and 90 min were needed to extract 90% of the polyaromatic compounds.

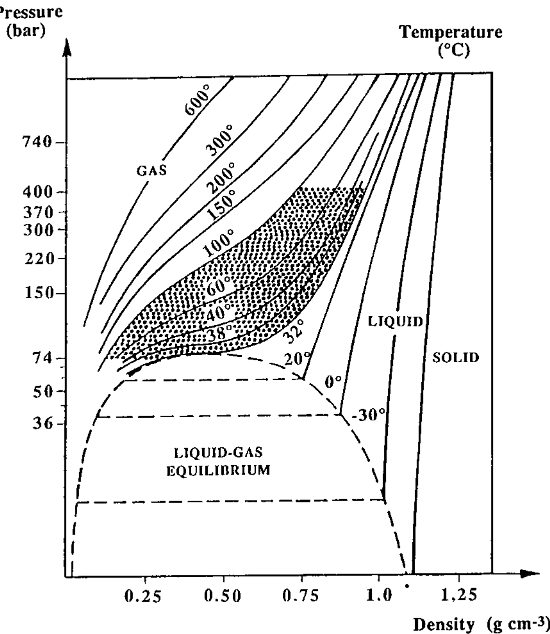


Fig. 1. Pressure–density diagram (P, ρ) for carbon dioxide. The shaded area corresponds to the experimental domain of supercritical phase extraction and chromatography. From ref. 4.

By far the most widely used extraction fluid has been supercritical CO_2. Its preferential use is due to the fact that it is chemically inert, inexpensive, non-toxic, non-flammable and has an easily accessible critical point at 31.1°C and 72.8 bar with a density of 0.468 g ml^{-1}; its low critical temperature allows extraction to be performed under mild thermal conditions. Carbon dioxide is also available in pure form [6,7] and, if desired, can be purified further. In addition, supercritical CO_2 vaporizes on reaching the atmosphere, thereby enabling extracted solutes to be easily isolated for further analysis and/or the direct coupling to analytical techniques such as gas chromatography (SFE–GC), high-performance liquid chromatography (SFE–HPLC) and supercritical fluid chromatography (SFE–SFC).

However, many other supercritical fluids have been used *e.g.*, N_2O, SF_6, CH_3OH, H_2O and $CHClF_2$ (Freon-22). Some of them may be useful for extracting specific compounds (*e.g.*, N_2O has been found to be more efficient than CO_2 for the extraction of amines from contaminated soils (see Fig. 2) [8].

3. CHOICE OF OPERATING CONDITIONS

Supercritical fluid extraction is carried out by placing the sample in contact with a static supercritical fluid in a closed container ("static" mode) or by percolating a supercritical fluid through the sample ("dynamic" mode) [9]. The static mode allows a better penetration of the matrix by the fluid [10], but the latter method permits saturation of the extraction fluid to be avoided, leading to better recoveries and shorter extraction times. Thus, in most instances, extractions combine these two modes: a short period of time is allowed for static equilibration, before enabling the sample to be dynamically extracted [11].

Once extracted from the matrix, the solute of in-

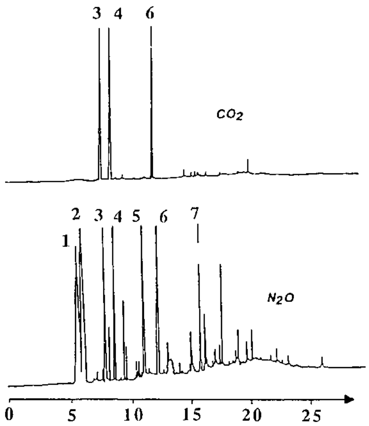


Fig. 2. Supercritical fluid extraction and on-line analysis by gas chromatography of amines from spiked soil using supercritical N_2O and CO_2 for extraction. Peaks: 1 = heptylamine; 2 = N-methylheptylamine; 3 = 2-ethylaniline; 4 = tributylamine; 5 = dodecylamine; 6 = diphenylamine; 7 = octadecylamine. Extraction conditions: 400 bar, 60°C, 20 min static followed by 20 min dynamic (130 μl min^{-1} of compressed fluid through the cell). Chromatographic conditions: HP-1 cross-linked methylsilicone capillary column (20 m × 0,32 mm I.D.); column temperature, 0°C during the extraction, then increased to 300°C at 15°C min^{-1} and held at 300°C for 10 min; flame ionization detection. From ref. 8.

terest needs to be trapped prior to analysis. The success of SFE depends on these two independent steps.

3.1. *Optimization of extraction conditions*

The two basic parameters in SFE are the extraction recovery (the proportion of the amount of solute extracted with respect to its initial amount, usually expressed as a mass percentage) and the extraction rate (extraction recovery per unit time at a given velocity of the supercritical fluid through the cell), the latter decreasing exponentially with time.

The realization of the extraction of a specified solute from a matrix necessitates the optimization of several parameters, mainly the pressure, temperature, the possible addition of an organic modifier to the fluid and flow-rate.

3.1.1. *Influence of pressure*

Four parameters are extremely helpful in the understanding of solute behaviour in supercritical media, and thus in executing successful analytical supercritical fluid extractions [12,13]: (i) the miscibility or threshold pressure [14,15], which corresponds to the pressure at which the solute partitions into the supercritical fluid; (ii) the pressure at which the solute reaches its maximum solubility; (iii) the fractionation pressure range, which is the pressure region between the miscibility and solubility maximum pressures (in this interval it is possible to extract selectively one solute by choosing the correct pressure); and (iv) a knowledge of the physical properties of the solute, particularly its melting point (in fact most solutes dissolve better when they are in their liquid state, *i.e.*, above their melting point).

To illustrate the difference between the threshold pressure and the solubility maximum pressure, the solubility–pressure curve of naphthalene is given in Fig. 3 [13]: this solute is slightly soluble in CO_2 at 75 bar (threshold pressure); as the pressure increases the solubility rises, especially around 90 bar, up to its maximum value.

The fluid pressure is the main parameter that influences the extraction recovery. An elevation of this pressure at a given temperature results in an increase in the fluid density (Fig. 1), which means a better solubility of the solutes. Consequently, the

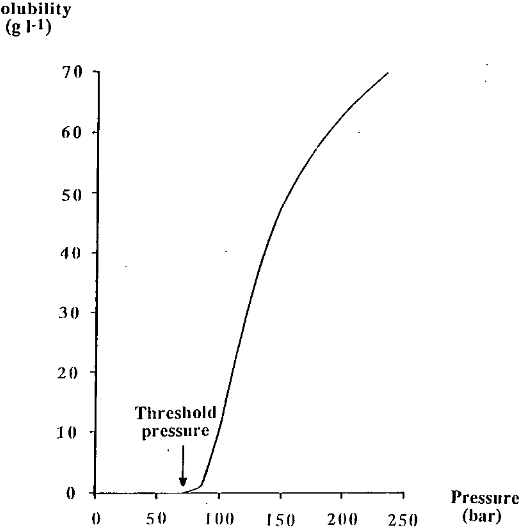


Fig. 3. Variation of the solubility of naphthalene with the pressure of supercritical CO_2 at 45°C. From ref. 13.

higher the extraction pressure, the smaller is the volume of fluid necessary for a given extraction; for example, one needs to double the volume of CO_2 in order to extract 70% of diuron herbicide from a contaminated soil when working at 110 bar instead of 338 bar (Fig. 4) [16]. However, high pressure is not always recommended for complex matrices owing to the higher solubility of solutes when the pressure is elevated; thus the extract can become very complex and, consequently, its analysis becomes very difficult. On the other hand, it must be borne in mind that the presence of co-extracted solutes can dramatically change the solubility level of the solute of interest.

3.1.2. Influence of temperature

At a constant pressure the density of CO_2 decreases when the temperature rises. This effect becomes more pronounced as the compressibility increases, as shown in Fig. 1. The temperature also affects the volatility of the solute. Hence the effect of a temperature elevation is difficult to predict because of its dependence on the nature of the sample. For a non-volatile solute, a higher temperature would result in lower extraction recovery owing to a decrease in solubility; thus the distribution coefficient of phenol between water and supercritical CO_2 decreases when the fluid temperature rises from 25 to 30°C [17]. On the other hand, for a volatile solute, there is a competition between its solubility in CO_2 (which decreases as the temperature increases) and its volatility (which rises with increasing temperature). For example, when the temperature increases from 80 to 120°C, the extraction recovery of diuron from soil with methanol-modified CO_2 is enhanced from 75% to 99% [9].

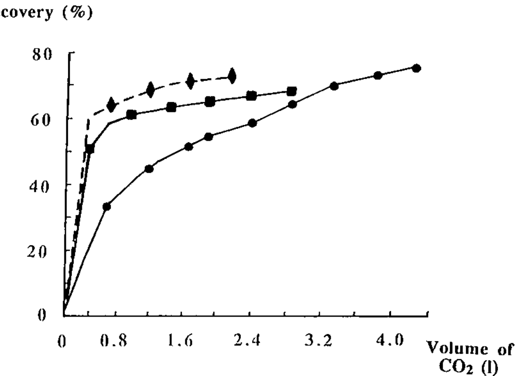


Fig. 4. Variation of the extraction yield of diuron from a polluted soil with the volume of CO_2 percolated at different pressures: ● = 110; ■ = 235; ◆ = 338 bar. Extraction conditions: extractant, CO_2–CH_3CN (90:10, v/v); extraction cell, 25 cm × 4.6 mm I.D.; temperature, 100°C; flow-rate of liquid CO_2, 16.5 ml min^{-1}. From ref. 16.

3.1.3. Addition of a modifier

The low polarity of CO_2 limits its use to the extraction of relatively apolar or moderately polar solutes. Thus, a small amount of a polar organic solvent (methanol, acetonitrile, water, etc.), called a "modifier" or "entrainer", is usually added to the supercritical fluid for the extraction of more polar solutes. The nature of the modifier depends on the nature of the solute to be extracted [18]; for example, the extraction of diuron is greatly enhanced with methanol instead of acetonitrile as a modifier, probably because of hydrogen bonding which could exist between diuron and methanol [16]. A reasonable starting point consists of selecting a modifier that is a good solvent in its liquid state for the target analyte.

It should be noted that the addition of large amounts of modifier will considerably change the critical parameters of the mixture [19,20], as shown in Fig. 5 for methanol–carbon dioxide mixtures [19]. As a result, binary mixtures of carbon dioxide and an organic solvent are often used in a subcritical state, where the diffusion coefficients are smaller than in a supercritical state.

Modifiers can be introduced as mixed fluids in the pumping system with a second pump and a mixing chamber [21], or by simply injecting the modifier as a liquid into the sample before extraction [9,22] (the latter way being less successful because it leads to concentration gradients within the matrix). Alternatively, one may use directly a cylinder tank of modified CO_2, but this is much more expensive; besides, as the tank gets empty, the content of modifier tends to increase.

3.1.4. Influence of fluid velocity

The speed of the supercritical fluid flowing through the cell has a strong influence on the extraction efficiencies. The slower the fluid speed, the deeper it penetrates the matrix. The fluid speed can be expressed by the linear velocity, which is strongly dependent on the flow-rate and the cell geometry.

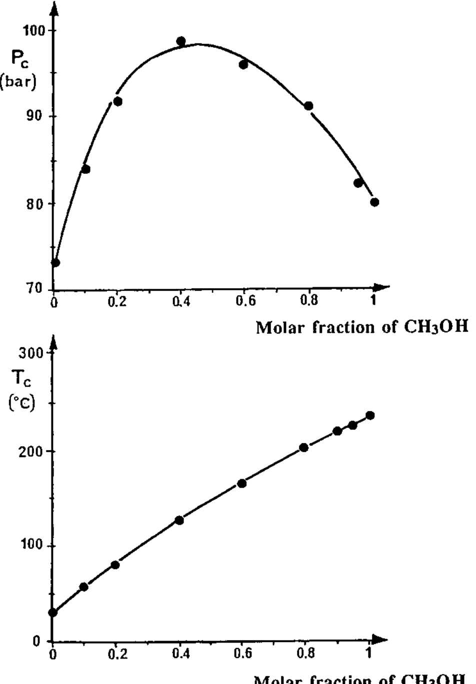


Fig. 5. Variations of the critical pressure and temperature of CO_2–CH_3OH mixtures with the molar fraction of methanol. From ref. 19.

3.1.4.1. Influence of flow-rate. For a given extraction cell, the flow-rate can be easily changed by using a new restrictor with a different inside diameter. Decreasing the flow-rate results in a lower linear velocity and usually in increased extraction recoveries (as a result of an extended contact between the supercritical fluid and the sample); for example ^{14}C-labelled linear alkylbenzenesulphonates were better extracted by supercritical CO_2 modified with methanol (40 mol%) (at 380 bar and 125°C) from a sludge-amended soil with a liquid CO_2 flow-rate of 0.45 ml min^{-1} (mean recovery 90.8 ± 1.3%) instead of 1.2 ml min^{-1} (mean recovery 75.6 ± 1.1%), the same volume of fluid being used in each instance [23]. However, this entails longer extraction times. On the other hand, high flow-rates can result in a decrease in the recovery either by inducing an elevated pressure drop through the extraction cell {this phenomenon probably occurred during the extraction of diuron from a contaminated soil with a CO_2–methanol mixture (90:10, v/v) [16]}, or by increasing analyte loss during decompression of the fluid. Thus an optimum flow-rate has to be found. Typical values range around 1 ml min^{-1} of compressed fluid (with extraction cells of I.D. *ca.* 1 cm), which corresponds to *ca.* 500 ml min^{-1} of gas after decompression.

3.1.4.2. Influence of cell geometry. For a given flow-rate, the fluid linear velocity can be changed by using several cells having the same volume but different inside diameters. Higher extraction efficiencies are expected with short, broad cells because the fluid linear velocity decreases as the cell diameter increases.

This has been observed during the extraction of polychlorinated biphenyls (PCBs), spiked onto octadecylsilane (C_{18}) sorbents, by supercritical CO_2 at 60°C and 140 bar (density 0.549 g ml^{-1}), with a flow-rate of 0.075 ml min^{-1} and a total fluid volume of 1.5 ml [24]. Two different cells containing 0.510 g of the standard packing were investigated in this study: a long, narrow cell with dimensions 4.4 × 50.0 mm (I.D. × length of bed) having a 1:11 diameter to length ratio, and a short, broad cell with dimensions 9.9 × 9.9 mm (I.D. × length of bed) having a 1:1 diameter to length ratio. Changing the cell geometry from 1:11 to 1:1 resulted in an average relative increase in the extraction recovery of nearly 50%; in addition, the recoveries decrease with increasing chlorine content, which correlates with chromatographic data obtained employing similar sorbents.

Similar results have been obtained for polycyclic aromatic hydrocarbons (PAHs) from a C_{18} sorbent using two different cells containing a 0.8-ml sorbent bed [a long, narrow cell with dimensions 0.37 × 7.3 cm (I.D. × length of bed) having a 1:20 diameter to length ratio, and a short, broad cell with dimensions 1.0 × 1.0 cm (I.D. × length of bed) having a 1:1 diameter to length ratio] [25–27]. Using supercritical CO_2 at 100°C and 315 bar (density 0.675 g/ml), with a flow-rate of 0.6 ml min^{-1} and a total fluid volume of 7.5 ml, the average recoveries ± standard deviations for the analytes pyrene, perylene, benzo[*ghi*]perylene and coronene using the 1:20 cell were 60.4 ± 0.9, 17.1 ± 0.6, 6.5 ± 0.7 and 1.7 ± 0.2%, respectively. With the 1:1 cell they were 80.4 ± 1.6, 35.8 ± 0.8, 15.4 ± 1.4 and 5.2 ± 0.3%, respectively [25–27].

On the other hand, neither the extraction cell dimensions nor the chlorine content had any signif-

icant effect on the recoveries of PCBs from the common adsorbent Florisil [24]. Considering the extraction by supercritical CO_2 of native PAH from railroad bed soil, neither the flow-rate nor the cell geometry had a significant influence on the extraction efficiency [28]. These results show the importance of the matrix effect in SFE; an adsorbent matrix such as Florisil behaves similarly to soil matrices because the analyte–matrix interactions are the same (solutes are primarily adsorbed on the surface or within the pores of the solid material).

In some particular instances, the extraction recovery can also be improved by decreasing the extraction cell diameter (*i.e.*, by increasing the linear velocity). This result can be explained by a better mass transfer within the cell due to an increase in turbulence in the fluid flow pattern inside the extraction cell. As an example, larger peak areas have been obtained for *n*-octadecane and *n*-eicosane using a 4 mm I.D. cell ($610 \cdot 10^3$ and $202 \cdot 10^3$ counts, respectively) instead of a 6 mm I.D. cell ($478 \cdot 10^3$ and $140 \cdot 10^3$ counts, respectively) [13].

3.1.5. Influence of the nature of the matrix

Factors such as the particle size, shape, surface area, porosity, moisture, level of extractable solutes and the nature of the matrix will affect the analytical results and are considered later. In the same way the interactions between solutes and active sites of the matrix can necessitate strict extraction conditions.

3.2. Efficiency of the solute trapping system

Once the compounds of interest are in the supercritical extraction fluid, the next step is to isolate them for further analyses. Generally, this is accomplished by decompression of the fluid through a restrictor (often heated to prevent the formation of pieces of ice that could plug the restrictor). Trapping becomes more difficult when either the solute is more volatile or the flow-rate is higher. The collection technique therefore needs to be efficient.

Most often, SFE is coupled to chromatographic techniques, either "off-line" or "on-line" [29]. The former coupling is much simpler to perform and gives an extract available for other analyses, whereas the latter provides the means to achieve maximum accuracy and sensitivity and to minimize losses.

3.2.1. Trapping systems for off-line SFE

Two different methods are commonly used for collecting the extract on depressurization of the CO_2, using a liquid trap or a solid surface.

With a liquid trap, the restrictor is simply placed in a vial containing a suitable liquid. The analyte is gradually dissolved in the solvent while the CO_2 is being discharged to the atmosphere. Sometimes the large volume of gas on decompression results in violent bubbling of the solvent, thus leading to analyte losses.

In the solid surface method, the extracted analytes are trapped on a solid surface (glass vials, stainless-steel beads or glass beads) cryogenically cooled either by the expanding supercritical fluid or by another source (CO_2 or liquid N_2); they are then rinsed from the surface for further analysis. In some instances, the trapping may involve a solid-phase sorbent (usually chromatographic packing material): after being cryogenically and chemically trapped, the solutes are eluted from the sorbent with a small volume of solvent. The latter system provides two trapping mechanisms: cryogenic trapping and adsorption; hence it presents the inconvenience of giving relatively slow desorption rates for some polar solutes from adsorptive materials [30].

3.2.2. Systems for coupling SFE on-line to chromatographic techniques

Very often SFE is coupled to gas chromatography (SFE–GC) or supercritical fluid chromatography (SFE–SFC) [29,31]. The success of such on-line couplings depends on the efficiency of the extract collection and of the quantitative transfer inside the chromatographic column within a narrow band (otherwise it would affect the chromatographic performances). Several interfaces have been tested.

For on-line SFE–capillary GC, the simplest method involves and on-column injector: the restrictor is inserted directly into the GC column through the injector [5,32–36]. This gives the best sensitivity with diluted samples. For concentrated or larger sized samples, the supercritical fluid is depressurized into a conventional split–splitless injection port, via a heated transfer line [8,37–40]. Other interfaces have been used: a T-piece between the restrictor and the column (with possibly a short retention gap) [41,42], a thermodesorption–cold trap injection system [43], a programmed-temperature

vaporizer injector [44,45] and a six-port valve [46–48].

SFE has also been coupled with both capillary and packed-column SFC (SFE–CSFC and SFE–PSFC).

With capillary columns, the simplest method involves an injector loop: part of the extract is injected directly into the column [9,49]. This enables the precipitation of the solutes or other related problems to be avoided. Other interfaces usually require a preconcentration of the analytes after depressurization of the supercritical fluid. This is carried out in an injector loop [50,51], either in a cryogenically cooled capillary [52,53] or in a column [54].

With packed columns, the injector loop has been successfully used, as it allows part of or all the extract to be injected into the column [55–58]. Other methods require a preconcentration step which is carried out in the chromatographic column itself [59], in a precolumn [60,61] or in a cryogenic trap [62,63].

4. ENVIRONMENTAL MATRICES EXTRACTED BY SFE

SFE has been applied to a broad range of environmental samples. The efficiency of this technique depends both on the nature of the solute to be extracted an on the characteristics of the matrix. Several pollutants have been successfully extracted using this technique, including PAHs, PCBs, dioxins, furans, phenols, pesticides and herbicides [64].

Polar solutes generally require modified carbon dioxide as the extractant. In some instances they can be derivatized *in situ* by the addition of a reagent directly to the sample matrix prior to extraction [65–67].

Although SFE is best suited for solids, other matrices (air samples and liquids) have also been investigated.

4.1. Air samples

Classical methods for the identification and determination of organic compounds from fumes involve two independent steps. Pollutants are first trapped on a solid-phase sorbent; then they are either thermally desorbed and analysed by GC, or eluted with a suitable liquid solvent before their separation by HPLC. Both methods may result in incorrect quantification: thermal desorption can entail the decomposition of thermally unstable volatile solutes, whereas solvent elution produces a dilute sample which hinders the determination of trace components. SFE has been applied as an alternative in order to overcome these problems.

Few studies have shown the efficiency of supercritical fluids in extracting compounds with different volatility from solid-phase sorbents [68–71]. These supports provide an easy way to collect volatile toxicants presents in gases before desorbing them by SFE for further analysis. Several samples have been successfully extracted following this procedure; some examples are presented in Table 2. Fig. 6 illustrates the on-line SFE–GC analysis (with flame ionization detection) of hardwood smoke phenolics collected from a residential chimney on a polyurethane foam sorbent plug (identification of individual species was based on a SFE–GC–mass spectrometric (MS) analysis of a replicate sample); quantitative recovery was obtained after a 10-min extraction with supercritical CO_2 at 300 bar and 45°C and a cryogenic trapping temperature of −30°C [35].

4.2. Liquids

The SFE of solutes in liquid samples has been confronted with technological complications related to the insulation of the matrix itself and the CO_2–liquid miscibility, like the design of a special extraction cell [74–76]. As a consequence, only a few studies have dealt with the direct SFE of liquids (Table 3). Most of the time liquid samples are first adsorbed on a solid material before being extracted by the supercritical fluid (Table 4). For example, Fig. 7 shows the chromatogram obtained after on-line SFE–GC (flame ionization detection) of a polyurethane foam sorbent resin that had been soaked in a coal gasification wastewater [37].

4.3. Solids

The properties of supercritical fluids make them very useful for the rapid and quantitative extraction of organic pollutants from environmental solids. In addition to solubility considerations, several characteristics exert a powerful influence and need to be taken into account.

TABLE 2

EXAMPLES OF SUPERCRITICAL FLUID EXTRACTIONS OF FUMES COLLECTED ON DIFFERENT SOLID-PHASE SORBENTS

Air samples collected on different adsorbents	Extracted solutes	Super- or subcritical fluid or fluid mixture	Off-line coupling		On-line coupling			Ref.
			Collection	Analysis	GC	CSFC	PSFC	
Polyurethane foam								
Urban air	Alkanes and aromatic hydrocarbons	CO_2			*			35
Diesel exhaust	Alkanes and PAHs	CO_2	Solvent	GC	*			36, 72
Roofing tar volatiles	Alkanes, benzenes, PAHs, phenols, S-heterocyclic compounds	CO_2			*			36, 72
Polyimide 109								
Air of a laboratory building	Organic compounds	CO_2	Adsorption	GC				73
Tenax								
Vehicle exhaust	Alkanes, benzenes, PAHs, oxy-PAHs	CO_2			*			5, 36
Glass-fibre filter								
Diesel exhaust	PAHs	CO_2	Solvent	GC				5

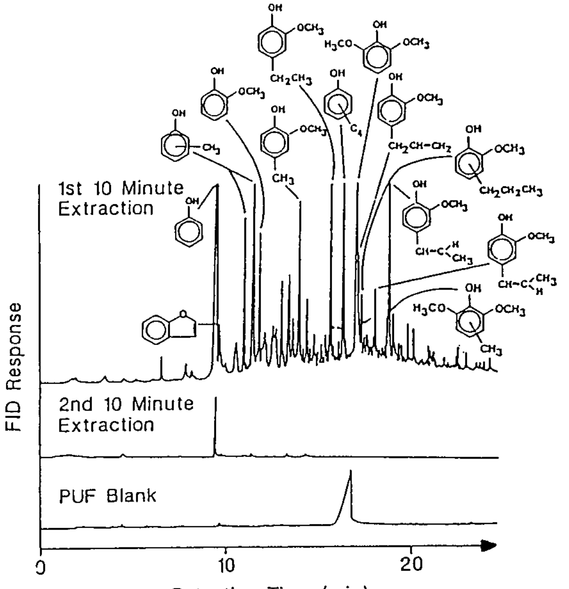


Fig. 6. Supercritical fluid extraction and on-line analysis by gas chromatography of hardwood smoke phenolics collected from a residential chimney on a polyurethane foam (PUF) sorbent plug. Extraction conditons: CO_2, 400 bar, 45°C, 10 min, on-column deposition at −30°C. Chromatographic conditions: DB-5 fused-silica capillary column (30 m × 0.32 mm I.D.) (1 μm film thickness); column temperature, −30°C during the extraction, before being rapidly elevated to 30°C at 30°C min^{-1} then to 320°C at 8°C min^{-1}; carrier gas, hydrogen; flame ionization detection. Identification of individual species was based on the SFE and on-line analysis by GC–MS of a replicate sample. From ref. 35.

4.3.1. *Influence of physical nature of the matrix*

Very often, grinding solid samples before use enhances extraction due to an increase in the surface area exposed to the supercritical fluid and thereby allowing better diffusion of the solute out of the sample matrix. Nevertheless, precautions must be taken in order to avoid large pressure drops in the extraction cell due to sample compaction (thus, fine grinding of the matrix is not recommended) and plugging (very often the addition of either small pieces of filter-paper or glass-wool or layers of glass beads at both ends of the cell prevents particles from being swept out).

4.3.2. *Influence of chemical nature of the matrix*

Extraction of an analyte depends on its distribution between the supercritical fluid and the sorptive sites of the sample matrix. The recovery rate is therefore a function of both the chemical nature of the solute and of the matrix itself. A solid with numerous active sites will highly adsorb polar solutes, resulting in a poor extraction rate. For example, the recovery of explosive from soil samples decreases when the organic content of the soil increases [52]. Some matrices are particularly difficult to extract (fly ash probably being one of the best known [80]).

The presence of organics inside the material can also affect the extraction as they could be more eas-

TABLE 3

EXAMPLES OF SUPERCRITICAL FLUID EXTRACTIONS OF LIQUIDS

Liquid matrix	Extracted solutes	Super- or subcritical fluid or fluid mixture	Off-line coupling		On-line coupling			Ref.
			Collection	Analysis	GC	CSFC	PSFC	
Standard in carbon disulphide or methylene chloride	Alkanes	CO_2					*	62
Contaminated water	Phenol	CO_2 CO_2–CH_3OH	Solvent	GC				17, 66
Municipal waste water treatment	Linear alkylbenzenesulphonates	CO_2, CO_2–CH_3OH, N_2O	Solvent	HPLC				77
Aqueous media	N-containing bases	CO_2	Adsorption	GC, HPLC			*	74, 76
	Phosphonate	CO_2					*	74
	Phenol	CO_2	Solvent, adsorption	HPLC				75
Viscous engine oil	Aromatics	CO_2					*	57

TABLE 4

EXAMPLES OF SUPERCRITICAL FLUID EXTRACTIONS OF LIQUIDS COLLECTED ON DIFFERENT SOLID-PHASE SORBENTS

Liquids adsorbed on different sorbents	Extracted solutes	Super- or subcritical fluid or fluid mixture	Off-line coupling		On-line coupling			Ref.
			Collection	Analysis	GC	CSFC	PSFC	
Polyurethane foam								
Coal gasification wastewater	Phenolics and N-containing aromatics	CO_2			*			37
Tenax								
Standard in acetone	PCBs	CO_2			*			43
Glass-wool								
Standard in methanol	Pesticides	CO_2	Adsorption	HPLC				78
Glass beads								
Standard in methylene chloride	PAHs, pesticides	CO_2	Solvent	GC				79
Mixture of a coal tar and a coal liquid	PAHs	CO_2			*			41
Alumina beads								
Standard in carbon disulphide	Alkanes, aromatics	CO_2, N_2O, SF_6			*			38, 39
Standard	*n*-Alkanes	CO_2			*			40
Octadecyl-bonded silica								
Standard in chloroform	PAHs, pesticides	CO_2	Solvent	GC				25–27
Standard in cyclohexane	*n*-Alkanes, nicotine, dicyclohexylamine	CO_2–C_2H_5OH			*			44

ily extracted than the solute of interest and/or plug the restrictor. This was evidenced during the extraction of PAHs from a reference sample (marine sediment SRM 1941) containing 2% (w/w) of elemental sulphur: the deposition of elemental sulphur as S_8 plugged the restrictor [19].

4.3.3. Influence of moisture

Depending on the nature of the solute, the presence of water affects the extraction efficiency. For example, the removal of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin from a wet spiked sediment [19.8% (w/w) water content] by modified carbon dioxide (containing 2% of methanol) requires twice the time for achieving the same extraction recovery compared with a dry spiked sediment [0.3% (w/w) water content] [81]. Similar results have been obtained during the carbon dioxide extraction of PAHs from petroleum waste sludge [82]. Water usually hinders the extraction of apolar compounds by sheathing the surface of the matrix and acting as a barrier to CO_2 penetration. This assumption is supported by the recent comparison between $CHClF_2$ (Freon-22) and CO_2 used as a supercritical fluid for the extraction of PAHs from petroleum waste sludge: $CHClF_2$ removes much more water from the sample than CO_2, thus increasing the amount of analytes exposed to the supercritical fluid and thereby leading to higher extraction efficiencies [82].

In contrast, water helps to bring polar compounds into solution owing to competition for active matrix sites.

Moisture also exerts an influence on the action of a modifier as illustrated in Table 5 [83]: methanol facilitates the extraction of phenol from a dry soil sample owing to an increase in the polarity of the supercritical fluid mixture (hydrogen bondings occur between phenol and methanol); however, this organic solvent has no effect when a wetted soil is considered, probably as a result of dissolution of

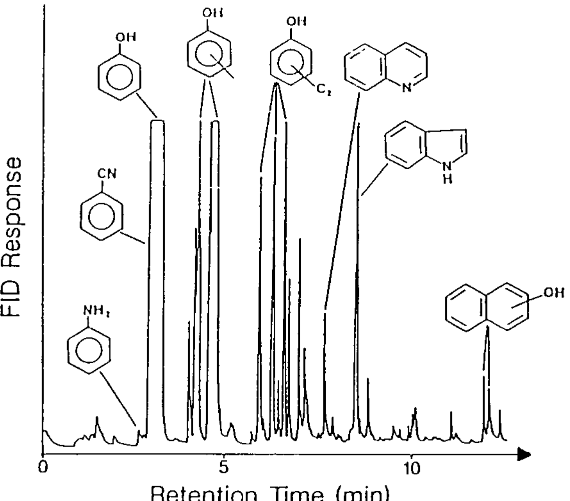


Fig. 7. Supercritical fluid extraction and on-line analysis by gas chromatography of coal gasification wastewater impregnated on polyurethane foam sorbent. Extraction conditons: CO_2, 300 bar, 45°C, 10 min, on-column deposition at 10°C. Chromatographic conditions: DB-5 fused-silica capillary column (30 m × 0.32 mm I.D.) (1 μm film thickness); column temperature, 10°C during the extraction, then rapidly increased to 70°C and subsequently to 320°C at 8°C min^{-1}; carrier gas, hydrogen; splitting ratio, 1:50; flame ionization detection. Identifications were based on the SFE and on-line analysis by GC–MS of a duplicate sample. From ref. 37.

methanol in the water contained in the soil, so the supercritical phase polarity is no longer increased. In contrast, as benzene is virtually insoluble in water, it favours the supercritical phase over the wetted soil phase; hence almost all the phenol can be extracted from the wetted soil owing to π–π interactions between benzene and phenol.

4.3.4. *Influence of contamination*

The way in which analytes are incorporated within the matrix is of prime importance. The longer a solute stays in a solid, the better it will diffuse through its pores and react with the eventual active sites contained in it. Spiked solute often resides superficially in the matrix, and it is therefore extracted more easily by the supercritical fluid; besides, if the spiking solvent is not removed before extraction (to minimize the loss of volatiles) it could act as a modifier and affect the extraction conditions [84].

In real contaminated samples, chemical reactions may also take place between the solute and other organic materials or active sites of the matrix, resulting in other pollutants that could be more difficult to extract. For example, parathion (an organophosphate pesticide) tends to form toxic products such as 4-nitrophenol, aminoparathion and diethyl thiophosphate [85]. In addition, some solutes may be hardly extracted from real contaminated samples because of associations between the solute molecule and matrix components; thus pesticides often lead to unextractable or "bound" pesticide residues in plant matrices and soils.

To optimize the SFE conditions for a specific sample, an analytical chemist needs to follow logically ordered steps [10]. Very often, standard solutions of the analytes must be first deposited on a non-sorptive matrix such as filter-paper, sand or deactivated glass beads. As there is no solute–matrix interaction in that case, this step provides the analyst with the minimal extraction conditions before extracting the solutes from more difficult matrices. For example, Fig. 8 shows the chromatogram obtained after the on-line SFE–GC of deactivated glass beads (45–150 μm I.D.) spiked with a small amount (0.5 ppm) of PAH compounds [41]. The extraction was conducted for 2 min with CO_2 at 50°C and 250 bar (density 0.83 g ml^{-1}). All the solutes were quantitatively recovered, except naphthalene (its high volatility probably entailed losses during sample preparation).

Matrix effects can then be studied by performing

TABLE 5

INFLUENCE OF POLAR MODIFIER ADDITION (2 mol%) TO SUPERCRITICAL CO_2 ON THE DISTRIBUTION COEFFICIENT OF PHENOL

Extraction conditions: extraction cell, 300 ml; initial mass, 100 g + 1% (w/w) phenol; pressure, 150 bar; temperature, 25°C; extraction time, 60 min (static); extractant: CO_2, CO_2–benzene or CO_2–CH_3OH (reprinted from ref. 83).

Nature of supercritical fluid	Distribution coefficient of phenol	
	Dry soil	Wetted soil
CO_2	0.35	0.35
CO_2 + benzene (2 mol%)	0.8	>7
CO_2 + methanol (2 mol%)	>7	0.35

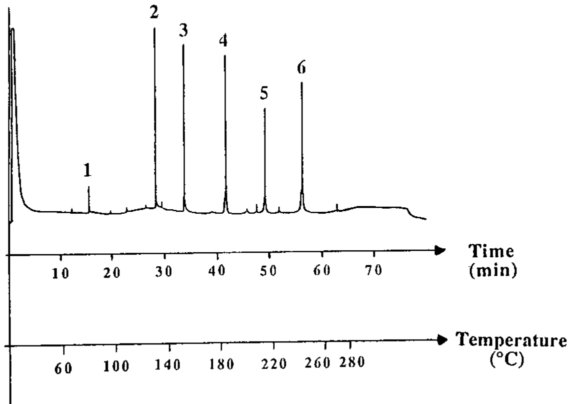


Fig. 8. Supercritical fluid extraction and on-line analysis by gas chromatography of PAHs deposited on deactivated glass beads (45–150 μm diameter) at a low level (0.5 ppm). Peaks: 1 = naphthalene; 2 = fluorene; 3 = phenanthrene; 4 = pyrene; 5 = chrysene; 6 = benzo[*e*]pyrene. Extraction conditions: CO_2, 250 bar, 50°C, 2 min, on-column deposition. Chromatographic conditions: SE-54 fused silica-capillary column (15 m × 0.25 mm I.D.) (0.25 μm film thickness) with a short retention gap of deactivated fused-silica tubing (30 cm × 0.53 mm I.D.); column temperature, increased from 30 to 280°C at 4°C min^{-1}; carrier gas, helium (linear velocity *ca.* 40 cm s^{-1}); flame ionization detection. From ref. 41.

SFE on a simulated or spiked sample. The actual sample should be the last to be extracted as the extraction conditions in real samples are more severe than in spiked samples.

SFE has been successfully applied to numerous environmental matrices. Some of these applications are summarized in Table 6.

For example, several chemical warfare agent simulants have been extracted from a spiked soil with supercritical CO_2 modified with methanol (5%); these are dimethyl methylphosphonate (DMMP), diisopropyl methylphosphonate (DIMP), diisopropyl fluorophosphate (DIFP) and chloroethyl ethyl sulphide (CES). DMMP is the simulant for O-ethyl-S-2-(diisopropylaminoethyl methylphosphonothioate) (VX), DIFP and DIMP are the simulants for O-isopropylmethyl phosphonofluoridate (GB) and CES that for bis(2-chloroethyl) sulphide (HD). Fig. 9 shows that a single SFE is sufficient for extracting all the simulants. However, diethylaminoethanethiol hydrochloride (DEAT · HCl), a by-product from VX manufacture, is hardly extracted because of its low solubility and/or strong sorption by the soil [102].

SFE also permits class-selective extractions, as illustrated in Fig. 10 [51]. Different PAHs are extracted from coal tar pitch, depending on the supercritical CO_2 pressure. At 70 bar, the two solutes that are mainly extracted are assumed to be dibenzofuran and fluorene. As the pressure is increased, PAHs of higher molecular mass (lower solubility in supercritical CO_2) are extracted; hence the three-ring compounds appear in the 100-bar extract and the four-and five-ring PAHs require a pressure of 200 bar.

5. COMPARISON BETWEEN SFE AND LIQUID–LIQUID EXTRACTION

SFE compares favourably with classical solvent extraction methods such as Soxhlet extraction or sonication [80, 84, 116]; Table 7 gives a comparison of the three methods for the recovery of PAHs from river sediment and fly ash [80]. Indeed, the extraction times involved using SFE are considerably lower (typically, less than 1 h instead of several hours) and the concentration step that is usually required with liquid–liquid extractions when dealing with trace analytes is eliminated or at least reduced to only a few minutes [88]. In addition, SFE requires the use of only a few millimetres of solvent or no solvent at all in some instances.

Sometimes, better recoveries of analytes could be even obtained with supercritical fluids. For example, almost 100% of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin could be extracted from a sediment spiked with 200 μg kg^{-1} in 30 min with a supercritical CO_2 methanol (2%) mixture, compared with the standard Soxhlet method which took 18 h and extracted only 65% of the dioxin [81].

6. CONCLUSIONS

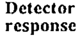
There is great concern regarding environmental pollution, especially the exposure of laboratory personnel to toxic solvents and the disposal costs associated with organic solvents. These two major concerns have given importance and preference to the use of supercritical fluids (mainly carbon dioxide) as an extractant medium. They allow the quantita-

TABLE 6

EXAMPLES OF SUPERCRITICAL FLUID EXTRACTIONS OF SOLID MATRICES

Solid matrix	Extracted solutes	Super- or subcritical fluid or fluid mixture	Off-line coupling		On-line coupling			Ref.
			Collection	Analysis	GC	CSFC	PSFC	
Soils and sediments	PAHs	CO_2, CO_2–CH_3OH, N_2O, N_2O–CH_3OH, C_2H_6, $CHClF_2$	Solvent	GC	*			29,32,34, 37,77,80, 82, 86–89
	Pesticides	CO_2, CO_2–CH_3OH, CH_3OH	Solvent, adsorption	GC, HPLC	*		*	52, 56, 66, 79, 85, 87
	Herbicides	CO_2, CO_2–CH_3OH, CO_2–C_2H_5OH, CO_2–CH_3CN, N_2O, N_2O–CH_3OH, C_2H_6	Solvent, adsorption	GC, PSFC, HPLC		*	*	9, 16, 22, 66, 88, 93–96
	PCBs	CO_2, CO_2–CH_3OH, CO_2–toluene, N_2O, $CHClF_2$	Solvent, adsorption	GC	*			30, 32, 48, 82, 92, 97, 98
	Phenols	CO_2, CO_2–CH_3OH, CO_2–benzene	Solvent adsorption	GC, HPLC	*			67, 83, 85, 86, 99
	Alkanes, alkylbenzenes	CO_2, CO_2–CH_3OH, H_2O	Adsorption	CSFC	*	*		35–37, 46, 47, 50, 100
	Linear alkylbenzenesulphonates	CO_2–CH_3OH	Solvent	HPLC				23
	Halocarbons	CO_2			*			40
	Condensed polyaromatics	CO_2, CO_2–CH_3OH	Solvent	GC				78, 89
	Polychlorinated dioxin	CO_2, CO_2–CH_3OH, N_2O, N_2O–CH_3OH	Solvent	GC				81
	Amines	CO_2, N_2O			*			8
	Fatty acids	CO_2–CH_3OH–HCO_2H	Adsorption	GC				101
	Chemical warfare agent simulants	CO_2, CO_2–CH_3OH	Solvent	GC				102
	Explosives	CO_2	Solvent	HPLC				52
Rocks	Hydrocarbons	CO_2, SF_6	Solvent	GC, SFC	*			39, 40, 88, 103

Air-dried biosludge, generated during the treatment of a coal gasification wastewater	*n*-Alkanes, PAHs	CO_2	Solvent	GC				5
Digester sludge from a municipal wastewater treatment facility	Linear alkyl-benzenesulphonates	CO_2, N_2O	Solvent	HPLC				23
Petroleum waste sludge	PAHs	CO_2, N_2O, $CHClF_2$	Solvent	GC				82
Air particulate matter (urban dust)	Alkanes, PAHs	CO_2, N_2O, N_2O–CH_3OH, C_4H_{10}	Solvent, adsorption	GC	*			32, 34, 38 41, 80, 97, 104
Internal combustion engine valve deposit	PAHs	CO_2	Adsorption	GC				105
Coal, coal tar, carbon black, lampblack, hydrocarbon waste, treated wood, fly ash	PAHs	CO_2, CO_2–CH_3OH, N_2O, N_2O–CH_3OH, CH_3OH, C_2H_6	Solvent adsorption	GC, HPLC	*	*	*	32, 34, 35, 40, 51, 57, 72, 80, 106–108
	Hydrocarbons	CO_2, CO_2–HCOOH			*			38, 46, 60
	Sesquiterpenes	CO_2			*			34
	Polychlorinated dioxins and furans	CO_2, CO_2–CH_3OH, CO_2–benzene, CO_2–toluene, N_2O, N_2O–CH_3OH, N_2O–toluene	Solvent	GC				109, 110
Semtex (plastic explosive)	Alkanes, nitrates, triazane	CO_2	Solvent	GC				111
Propellants	Stabilizers	CO_2					*	62, 112
Plant tissues	Pesticides	CO_2	Solvent, adsorption	GC, PSFC				113, 114
	2,4-Dichlorophenol	CO_2	Solvent	HPLC				115
	Herbicides	CO_2–CH_3OH, CO_2–C_2H_5OH				*	*	9, 94



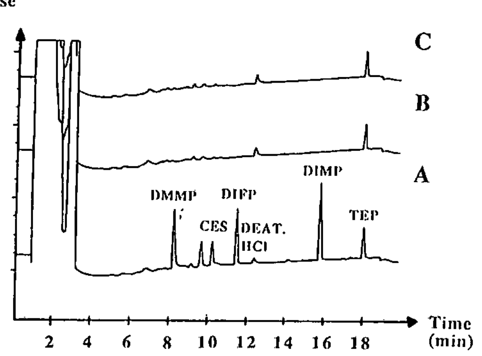


Fig. 9. Off-line analysis by gas chromatography of (A) first 10-min supercritical fluid extraction of spiked soil, (B) second 10-min supercritical fluid extraction of soil and (C) third 10-min supercritical fluid extraction of soil. DMPP = dimethyl methylphosphonate; CES = chloroethyl ethyl sulphide; DIFP = diisopropyl fluorophosphate; DEAT·HCl = diethylaminoethanethiol hydrochloride; DIMP = diisopropyl methylphosphonate; TEP = triethyl phosphate (internal standard). Extraction conditions: CO_2–CH_3OH (95:5), 300 bar, 60°C, 10 min, 1 g of soil, collection in methanol. Chromatographic conditions: DB-5 fused-silica capillary column (30 m × 0.53 mm I.D.) (1.5 μm film thickness); column temperature, 70°C for 4 min, then increased to 150°C at 8°C min^{-1}; carrier gas, helium (flow-rate 7 ml min^{-1}); injector held at 120°C; flame ionization detector held at 200°C. From ref. 102.

tive extraction of numerous pollutants in a very short time, and the addition of a small percentage of an organic solvent could extend the scope of SFE applications.

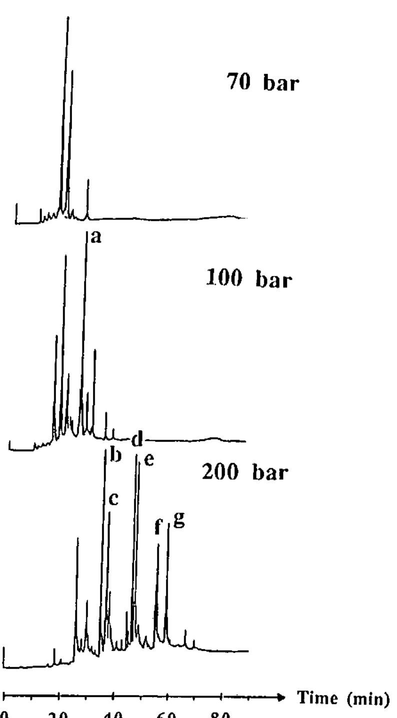


Fig. 10. On-line supercritical fluid chromatography of coal tar pitch extracted with supercritical carbon dioxide at different pressures. Peaks: (a) phenanthrene; (b) fluoranthene; (c) pyrene; (d) benz[*a*]anthracene; (e) chrysene; (f and g) benzofluoranthenes and benzopyrenes. Extraction conditions: CO_2, 43°C, 60 min, flow-rate 3 ml min^{-1} of gaseous CO_2 at 150 bar, 10 μg of coal tar pitch. Chromatographic conditions: SB-biphenyl-30 fused-silica capillary column (10 m × 0.05 mm I.D.) (0.25 μm film thickness); column temperature, 110°C; supercritical CO_2; initial density of 0.25 g ml^{-1} for 10 min, then increased to 0.74 g ml^{-1} at 0.006 g ml^{-1} min^{-1}; flame ionization detector held at 400°C. From ref. 51.

TABLE 7

COMPARISON OF SONICATION, SOXHLET AND SUPERCRITICAL FLUID EXTRACTION FOR THE EXTRACTION OF PAH COMPOUNDS FROM SPIKED RIVER SEDIMENT AND FLY ASH

Extraction conditions: sonication, 10 ml of CH_2Cl_2, 100 W, 4 h, 0.5 g of spiked sediment; Soxhlet extraction, 50 ml of CH_2Cl_2, 8 h, 1 g of spiked sediment; SFE, N_2O–CH_3OH (5 vol. %), 350 bar, 65°C, 30 or 60 min, total volume of fluid 20 or 40 ml of liquid, 50 mg of spiked sediment or fly ash, 25 μm I.D. outlet restrictor, collection in CH_2Cl_2, analysis by GC–MS (reprinted from ref. 80). Results are means ± standard deviation (n = 3).

Matrix	Compounds extracted	Recovery using different extraction methods (%)			
		Sonication (4 h)	Soxhlet (8 h)	SFE (30 min)	SFE (60 min)
River sediment	Phenanthrene	79 ± 5	98 ± 3	102 ± 4	103 ± 7
	Pyrene	77 ± 5	96 ± 3	95 ± 2	101 ± 8
	Perylene	61 ± 6	86 ± 6	92 ± 10	97 ± 6
Fly ash	Phenanthrene	30 ± 1	60 ± 1	72 ± 5	89 ± 8
	Pyrene	33 ± 1	71 ± 1	48 ± 8	67 ± 4
	Perylene	23 ± 1	77 ± 6	19 ± 5	28 ± 5

Analytical SFE is currently an evolving technique in which many experimental parameters and problems have yet to be properly defined. The influence of some parameters such as the pressure and the temperature of the extraction fluid are now well mastered; others (extraction cell configuration, fluid flow-rate through the extraction cell, period of extraction, sample matrix effects, etc.) need further studies. Similarly, the sample size needs to be optimized. Very often, a small sample size is used; unfortunately it may not be a true representative of the overall sample and, hence, may result in wrong analytical results.

In spite of the above-mentioned precautions, analytical-scale SFE has been successfully applied to a wide range of environmental samples and numerous interesting results have been obtained. This new technique, still in its development stage, will therefore without doubt become a routine sample preparation technique. Nevertheless, one needs to always bear in mind the advice of Bartle and Clifford, who say "SFE must not be considered as a magic technique but as an important weapon in the analyst's armory" [93].

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Review

Supercritical fluid extraction in environmental analysis

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ABSTRACT

Supercritical fluid extraction (SFE), usually with carbon dioxide and often with a modifier, is a rapid, selective and convenient method for sample clean-up in environmental analysis. Three inter-related factors influence analyte recovery in SFE: solubility in the fluid, diffusion through the matrix and adsorption in the matrix. SFE may be coupled on-line to various analytical methods: gas, liquid and supercritical fluid chromatography. A wide range applications of SFE from environmental samples is described: hydrocarbons, chlorobenzenes and chlorobiphenyls, dioxins and chlorinated pesticides, herbicides and ionic surfactants. Organic compounds may be concentrated from air and water and extracted from adsorbents by SFE. Direct SFE from water is also possible.

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1. INTRODUCTION TO SUPERCRITICAL FLUID EXTRACTION

Supercritical fluid extraction (SFE) is a novel sample preparation method which promises to have a profound influence on environmental analytical chemistry, since it affords rapid, selective and convenient sample clean up [1–4]. The main advantage of a supercritical fluid over an extracting liquid is that its properties, *viz.* density, solvating power, viscosity and solute diffusivity, can all be controlled by varying the applied pressure and temperature. This leads to greater selectivity, rapid mass-transfer and higher flow-rates as compared with liquids. Further, the separation of solvent from solute is simply achieved by decompression, since the solvent is usually gaseous at ambient temperature. SFE also has considerable advantages over liquid extraction in terms of sample size, cost and volume of solvent and analysis time. Extraction can be performed either off-line or on-line; coupling of SFE to gas, liquid and supercritical fluid chromatography (SFC) have all been demonstrated.

In this review, we outline some of the principles of SFE in the context of environmental analysis and describe some illustrative applications.

2. PRACTICE OF SFE

Carbon dioxide has so far been the most widely used extraction solvent because of its convenient critical properties [5], non-toxicity, cheapness and non-flammable character. It is usually classified as a non-polar solvent, but its large quadrupole moment leads to some affinity with polar solutes and many large polar organic molecules are soluble in it.

For the extraction of more polar molecules, polar modifiers such as those listed in Table 1 are usually

TABLE 1

MODIFIERS FOR CARBON DIOXIDE IN SFE

Modifier	T_c (°C)	P_c (atm)	Molecular mass	Dielectric constant at 20°C	Polarity index
Methanol	239.4	79.9	32.04	32.70	5.1
Ethanol	243.0	63.0	46.07	24.3	4.3
Propan-1-ol	263.5	51.0	60.10	20.33	4.0
Propan-2-ol	235.1	47.0	60.10	19.3	3.9
Hexan-1-ol	336.8	40.0	102.18	13.3	3.5
2-Methoxyethanol	302	52.2	76.10	16.93	5.5
Tetrahydrofuran	267.0	51.2	72.11	7.58	4.0
1,4-Dioxane	314	51.4	88.11	2.25	4.8
Acetonitrile	275	47.7	41.05	37.5	5.8
Dichloromethane	237	60.0	84.93	8.93	3.1
Chloroform	263.2	54.2	119.38	4.81	4.1
Propylene carbonate	352.0		102.09	69.0	6.1
N,N-Dimethylacetamide	384		87.12	37.78	6.5
Dimethyl sulphoxide	465.0		78.13	46.68	7.2
Formic acid	307		46.02	58.5	
Water	374.1	217.6	18.01	80.1	10.2
Carbon disulphide	279	78.0	76.13	2.64	

added to the CO_2 [6]. The modifier–phase diagram must be considered to ensure that there will be only one phase under the conditions of the extraction. Thus for methanol–CO_2 at 50°C, there is only one phase above 95 atm (1 atm = $1.01 \cdot 10^5$ Pa) whatever the composition, but below this pressure, two phases are possible. A comprehensive compilation of phase data for CO_2 mixed with numerous modifiers has been published [7].

Experimental SFE is conceptually simple [4]: a pump is used to supply a known pressure of the extraction fluid to an extraction cell held at a temperature above the critical temperature of the fluid, which then flows through the sample and out through a pressure restrictor to a collecting device at atmospheric pressure (Fig. 1).

The pump is either a syringe or a reciprocating type with a cooled head. Modifier, if required, is introduced either by means of a separate pump via a mixing device, or from a pre-mixed cylinder; it should be remembered however, that if using a cylinder, the modifier composition changes slightly as the contents are consumed. Alternatively, modifier may be added directly to the matrix. An on-off valve is located between pump and extraction vessel, and often a length of tubing in the cell oven to ensure that the fluid is at the correct temperature.

The solid sample to be extracted is held between frits in an extraction cell usually fabricated from

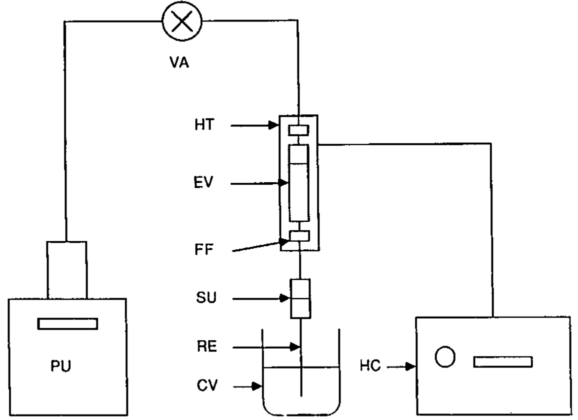

Fig. 1. Off-line SFE system. PU = CO_2 pump; VA = on/off valve; HT = thermostatted heating tube; FF = fingertight connectors; CV = collection vial; SU = swagelok union; RE = restrictor; HC = heater controller; EV = extraction vessel.

stainless-steel and available from a number of suppliers. The extraction cell dimensions may affect the rate of extraction [8], perhaps because of turbulence effects; diffusers and ultrasonic irradiation have also been employed. Cell orientation and fluid flow direction is important if the cell is not full, but less important if it is. A commercial SFE instrument allowing eight parallel extractions is available. Liquid or wet samples may be mixed [9] with an adsorbent such as pelletised Celite ("Hydromatrix") or a drying agent (*e.g.*, magnesium sulphate). Organic water pollutants may be adsorbed onto a solid adsorbent (either solid-phase extraction cartridges or filter discs) from which they are removed for chromatographic analysis by SFE [10]. Direct SFE of aqueous solutions has also been demonstrated [11].

The restrictor maintains the pressure within the cell. It may be, most simply, a length of fused-silica capillary tubing with an internal diameter between 20 and 50 μm or a crimped stainless-steel tube. More elaborate devices include back-pressure regulators and micrometer valves. The extracted material may be collected in a vial containing solvent or by direct cooling. The restrictor is usually heated to prevent blockage when extracting materials containing water which freezes as the supercritical fluid evaporates. This arrangement also prevents deposition of extracted material in the restrictor; during extraction of sediments containing elemental sulphur, locating a copper scavenge column between cell and restrictor is also recommended [12]. Solid traps containing glass beads, silica gel or a liquid chromatographic stationary phase have also been used to collect analytes. Alternatively, SFE equipment is directly coupled to an analytical instrument and such systems are discussed in Section 4.

3. FACTORS AFFECTING EXTRACTION FROM ENVIRONMENTAL SAMPLES

Three interrelated factors influence recovery during SFE as is shown in the so-called SFE triangle [13]. For successful extraction, the solute must firstly be sufficiently soluble in the supercritical fluid. This is particularly important at the start when extraction is occurring rapidly. The onset of extraction in a graph of % recovery in a given time against fluid pressure or density (Fig. 2a) is referred to as the

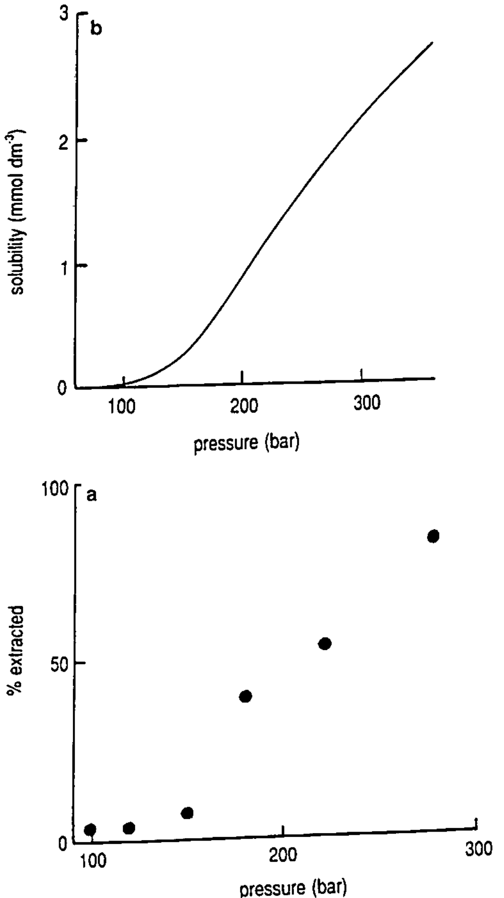

Fig. 2. (a) Percentage recovery of atrazine from soil by SFE with carbon dioxide at different pressures after 15 min at 80°C and constant flow-rate, compared (b) with calculated solubility at the same temperature.

"threshold" pressure. Control of solubility via applied pressure may allow stepwise extraction; for example [14] the extraction of two- and three-ring polycyclic aromatic hydrocarbons (PAHs) from a coal-derived solid occurred at a CO_2 pressure of 100 atm whereas five-ring PAHs required a pressure of 200 atm (Fig. 3). These observations have been correlated with calculated solubilities [15]. It is thus important to know the conditions under which the analyte is sufficiently soluble. In fact solubility of a substance in a supercritical fluid is the sum of two factors: the volatility of the substance and the solvating effect of the supercritical fluid, which is a

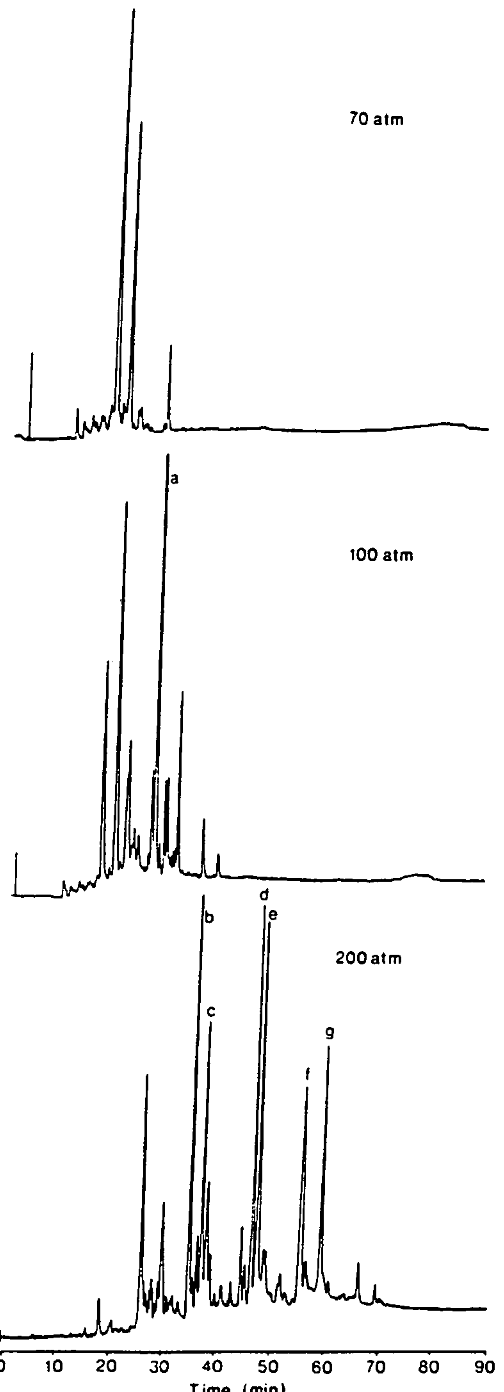

Fig. 3. SFC chromatogram of coal tar pitch extracted at different pressures. From ref. 14. Selected peak identities: a = phenanthrene; b = fluoranthene; c = pyrene; d = benz[*a*]anthracene; e = chrysene; f = benzofluoranthenes; g = benzopyrenes.

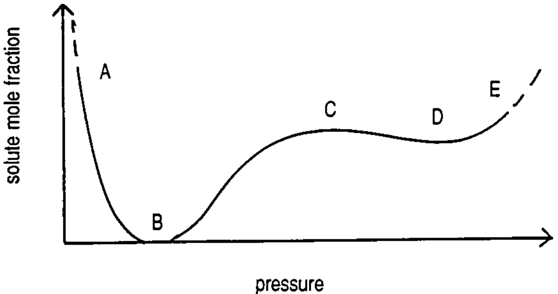

Fig. 4. Schematic diagram of solubility *versus* pressure at constant temperature.

function of fluid density [13]. The solubility has the general form shown schematically in Fig. 4; it is noteworthy that a decrease in solubility may occur (regions A–B and C–D) as a consequence of repulsive forces "squeezing" the solute out of solution. A number of compilations of solubility data for supercritical fluids have appeared (*e.g.*, ref. 16), from which the threshold pressure can be determined; such data are generally obtained by gravimetric measurements, although a more rapid chromatographic procedure has been used with some succes [17]. Alternatively, supercritical fluid solubilities may be predicted either by use of an equation of state such as the Peng–Robinson equation [18], or by means of various empirical correlations so as to extend existing data. The solubility data for atrazine (Fig. 2b) thus obtained [19] correlates well with experimental extraction behaviour (Fig. 2a): predicted solubilities begin to rise at 100 atm in agreement with the experimentally observed "threshold" solubilities. Janda *et al.* [20] observed that simazine was much less efficiently extracted from sediment than was atrazine. Calculated solubility curves [19] for these compounds are in quantitative agreement with these results; simazine is predicted to be much less soluble than atrazine in supercritical CO_2.

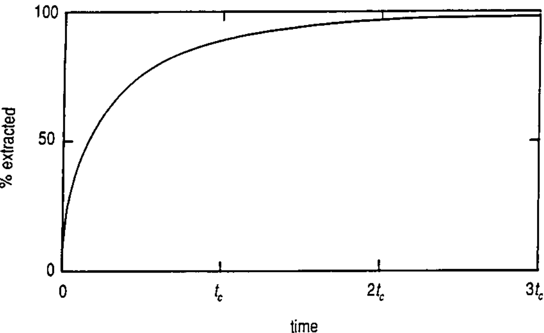

Fig. 5. Theoretical curve of pecentage extraction *versus* reduced time.

SFE usually exhibits [13] the time dependence shown in Fig. 5. If the concentration of analyte in a continuous flow of fluid is well below the solubility limit, the rate determining process is diffusion out of the matrix. An effective diffusion coefficient (D) and a particular matrix geometry are assumed, along with no solubility limitation; the solutions of the appropriate differential equations are obtained by the same methods as those applied to heat conduction. For a sphere, the solution is therefore described as the "hot-ball" model [21]. If the mass of solute, initially m_0, is m after time t, the ratio m/m_0 is given by

$$\frac{m}{m_0}=\frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-n^2\pi^2\frac{Dt}{r^2}\right) \quad (1)$$

where n is an integer. Making the substitution $\pi^2 \cdot \frac{D}{r^2}=a$, where r is particle radius

$$\frac{m}{m_0}=\frac{6}{\pi^2}\cdot\left[\exp(-at)+\frac{1}{4}\exp(-4at)+\frac{1}{9}\exp(-9at)+\ldots\right] \quad (2)$$

representing a sum of exponential decays and reproducing (Fig. 6a) observed behaviour. At long times, the later (faster decaying) terms decrease in importance in comparison with the first term; a graph of ln (m/m_0) *versus* t becomes linear (Fig. 6a). Although the initial steeper fall appears as a small feature, it represents the extraction of the majority of the material. Eqn. 1 shows a squared dependence on r, rationalising the well known fact that speed of extraction is increased by crushing or grinding solids, or coating liquids on a finely divided substrate. Real samples will contain particles of irregular shape and the curve has a large intercept compared with the value of -0.5 for spheres (Fig. 6b).

Fig. 7 shows experimental results [21] for the extraction of phenanthrene from rail-road bed soil at 50°C using CO_2 at 400 atm. The curve of ln

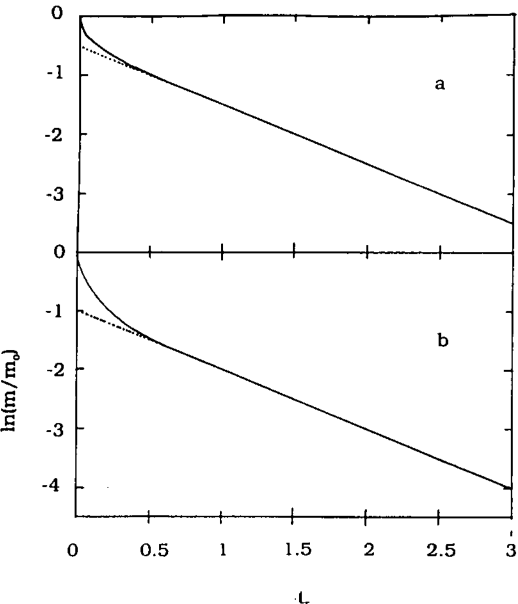

Fig. 6. ln (m/m_0) *versus* scaled time for the hot-ball model: (a) basic model; (b) with effect of irregular particle shape.

(m/m_0) *versus* t has the form of the "hot-ball" model although the intercept is close to -2, indicating irregular shapes for the soil particles. Similar curves have been found for the SFE of numerous analytes from environmental matrices, *e.g.*, PAH with molar mass 128 to 252 [20] and atrazine [19] from soils, and alkylbenzenesulphonates from organic digester sludge [21].

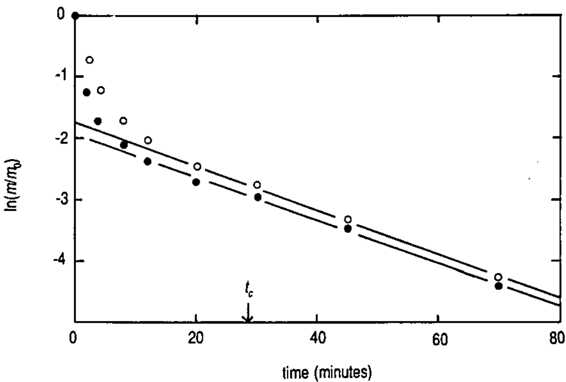

Fig. 7. SFE of phenanthrene from railroad soil with CO_2 at 50°C, two different pressures and constant flow-rate. From ref. 21.

TABLE 2

USE OF THE EXTRAPOLATION PROCEDURE DURING SFE OF VOLATILE ORGANICS FROM SOIL

	Measurement (arbitrary units)			
	m_1	m_2	m_3	m_0
	726	248	116	1190
	888	208	68	1200
	771	176	64	1050
	762	208	84	1110
	720	212	92	1100
	753	188	80	1080
	861	200	76	1180
	768	192	80	1100
	813	224	88	1180
Mean	785	206	83	1130
Standard deviation	58	21	15	55
R.S.D. (%)	7.4	10	18	4.9

The exponential behaviour of the extraction after the initial period means that extrapolation may be used to obtain quantitative analytical information without exhaustive extraction. If m_1 is the mass extracted in the initial non-exponential period and m_2 and m_3 are the masses extracted in two subsequent equal time periods, then the total mass, m_0 is given by

$$m_0 = m_1 + \frac{m_2^2}{m_2 - m_3} \tag{3}$$

This method has been tested by Liu *et al.* [22] by coupled SFE–GC of volatile organic compounds at sub-μg/g levels in soil. Table 2 contains results of repeated runs during which extraction was carried out for approximately 20 min but not to completion. An overall R.S.D. of 4.9% was observed. Table 3 compares the effect of different time intervals on the values of m_0 for the extraction of three compounds from soil. The calculated m_0 values were not affected as long as the first time period covers the entire non-linear region.

Solubility affects the kinetics of SFE since, as already stated, the "hot-ball" [21] model assumes no solubility limitations. If the concentration of solute in the fluid is finite (assumed to be proportional to

TABLE 3

EFFECT OF TIME INTERVAL USED IN COMPUTING m_0

Time intervals used (s)			Measured value of m_0 (arbitrary units)		
Δt_1	Δt_2	Δt_3	Toluene	*p*-Xylene	Benzylamine
0–200	200–400	400–600	116	196	251
0–400	400–600	600–800	116	196	279
0–400	400–800	800–1200	116	197	266
0–300	300–800	800–1300	116	197	262
0–300	300–700	700–1100	116	196	263
0–500	500–1000	1000–1500	116	198	262
Mean m_0			116	197	266
Standard deviation (S.D.)			–	0.82	7.2
R.S.D. (%)			–	0.42	2.7

the partition coefficient is proportional to the solubility of the solute in the fluid (S), a new version of eqn. 1 can be written [23]

$$\frac{m}{m_0} = 6 \sum_{n=1}^{\infty} \frac{\left(\frac{hr}{ar}\right)^2}{[hr(hr-1)+a_n^2]\exp(-a_n^2 DHr^2)} \tag{4}$$

where a_n are the roots of the equation

$$a \cot(a) = 1 - hr \tag{5}$$

and

$$h = \frac{KSF}{AD} \tag{6}$$

K is a constant for a particular matrix, A is its surface area and F is the volume flow-rate of fluid. Plots of ln (m/m_0) against time now form a family of curves (Fig. 8) for which the gradients of the linear portions become increasingly steep as solubility increases, with a limiting value for infinite solubility —the previously mentioned "hot-ball" model.

These considerations are in keeping with effects observed during the SFE at 50°C and constant flow-rate of phenanthrene from soil (Fig. 7). At 180 atm (open circles) the ln (m/m_0) *versus* time curve falls less steeply initially than the 400 atm curve (closed circles) and the linear portion is displaced upwards. The lower rate of extraction at 180 atm can be explained by the difference in solubility at the two pressures. At 50°C, the saturated mole fraction of phenanthrene in CO_2 is 0.0015 at 180 atm but 0.003 at 400 atm. Although the two curves appear similar, the amount extracted after 4 min differs by over 10%. The effect of adsorption on active sites in the matrix may be overcome by adding modifier to the CO_2 to increase the rate; spiked samples may be a poor guide to the necessary conditions (Fig. 9).

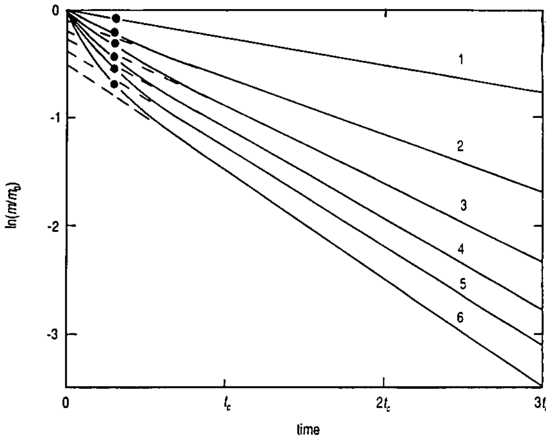

Fig. 8. Solubility-limited hot-ball model plots. Solubility increases from curve 1 to curve 6.

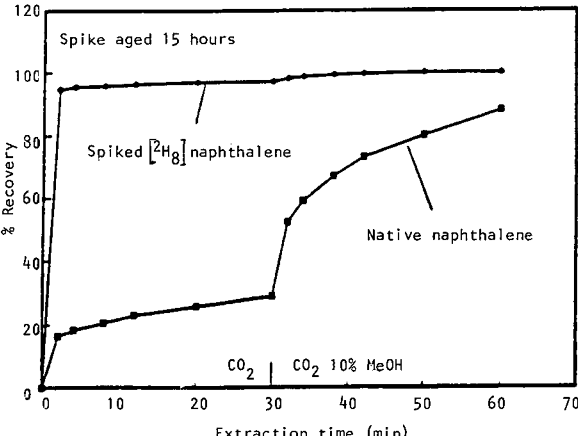

Fig. 9. SFE recovery rates of naphthalene and spiked [$^2H_8$]naphthalene from the same sample of air particulates at 400 atm and 80°C. From ref. 49.

4. ON-LINE COUPLING OF SFE TO CHROMATOGRAPHIC ANALYSIS

Since the extraction solvent is easily removed, the analyte may be trapped for subsequent separation, and trace analysis is possible with on-line GC, SFC and HPLC. In direct coupling, the solvent peak is eliminated and the analysis of compounds which may elute with the solvent becomes possible. On-line coupling also reduces sample handling, the possibility of sample loss and contamination. The process is attractive if only limited amounts of sample are available since all of the analyte can be transferred.

A number of application areas of SFE–GC and SFE–SFC have been described [1,4]. If analytes are both thermally stable and volatile, then GC is the preferred separation technique; thus fuels, polychlorinated biphenyls and PAH in environmental samples can all be analysed by on-line SFE–GC. When the sample contains thermally unstable or reactive compounds, SFE–SFC is recommended; SFE–SFC is particularly attractive [24] since the extracting fluid may be the same as the mobile phase. SFE–SFC can be applied to the analysis of soils and sediments for compounds such as thermally unstable pesticides.

4.1. SFE–GC

On-line SFE–GC has the following steps: extraction, depressurisation and venting of the supercritical fluid, collection and focusing of the extract on to a GC column and GC analysis. Two broad methods of collection and focusing have been reported. In the first, the extract is collected in an external device, *e.g.*, by depressurising into a cold trap located before the GC; the trap is heated in a flow of carrier gas to transfer the extract to the column. Alternatively, the GC system may be used as the trap by depressurising into a retention gap before the column or depositing the extract directly inside the column [4,24] (Fig. 10).

The cold trapping procedure prevents any deleterious effects of the supercritical fluid on the GC column or detector but is limited by the efficiency of collection of volatiles in the extract. Use of a suitable adsorbent, *e.g.*, Tenax, with thermal desorption may be used. SFE–GC with column collection is more efficient since the stationary phase helps concentrate the extract in a narrow band; both on-column and split injection versions have been reported for quantitative analysis. The former permits ng/g detection limits for milligram amounts of sample extracted. Split injection is preferred if the sample is wet, since freezing and plugging of the restrictor outlet or GC column may occur with on-column injection.

The coupling of SFE with GC provides a rapid and convenient method for the analysis of air and water matrices. Trace components in air may be

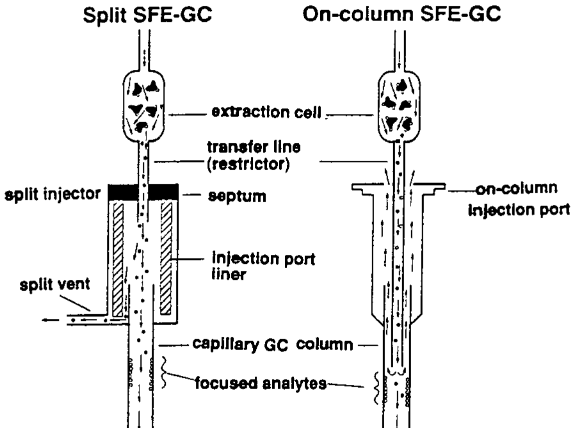

Fig. 10. On-line coupling of SFE to GC. From ref. 3.

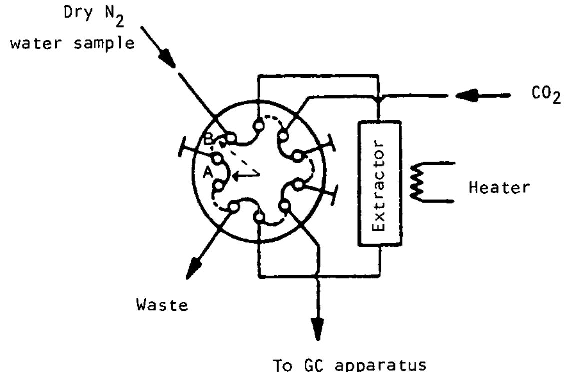

Fig. 11. Apparatus for automated SFE–GC analysis of water. From ref. 25.

concentrated onto adsorbents such as Tenax, polyurethane foam, charcoal or silica/alumina, from which they may be extracted by SFE and transferred quantitatively to the GC via an SFE–GC interface. Similarly, pollutants in water may be concentrated on polyurethane foam or solid-phase extraction cartridges and Empore discs and then analysed by SFE–GC.

An automated version of the latter equipment has been described [25] (Fig. 11), in which water is passed through a Tenax column which, after drying, is subjected to SFE. Contaminants are transferred to the capillary GC column via a length of small internal diameter fused-silica capillary. The whole isolation process is controlled by a single ten-port valve.

4.2. SFE–SFC

The instrumentation [24] for on-line SFE–SFC ranges from relatively simple systems to more complex arrangements involving switching valves and multiple pumps. Most systems include an extraction cell which is temperature controlled or held in an oven, a switching valve, a cryogenically cooled trap and a chromatographic oven housing the column —usually a capillary— and a detector. The fluid may be delivered to both the cell and column using a single pump. When different conditions are required for simultaneous extraction and chromatography, a dual pumping system is required.

A typical SFE–SFC system is illustrated in Fig. 12. The outlet of the cell is connected to a flow restrictor which is in turn connected to an accumulating trapping system; this may be a coated or more usually uncoated fused-silica retention gap or transfer line, or an adsorbent trap housed in a cryogenically cooled tee. During extraction, the tee is vented to atmosphere and the extract is concentrated within the transfer line or trap. After extraction is complete, the valve is switched and supercritical fluid is introduced into the side arm of the tee to transfer the extract on to the SFC column. If uncoated fused-silica tubing is used for the retention gap, the extract is rapidly transferred to the analytical column and there concentrated by phase-ratio focusing. The process is aided by keeping the mobile phase at low density. Other more complicated systems have been reported, using on–off and multiport switching

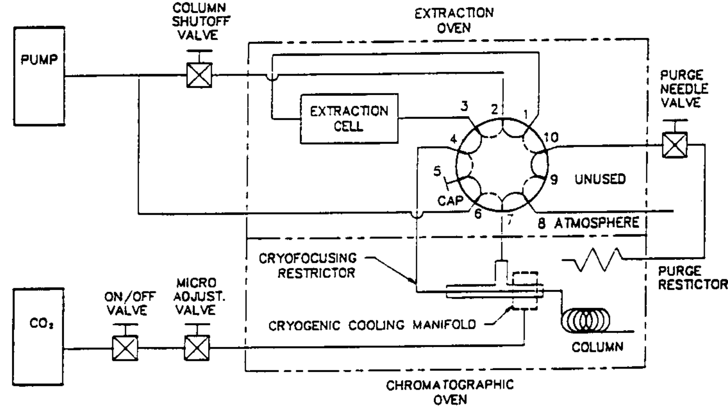

Fig. 12. On-line coupling of SFE to SFC.

valves to allow continuous extraction or to permit venting of the extraction cell during simultaneous chromatographic analysis.

4.3. SFE–HPLC

Coupled SFE–HPLC has also been described [26]. For example, a system for the determination of chlorinated phenols in various solid matrices permits direct introduction of supercritical fluid extracts into an HPLC, allowing quantitative determinations down to the sub-μg/g level without clean-up.

5. APPLICATIONS

5.1. Purgeable halocarbons

Eleven purgeable halocarbons were isolated from a sediment matrix by on-line SFE with carbon dioxide [27]. The restrictor from the SFE apparatus was directly inserted into a split/splitless injector (Fig. 10). The injector was kept at 250–325°C to minimise cooling when supercritical fluid decompressed. To focus the analytes, it was necessary to cryogenically cool the gas chromatographic oven during the SFE. The halocarbons deposited in the capillary chromatographic column were then analysed by GC–electron-capture detection (ECD). The SFE was performed at 250 atm and 40°C for 10 min. Volume of the extraction cell with the sediment was 2 cm^3. Recovery of the purgeable halocarbons from the sediment was in the 90% region at low μg/g levels.

5.2. Chlorobenzenes and chlorophenols

Hong-Xu [28] described SFE of ten chlorobenzenes from sediment samples. The SFE was performed off-line. After the SFE, the analytes were concentrated in the trap packed with C_{18} reversed-phase. Chlorobenzenes were then eluted from the trap by isooctane and after a clean-up procedure analysed by GC–ECD. Optimum SFE conditions were as follows: extraction temperature 80°C, extraction time 20 min, sample amount 1 g placed in 7 cm^3 extraction chamber, extraction pressure of pure CO_2 168 atm, flow-rate of liquid CO_2 2 cm^3/min. The method was tested using sediments with certified concentrations of chlorobenzenes and by comparisons with Soxhlet extraction. In the concentration range 5–200 ng/g for the different chlorobenzenes, the SFE provided results coincidental with the certified values and/or Soxhlet extraction.

Richards and Campbell [29] investigated recovery of some priority pollutants from spiked soil samples by SFE, Soxhlet extraction and sonication liquid extraction. They found that SFE is more efficient than other extraction methods for 13 of 16 compounds tested. SFE averaged 80.2% and individual values ranged from 70.4 to 95%. Only phenolic and chlorophenolic compounds had equivalent recoveries in the SFE and Soxhlet extraction. Sonication was less efficient than the SFE for all compounds studied except 2,4-dichlorophenol, which was recovered in equal amounts by both extraction methods. Groups of compounds tested were: phenols and chlorophenols, chlorobenzenes and naphthalene. The best results were obtained by using carbon dioxide with 2% of methanol as a supercritical fluid. The extraction was performed at 39.5 MPa and 80°C. The spiked soil (2 g) (spiking level 25 μg/g for each compound) was extracted in a 1.67 cm^3 chamber for 30–40 min (20 cm^3 of the extraction fluid was consumed; measured as a liquid using pump displacement). The SFE was performed off-line; the compounds extracted by the SFE were trapped at the outlet of the restrictor into methylene chloride. The methylene chloride solution was concentrated to 1 cm^3 under gentle stream of nitrogen and analysed by GC–MS.

Recovery of chlorophenols from spiked sediment was also measured in other work using off-line SFE [30]: supercritical fluid CO_2 at 23 MPa and 48°C, amount of sediment 0.5 g, time of the SFE 15 min, restrictor used was 20 cm long, 25 μm I.D. Analytes leaving the restrictor were trapped in 0.5 cm^3 of methanol. After derivatisation to chlorophenol acetates, the compounds were analysed by capillary GC. Recoveries of *o*- and *p*-chlorophenol, 3,4-dichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol were equal to or higher than 90%. Under the SFE conditions described there were no differences between recoveries from dry and wet (20% of water) sediment except *o*-chlorophenol and *p*-chlorophenol, which were partially lost during the drying.

5.3. Polychlorinated biphenyls

The possibility of isolation of polychlorinated biphenyls (PCBs) from sediment using simple off-line SFE apparatus was shown already in 1986 [31]. More recent papers deal with on-line coupled techniques, where SFE is directly coupled usually to GC–ECD.

Hawthorne and Miller [32] accomplished coupling SFE to GC–ECD by inserting the SFE restrictor outlet through the on-column injection port. The GC oven was cooled during the extraction. Extracted analytes were thermally focused inside the capillary chromatographic column at the outlet of the restrictor. Since nitrous oxide, which was used as a supercritical fluid, gives a relatively high ECD response, after the extraction period and withdrawing the restrictor from the injector the chromatographic column was flushed for 2–3 min with a carrier gas still at low temperature (5°C). PCBs were then analysed using a temperature program. Parameters of the SFE were as follows: extraction period 10 min, extraction pressure 300 bar, temperature 45°C, extraction fluid nitrous oxide. A restrictor with 25 μm I.D. (corresponding to a flow-rate of the gaseous nitrous oxide of *ca.* 240 cm^3/min) was used. This restrictor provided good SFE recoveries, but required wide-bore thick-phase GC columns to provide good low-temperature focusing. The amount of the sediment taken for one analysis was 10 mg. The on-line SFE–GC–ECD analysis of the spiked sediment (8 μg/g of Aroclor 1254) gave essentially quantitative extraction and recovery of the PCBs.

In the paper published by Onuska and Terry [33], the PCBs extracted from the sediment by SFE were collected in an "accumulator" (2 m × 0.32 mm I.D. fused-silica capillary coated with cross-linked SE-54 stationary phase). The analytes emerging from the restrictor are partitioned in the accumulator, which is kept at lower temperature (5°C). Desorption from the accumulator is achieved by a rapid temperature ramp to 140°C and with hydrogen as a carrier gas. Direct on-line coupling of the accumulator to the analytical capillary column is made by switching a six-port valve to another position (the first position of the six-port valve is utilised during the SFE). Recommended parameters of the SFE were: supercritical fluid CO_2 + 2% methanol (kinetics of SFE with pure CO_2 were proved to be slower), pressure 20.7 MPa, temperature 40°C. The restrictor was 10 cm long, 25 μm I.D. (flow-rate of the supercritical fluid 0.35 g/min). The SFE was performed in static mode: the extraction cell was pressurised and the pressure was maintained in the cell for 2 min to reach equilibrium. Extracted analytes were then transferred to the accumulator by opening a valve in the extraction cell outlet for 30 s. The valve was then closed and the cycle was repeated five times. After the fifth step the six-port valve was switched to another position, residual methanol in the accumulator was flushed by the carrier gas at 5°C, and GC–ECD analysis started. From 10 to 100 mg of the sediment with a certified value of 2.02 μg/g of PCBs were extracted and analysed. Statistic comparison of the certified and determined values revealed excellent coincidence.

Recent data provided by Onuska and Terry [34] show that difluorochloromethane (freon-22) is the most efficient fluid for SFE of PCBs from sediment (most likely because of its high dipole moment) in comparison with pure CO_2. Methanol-modified CO_2 yielded also acceptable recoveries. The off-line SFE using freon-22 was performed mostly at 100°C and 400 atm and PCBs were trapped by inserting the restrictor outlet into several cm^3 of acetone in a vial. Although the widespread use of freons for industrial purposes is being reduced, which would affect their future analytical uses, the hydrogen-containing fluorochlorohydrocarbons have much lower influence on ozone depletion, and are more suitable for SFE.

5.4. Tetrachlorodibenzo-p-dioxins

Onuska and Terry [35] tested SFE for isolation of tetrachlorodibenzodioxin (2,3,7,8-TCDD) from sediment matrix. They used off-line SFE. The extraction cell had a volume of 0.5 cm^3 and 50 mg of the sediment were extracted. The sediment was spiked by the tested compound to a concentration of 0.2 μg/g. The SFE extracts were collected in hexane, which was, after the procedure completion and concentration of the hexane solution, analysed by GC–MS. The results of the study showed, that pure CO_2 is not able to extract tetrachlorodibenzodioxin effectively. After 30 min of extraction at 310 atm and 40°C the recovery was only about 50%. Much better results were obtained using pure nitrous oxide (91% recovery). The best results involved modification of

the fluids by 2% of methanol. During 30 min of SFE, recovery by carbon dioxide + 2% methanol of over 93%, and by nitrous oxide + 2% methanol of 100% was achieved. Moisture content in the sediment dramatically decreased the recovery by 10–15%. To achieve the same recovery with wet sediment the time of SFE must be doubled. For comparison, classical Soxhlet extraction of 1 g of the sediment by a hexane–acetone–trimethylpentane mixture for 18 h provided only 65% recovery of TCDD.

The SFE was also used for enrichment and isolation of TCDD from municipal incinerator fly ash [36]. For the SFE pure nitrous oxide and its mixtures with methanol and toluene were used. Experiments were carried out at 45°C with 25 mg sample. The best recovery was obtained with nitrous oxide + 2% methanol at 400 bar. Under these conditions the SFE was more efficient than the Soxhlet technique.

Suitability of nitrous oxide for the SFE of dibenzo-*p*-dioxins and dibenzofurans from incinerator fly ash was also described by Alexandrou *et al.* [37]. They found also that carbon dioxide + 10% benzene is able to provide high recoveries of these compounds. For the fractionation and clean-up of the complex organic mixtures in an organic solution obtained after the off-line SFE of the fly ash sample, a further SFE step can be used (instead of the usual clean-up procedure by column liquid chromatography). For example, the liquid extract can be collected in a Florisil column. The column is then extracted for 15 min with CO_2 at 40°C and 200 atm. During this extraction over 75% of PCBs and chlorobenzenes are removed from the column. Full recoveries of polychlorodibenzo-*p*-dioxins are obtained by extracting of the column with nitrous oxide for 90 min at 400 atm [38].

5.5. Organochlorine pesticides

More than 20 organochlorine pesticides were isolated from sediment matrix by off-line SFE [39]. Spiked sediment (1–10 g) was extracted at 60°C and 250 atm for 5 min in static mode and 20 min in dynamic mode. Flow-rate of supercritical fluid (CO_2) was 0.5 cm^3/min. Methanol was used as polarity modifier. Recovery of chlorinated pesticides under these conditions was higher than 90%. It was also shown that the SFE with carbon dioxide is inefficient for extracting elemental sulphur from environmental solids [40]. Elemental sulphur is a serious problem when an electron-capture detector or a flame photometric detector is used for the gas chromatographic analysis. The extracts of environmental solids after liquid extraction usually require treatment with metallic mercury or copper, which results in conversion of soluble sulphur to insoluble sulphides. The method is effective; however the treatment leads to degradation of a number of pesticides. In contrast to Soxhlet extracts, less than 2% of the elemental sulplur was present in the SFE extracts, while the majority of pesticides from the spiked sediments were recovered. Thiophosphate and chlorinated pesticides were used in this work [41].

5.6. s-Triazine herbicides

SFE recoveries higher than 90% from sediment can be achieved by pure CO_2 for propazine, terbutylazine, atrazine and cyanazine [42]. Simazine, as a very poorly soluble compound in pure CO_2, requires addition of methanol to the supercritical fluid. The entrainer can be added directly to the sediment in the extraction chamber [43]. The SFE was performed under the following conditions: off-line SFE, amount of the spiked sediment 0.5 g, spiking level 4 μg/g–40 ng/g, extraction pressure 230 atm and temperature 48°C, time of the SFE 30 min, consumption of the liquid CO_2 during the extraction period 18 cm^3. Compounds leaving the restrictor from the SFE apparatus were trapped into a few cm^3 of methanol. Methanolic solution was (after concentration under gentle stream of nitrogen) analysed by capillary GC–flame ionization detection (FID) and/or HPLC–diode-array detection (DAD) at 225 nm. Due to background of natural hydrocarbons in the sediment, which are easily recovered by SFE and interfere with *s*-triazines during the GC, the HPLC method was found to be more suitable for the final analysis than GC–FID. It was also confirmed in another study with actual soil samples [44], that presence of methanol or acetone in supecritical CO_2 improved recovery of atrazine.

5.7. Phenoxycarboxylic acid herbicides

Experiments on the solubility of some pesticides in supercritical carbon dioxide [39] and model experiments with their SFE from a glass wool [40] showed the potential of the method for environmental samples containing phenoxycarboxylic acids or their derivatives.

Soil spiked with 1.8–7.5 μg/g of isooctyl ester of 2,4,5-trichlorophenoxyacetic acid was extracted in off-line apparatus under the following conditions [41]: sample amount 10 g (before the SFE the soil was mixed with water and methanol, 5% and 3% (w/w), respectively, in order to increase polarity of the passing supercritical fluid), pressure 100–316 atm, temperature 40–45°C, extraction time 40 min, flow-rate of the liquid CO_2 2–2.5 cm^3/min. It was shown that the higher the pressure, the higher the recovery of the herbicide. However, at the highest pressure the recovery did not exceed 80%.

5.8. Fuels and crude oil

Fuel and crude oil contamination of environmental solids can be relatively easily isolated from the environmental solids by SFE using pure CO_2. It was, for example, shown [45], that recovery of C_5–C_{40} *n*-hydrocarbons from spiked alumina by the SFE approached 100% (CO_2 at 325 atm; extraction temperature 60°C and time 20 min). The method was also used for the determination of biomarkers (pristane and phytane) and carbon number distribution in crude oil source rocks (extraction pressure 400 bar, time 20 min, temperature 60°C, amount extracted 200 mg, on-line SFE–GC–FID).

It was shown [46] for the example of a wet sediment (20% water) contaminated by fuel, that C_8–C_{30} *n*-hydrocarbons, alkylbenzene and alkylnaphthalene isomers were removed from the sample (1.3 g) by pure CO_2 (380 atm and 50°C) in 10 min completely. It is important, that the SFE is efficient also for wet samples, because lower *n*-alkanes otherwise disappear during the drying procedure. Quantitative analysis was performed using on-line SFE–capillary GC–MS with a conventional split/splitless injector. The SFE–GC coupling was achieved by inserting the restrictor (10 cm long, 25 μm I.D.) directly into the split/splitless injector.

Pure supercritical CO_2 was also found to be an excellent fluid for extraction of diesel fuel from clay and soil [47] (similar results are also described in ref. 48). A range of hydrocarbons from C_{14} to C_{20} was monitored. The off-line SFE was performed at 314 atm and 70°C. Matrix sample size was 1 g and flow-rate of the liquid CO_2 was 2 cm^3/min. Up to 100 volumes of the SFE cartridge of the liquid CO_2 were used for the SFE. The analytes extracted were then trapped in the small C_{18} column and after the SFE eluted with 3 cm^3 of methylene chloride. The eluate was analysed by GC–FID.

5.9. Polycyclic aromatic hydrocarbons

PAHs are one of the most troublesome group of compounds, because they are poorly soluble in supercritical fluids, especially the compounds with more condensed benzene rings (also for ordinary liquid solvents). Their recovery also strongly depends on the type of matrix. Recovery of the PAHs also usually decreases with increasing molecular mass (decreasing solubility). At matrices such as fly ash and sediments they are adsorbed very strongly and their release by a supercritical fluid is sometimes difficult to obtain complete recovery. One should take care about the type of matrix to be analysed for PAHs. For example, it was shown [42], that PAHs were not completely recovered from urban dust by CO_2 at 80 atm (density 0.23 g/cm^3), whilst the SFE of glass beads spiked with the PAHs removed a significant amount of analytes.

Different solubilities of various groups of hydrocarbons in supercritical fluids can be used for removal of compounds, which could interfere during final chromatographic analysis. The SFE thus provides a certain selectivity. It was shown by Hawthorne and Miller [43], that the major portion of *n*-alkanes (nonadecane–hexacosane) was removed from diesel exhaust particulates by CO_2 at 75 atm, while PAHs were not significantly extracted. The PAHs were then extracted at 300 atm with recovery mostly above 90%.

There has not been yet established an unambiguous analytical scheme for SFE of PAHs from various matrices. However, probably the best results were obtained with nitrous oxide with 5% methanol as a supercritical fluid. Recovery of PAHs using this fluid is much higher than recoveries obtained with CO_2 or ethane and even higher than with CO_2 with

TABLE 4

SFE OF PAHs FROM ENVIRONMENTAL SOLIDS

Matrix, amount	Fluid	Time of SFE	Temp. (°C)	Pressure (MPa)	Conc. order	SFE setup	Ref.
Carbon black, 450 mg	CO_2	Static 5 min	50	32.8	μg/g	On-line	27
Urban dust, 6 g	CO_2	4 h	40	36.2	μg/g	Off-line	31
Sediment, 10 mg	N_2O	10 min	45	30.6	μg/g	On-line	32
Urban dust, 15 cm^3 SFE cell	CO_2	Static and 1 min dynamic	50	20	μg/g	On-line	42
Dust, 5–30 mg	CO_2	90 min	45	30.6	μg/g	Off-line	43
	CO_2–CH_3OH	30 min	65	30.6	μg/g	Off-line	43
Dust, sediment, fly ash, 20–50 mg	Ethane, N_2O, CO_2, N_2O–CH_3OH	30 min	45–65	30.6	μg/g	On-line	44
Petroleum waste sludge, 310 mg	$CHClF_2$	40 min	100	40	μg/g	Off-line	49

5% methanol [44]. It is probably due to higher polarity of the nitrous oxide–methanol and resulting higher solubility of the PAHs in this fluid. Typical conditions which were used for the SFE of PAHs from various matrices are given in Table 4.

A very promising supercritical fluid for the SFE of PAHs is difluorochloromethane (freon-22) [49]. It has been recently shown that extraction rates of individual PAHs from a petroleum sludge were similar with freon-22, whilst the rates decreased with increasing molecular mass using CO_2 and nitrous oxide. The SFE with difluorochloromethane for 40 min was much more efficient than methylene chloride sonication for 18 h, especially for higher PAHs. Also trifluoromethane provides good recoveries of PAHs from soil [50].

5.10. Linear alkylbenzenesulphonates

Quantitative extraction of anionic surfactants from soil, sediment, and municipal waste water treatment sludge was achieved by supercritical CO_2, whose polarity was increased by high content (*ca.* 40 mol%) of methanol [51]. Recovery of alkylbenzenesulphonates was higher than 90% after 30 min of the SFE at 380 atm and 125°C. Amount of the sample extracted was usually 1 g. Flow of the extraction fluid through the extraction cartridge was 1.2 or 0.45 cm^3/min, using 10 cm lengths of either 30 or 25 μm I.D. fused-silica tubing as a restrictor. The compounds extracted by the SFE were collected in a vial containing 5 cm^3 of ethanol. For the recovery measurement ^{14}C-labelled compounds were used. The native alkylbenzenesulphonates were analysed by HPLC with fluorescence detector. The off-line SFE apparatus used comprised a simple vessel for the preparation of high content of polarity modifier in the supercritical fluid.

5.11. SFE of organic compounds from adsorbent materials

SFE can be also used for extraction of organic compounds from Tenax, XAD resins, reversed-phase based sorbents, charcoal or polyurethane foams after previous enrichment of organic compounds from water, air, etc.

It was shown [43] that PAHs can be effectively removed from Tenax by CO_2: 80 mg of Tenax, which was used for enrichment of PAHs from diesel exhaust, were extracted by CO_2 at 45°C and 20 MPa for 5 min. Naphthalene, 9-fluorenone, phenanthrene, pyrene, benz[*a*]anthracene, benzo[*ghi*]perylene and coronene provide recoveries higher than 90%. Similar results were obtained for XAD-2 resin and polyurethane foam [52]. On the other hand, PAHs were not recovered from Spherocarb. However, nor was Soxhlet extraction successful in this case [52].

SFE was also used for determination of semivolatile mutagens in air using solid adsorbents [53]. Adsorbents (finally XAD-4 resin was utilised), which were used for the trapping of the mutagens from air, were extracted by pure and entrainer-modified car-

bon dioxide. Mutagens, such as 4-nitrobiphenyl, 2-nitrofluorene and fluoranthene, were recovered from the XAD-4 trap in 180 min with efficiency 88.5, 92,3 and 60.6%, respectively, by using CO_2 with 12% of hexane at 400 atm and 50°C [53].

Chlorinated benzenes (tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene) can be isolated from water by solid-phase extraction (Amberlite XAD resins, Tenax GC and reversed-phase HPLC C_{18} were used). The analytes can be, after drying of the sorbent by means of a stream of nitrogen at 60°C, released from the adsorbent material by supercritical fluid extraction. In this work [25], carbon dioxide with 10% of methanol at 400 atm and 60°C was used. The restrictor used was a piece of 20 μm I.D. fused-silica capillary. Time of the SFE was 45 min. Overall recovery of chlorobenzenes was over 97% for their concentration of 1 $\mu g/dm^3$ in water.

Sulphonyl urea herbicides can be isolated from water samples by solid phase extraction [54]. The analytes were then eluted from the extraction disk by SFE using 5% methanol + CO_2. For the final analysis HPLC–UV (235 nm) was used. Compounds tested were: sulphachloropyridazine, thifensulphuron methyl, metsulphuron methyl, sulphometuron methyl, chlorsulphuron, tribenuron methyl, benzsulphuron methyl and chlorimuron ethyl. Recovery of the herbicides was mostly higher than 80%. Only tribenuron methyl and chlorimuron ethyl had recoveries lower than 80%. Recoveries were measured for 1 dm^3 water sample and concentration level 50 $\mu g/dm^3$.

Solid-phase extraction with SFE elution was also tested for analysis of explosives in water [55]. 2,6-Dinitrotoluene, 2,4-dinitrotoluene, and trinitrotoluene were adsorbed on phenyl stationary phase. After the nitrotoluenes were trapped on the solid sorbent, they were eluted by supercritical CO_2. Before the SFE, the sorbent was doped with toluene. After the off-line experiments, on-line SPE–SFE–GC—ECD was used for analysis of water samples. Recovery of the SFE (400 atm, 75°C) achieved 100%. The method was tested for low $\mu g/dm^3$ and ng/dm^3 levels.

5.12. Direct SFE of organics from water solutions

There are several problems associated with the SFE of compounds from water solution. Probably the main problem is relatively high solubility of water in the supercritical CO_2 (approximately 0.3% [56]). For a dynamic SFE, which is commonly used for the extraction of solids, the removal of water phase and enrichment of the supercritical fluid by water can cause "principal" (the water phase is transferred through the restrictor into the collection vessel or accumulator in off-line methods; and/or would enter the chromatographic system, which is not desirable in most cases) and technical (plugging of the restrictor by ice during the supercritical fluid expansion) problems. The problems have been recently partly solved, but the present state-of-art of the SFE of organics from water is not as acceptable for routine analysis as the SFE of environmental solids.

The first papers about the SFE of water samples probably appeared in late eighties [11,57]. The first method [11] is based on "closed-loop stripping" principle, when the supercritical fluid is (after pressurising of the system) recycled by a pump from the outlet of the extraction cell back into the water sample. After equilibrium in the whole system is achieved, a sample of the supercritical phase is taken by means of a valve with loop. The content of the loop is then analysed by supercritical fluid chromatography.

The system described above was tested for analysis of diisopropyl methylphosphonate (concentration 834 $\mu g/dm^3$–834 mg/dm^3) in water. Volume of water sample was 8 cm^3. Time necessary to reach the equilibrium was 1.5 h. When 0.1 mg of NaCl was added to the water sample before the extraction, the equilibrium time was reduced to less than 5 min. The relative standard deviation was 15% for concentration 834 $\mu g/dm^3$ and 1.5% for concentration higher than 8.34 mg/dm^3. The method was also used for SFE of phenol from aqueous solutions [58].

The other system [57] is based on a sandwich type phase separator, in which supercritical CO_2 and water phase, after passing extraction coil, are separated by means of a hydrophobic membrane. Two membranes were able to withstand higher pressure: PVDF $[(-CH_2-CF_2-)_n]$ and Delrin $[(-CH_2-O-)_n]$. A sample of the separated supercritical fluid was taken by a valve with loop for SFC analysis. Phenol and 4-chlorophenol were utilised as test compounds.

Water solutions can be also extracted by a super-

critical fluid when a smaller amount of water is added to the inert material (sand, glass beads, etc.). The film of the water phase on the surface of the carrier material can be subjected to SFE. In this case the restrictor has to be attached in the upper part of the extraction chamber, to prevent liquid water entering the restrictor. This method was used for the SFE of phenol from water [45].

5.13. Simultaneous SFE and derivatisation

The *in-situ* derivatisation of the polar compounds is quite challenging. Under the SFE conditions (especially at higher pressures) the derivatisation reactions of highly polar compounds could take place easily [59,60], resulting in less polar products, which are more suitable for the SFE and subsequent chromatographic analysis.

An interesting paper has been recently published by Hawthorne and co-workers [45,61]. For the derivatisation of polar compounds (2,4-dichlorophenoxyacetic acid and Dicamba, phospholipid fatty acids in the whole cells and phenolics in waste water) trimethylphenylammonium hydroxide and boron trifluoride in methanol were used. The procedure consists of four steps: (1) the sample is placed into the extraction cell together with the derivatising reagent, (2) the cell is placed into the heater and pressurised by CO_2 to 400 atm, (3) the derivatisation takes place for 5–45 min under static conditions (the outlet of the extraction cell is closed), and (4) the outlet of the cell is opened and the sample is extracted dynamically for 5–15 min. Derivatised compounds —methyl esters and/or anisoles— were trapped at the outlet of the restrictor into a small amount of methanol or dichloromethane. The solutions were analysed by GC–FID, -ECD and -MS. Recovery for all compounds is >90%.

In-situ derivatisation–SFE was also used for acetylation of phenols isolated by solid-phase extraction from water samples [62]. The pH value of the water sample was adjusted to 12. The water sample was then passed through the conditioned anion-exchange disk. Anionic form of the phenols was thus trapped in the exchanger. After the solid-phase extraction, 0.5 cm^3 of acetic anhydride was added and the derivatisation to the acetylated phenols took place under the static SFE conditions (400 atm, 50°C). After the derivatisation, the phenolic acetates were eluted with 30 cm^3 of supercritical CO_2. The SFE extract was trapped in 2 cm^3 of acetone and analysed by GC–MS. Recovery of all phenols tested (2- and 4-nitrophenol and 1-naphthol) was higher than 75% at 25–50 $\mu g/dm^3$ level. Total time for the derivatisation–SFE was about 30 min.

6. CONCLUSIONS

Supercritical fluid extraction is rapidly becoming an important technique in the preparation of samples for analysis. Much of the work published is related to environmental problems. It has been shown to be rapid, less laborious and usually less toxic and hazardous but more environmentally friendly than liquid extraction.

A problem remaining with this technique is the effect of the matrix on extraction. This can sometimes result in a large fraction of the solute being slowly extracted after a rapid initial extraction. Furher efforts are required in the understanding of this phenomenon and in the development of techniques to overcome it.

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Factors controlling quantitative supercritical fluid extraction of environmental samples

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ABSTRACT

The development of quantitative supercritical fluid extraction (SFE) methods for the recovery of organic pollutants from environmental samples requires three steps: quantitative partitioning of the analytes from the sample into the extraction fluid, quantitative removal from the extraction vessel, and quantitative collection of the extracted analytes. While spike recovery studies are an excellent method to develop the final two steps, they are often not valid for determining extraction efficiencies from complex real-world samples such as soils and sediments, exchaust particulates, and sludges. SFE conditions that yield quantitative recoveries of spiked analytes may recover <10% of the same analytes from real-world samples, because spiked pollutants are not exposed to the same active sites as the native pollutants. Because of the heterogeneous nature of environmental samples, the partitioning step may be controlled by analyte solubility in the extraction fluid, kinetic limitations, and/or the ability of the extraction fluid to interrupt matrix–analyte interactions. While the interactions that control SFE rates from heterogenous environmental samples are not well understood, a generalized scheme for developing quantitative SFE methods is proposed based on interactive considerations of the collection efficiencies after SFE, fluid flow parameters in the extraction cell, analyte solubility, extraction kinetics, and analyte–matrix–extraction fluid interactions. The proposed development scheme includes increasing SFE extraction rates by the use of more polar fluids than CO_2 such as $CHClF_2$, the addition of organic modifiers to CO_2, and the use of high temperature extractions with pure CO_2. Validation of quantitative extractions based on multiple extraction methods (SFE followed by liquid solvent extractions) is also described.

INTRODUCTION

The interest in using supercritical fluids as a replacement for conventional liquid solvents for the extraction of organic pollutants from environmental samples has increased rapidly because of the need to reduce liquid solvent wastes as well as to perform more rapid sample preparations [1,2]. Acceptance of supercritical fluid extraction (SFE) by the regulatory community has begun as demonstrated by the recent approval of the first SFE method as a replacement for conventional Soxhlet extraction of total petroleum hydrocarbons (TPH) by the US Environmental Protection Agency [3].

While an increasing number of quantitative applications of SFE for the extraction of environmental pollutants has been reported in recent years, reported recoveries are often low and there has been little consistency in the SFE conditions (*e.g.*, fluid choice, presence and identity of modifiers, pressure, temperature, extraction flow-rate) among the various reports. Review of the available literature demonstrates that SFE conditions that successfully extract a specific pollutant from one environmental sample may not yield quantitative recovery from a different matrix. (Similar inconsistencies exist for conventional liquid solvent extraction methods, but are rarely investigated and discussed.) For example, extraction with pure CO_2 has yielded quantitative recovery of polychlorinated biphenyls (PCBs) from polyurethane foam (PUF) sorbent resins [4], while the use of similar SFE conditions only resulted in *ca.* 60% recoveries of the same PCB congeners from

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sediment [5]. It is becoming increasingly clear that high solubility of a particular species in the supercritical fluid is not a sufficient condition to yield high extraction efficiencies, and that the ability of the supercritical fluid to overcome matrix–analyte interactions is often more important than high solubility for achieving quantitative recoveries. While the interactions of pollutant molecules with a sorbent resin may be expected to be relatively homogeneous, pollutants may interact with several different binding sites (each having different binding strengths) with heterogeneous environmental solids. Even when pollutant molecules are efficiently extracted, volatile and semi-volatile organics can easily be lost during the collection step since the analytes are generally collected from the depressurized fluid at a high gas flow-rate.

The present paper describes factors that we have found to be important in developing quantitative SFE methods for extracting common pollutants from environmental matrices. The discussion will focus on the extraction of heterogeneous real-world samples (such as soil, sediments, sludges, and air particulates), since the potential analyte–matrix interactions are more complex than those expected for sorbent resins. Attempts are made to explain the differences in the extraction behavior of analytes from different matrices, and a general approach to developing quantitative SFE methods for complex environmental solids is presented. While the understanding of supercritical fluid–analyte–matrix interactions is far from complete, it is hoped that the present work will provide some useful guiding principals for the development of quantitative SFE methods for complex environmental samples.

EXPERIMENTAL

Except for the spike recovery studies, all samples contained native (not spiked) pollutants. Certified reference samples including urban air particulate matter (SRM 1649) and river sediment (SRM 1939) were used as received from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Contaminated soils and a petroleum waste sludge were used as received except that the soils were sieved through a 3.35-mm (6 mesh) screen to remove rocks and sticks before extraction.

All SFE extractions were performed using either ISCO Model 260D or 100D syringe pumps connected to either an ISCO Model SFX2-10 extraction unit or extraction cells from Keystone Scientific (Bellefonte, PA, USA) which were placed into a tube heater or a chromatographic oven to control temperature. Flow-rates through the extraction cells were controlled at the desired values of ca. 0.1–3 ml/min (measured as liquid flow at the pump) by 10–15 cm lengths of fused-silica tubing having inner diameters of 10–50 μm. Unless otherwise noted, all extracts were collected in 3–5 ml of a suitable organic solvent [acetone for GC–electron-capture detection (ECD) determinations of PCBs, perchloroethylene for infrared determinations, and methylene chloride for GC–MS and GC– flame ionization detection (FID) determinations].

Extracts were analyzed using Hewlett-Packard Model 5890 GCs equipped with FID and ECD systems. GC–MS analyses were performed with a Hewlett-Packard Model 5988 GC–MS system. Chromatographs were equipped with HP-5 (25 m × 320 μm I.D., 0.17 μm film thickness) or J&W DB-5 (60 m × 250 μm I.D., 0.25 μm film thickness) columns. TPH concentrations in extracts were determined using a Foxboro Model MIRAN-1A infrared spectrometer (Foxboro, MA, USA) as described earlier [6].

The relative extraction rates of spiked *versus* native polycyclic aromatic hydrocarbons (PAHs) were performed by spiking 1–10 μl of a methylene chloride solution containing [$^2H_8$]naphthalene, [$^2H_{10}$]phenanthrene, [$^2H_{10}$]pyrene, [$^2H_{12}$]chrysene, and [$^2H_{12}$]benzo[*b*]fluoranthene onto the sample of interest (each of which contained native PAHs), and either extracting after 10 min or after a 14-h waiting period. The relative extraction rates were determined by collecting timed fractions, adding 1-chloroanthracene as an internal standard, and determining the concentration of each deuterated PAH spike and each native PAH by monitoring their molecular ion by GC–MS.

RESULTS AND DISCUSSION

Conceptual steps in an SFE

The quantitative extraction of a particular pollutant from an environmental solid can be viewed as a three step process: First, the analyte must be efficiently (and rapidly) partitioned from the sample

matrix into the bulk supercritical fluid. Second, the analyte must be swept from the sample extraction cell. Finally, the analyte must be efficiently collected in a form that is compatible with the method used for analysis of the extract. While the first step is controlled by the chemistry of the system, steps two and three are essentially controlled by the mechanics or "plumbing" of the extractor including (but not limited to) the dimensions of the extraction cell (and sample size), the flow-rate of the supercritical fluid, and the efficiency of the collection device. Because the physiochemical processes that control SFE extraction (and collection) efficiencies are not well understood, particularly for heterogeneous environmental samples, and because of the large variety of SFE extraction and collection methods that are used, a discussion which includes all the relevant variables is beyond the scope of this paper (and indeed, is not possible with the present understanding of SFE processes). Therefore, the following discussion will attempt to present guidelines which we have found to be useful in evaluating and developing the important steps in SFE.

Trapping efficiencies. Since the first two steps in the SFE experiment listed above can not be accurately evaluated until the third step is quantitatively efficient, the collection of extracted analytes will be addressed first. Although trapping of analytes using SFE has been performed by "on-line" techniques including SFE–GC, SFE–supercritical fluid chromatograph (SFC), and SFE–LC [1,2,7–9], the vast majority of applications have utilized "off-line" trapping either in a cryogenic or sorbent trap, or (most commonly) by trapping in a small volume of liquid solvent [1]. Each collection method (with the possible exceptions of SFE–SFC and SFE–LC) depends on depressurizing the compressed supercritical fluid to ambient conditions with a coincident deposition of the analytes in (or on) the collection media. Since, for example, a 1-ml/min flow of supercritical CO_2 depressurizes to *ca.* 500 ml/min of gaseous CO_2, the collection step essentially becomes a problem in efficiently trapping the analytes from a high-flow gas stream. Although the analytes (and depressurized fluid) are cooled because of the expansion upon depressurization, trapping efficiencies can be very low depending upon the method used for collecting the extracted analytes.

While the loss of volatile analytes seems most likely, even relatively non-volatile species can be lost during the collection step. Each collection method has potential loss mechanisms associated with the phenomena used for trapping. For example, off-line trapping mechanisms have generally poor recoveries of very volatile analytes (*e.g.*, hexane, benzene), but the losses of volatiles with cryogenic trapping can be particularly severe. With certain designs of cryogenic traps, losses of $>95\%$ of compounds as non-volatile as chrysene (b.p. 440 °C) have been reported [9]. Sorbent traps must quantitatively retain all of the analytes of interest as well as allow them to be quantitatively recovered after collection. Quantitative retention on sorbent traps is particularly difficult when organically-modified CO_2 is used for the extraction, because the organic modifier (*e.g.*, methanol) becomes a liquid solvent upon depressurization and can itself elute the target analytes from the sorbent resin during the SFE step, resulting in low apparent recoveries [10]. The collection efficiencies (particularly of more volatile components) obtained using the most common collection method, liquid solvent trapping, can be affected by the identity of the collection solvent, solvent volume and temperature, collection vial design, the flow-rate of the extraction fluid, and the use of restrictor heaters (to reduce restrictor plugging, see refs. 11–13). Unfortunately, *all* trapping methods used for SFE can suffer from less-than-quantitative collection efficiencies which are often (and unfortunately) attributed to poor extraction rather than collection efficiencies. Because of the potential for poor collection of analytes after SFE (regardless of the collection method used), the first major step in developing any SFE method should be the testing (and if necessary) the development of quantitatively efficient collection methods. Appropriately designed spike recovery studies will not only aid in developing an efficient collection system, but will also determine if the target analytes have sufficient solubility to extract under the SFE conditions selected initially. However, as discussed below, they are not an appropriate method for developing quantitative extraction conditions because of large potential differences in the matrix–analyte interactions experienced by spiked and native molecules.

The extraction conditions to be used for the real-world samples should be used to extract the ana-

lytes of interest spiked at known concentrations onto a relatively inert matrix (*i.e.*, the spiked matrix should retain the spiked analytes until the SFE extraction is begun, but should not retain the spiked analytes during the SFE extraction since the goal is to evaluate only the collection method). For example, to evaluate the liquid solvent collection conditions used for the representative pollutants shown in Table I, *ca.* 18 μg of each species was spiked onto sand, extracted with the conditions that were expected to be used for real-world samples, and collected with the various test solvents [11]. Since the method was to be used for wet soil samples, the restrictors and solvent were mildly heated with a heat gun to avoid restrictor plugging expected from ice formation. As shown in Table I, recoveries of the analytes in hexane and methanol were particularly poor, and could have been mistakenly evaluated as poor extraction (rather than collection) efficiencies. Also note that, in addition to the identity of the collection solvent, additional collection conditions (including extraction fluid flow-rate, heating methods used to avoid restrictor plugging, collection solvent volume and temperature, and collection vial shape) may affect the collection efficiencies [11-13]. For example, heating the collection solvent and restrictor with a heat gun limited the collection efficiencies in the best solvent (methylene chloride) to *ca.* 75–90%. However when ice formation was avoided by keeping the collection solvent temperature from cooling below 5°C using a heating block (rather than with a heat gun), the collection efficiency increased to >98% for all of the test analytes using only 3 ml of methylene chloride as the collection solvent (Table I).

Losses that occur with collection in a liquid solvent may occur both because a particular analyte molecule is never trapped in the solvent, or because trapped molecules are purged from the collection solvent by the high gas flow of the depressurized extraction fluid. The purging losses of analytes from the collection solvent can easily be tested by preparing a suitable standard solution in the collection solvent and purging the solution with the extraction fluid in the same manner as that used for sample extractions. However, a previous study has demonstrated that the majority of losses can be attributed to inefficient partitioning of the analytes from the depressurized extraction fluid rather than to the purging of trapped analytes from the collection solvent [12], demonstrating that the best test of a collection system is the spike recovery study described above, rather than the purging study.

Each different commercially-available (and laboratory-built) collection system can have greatly different collection efficiencies, and the extraction conditions themselves (*e.g.*, flow-rate, type of restrictor used, extraction fluid identity, extraction temperature, and the presence and identity of an organic modifier) can affect the collection efficiencies. Because of the large number of experimental variables that can affect collection efficiencies, the

TABLE I

EFFECT OF SOLVENT ON COLLECTION EFFICIENCY OF REPRESENTATIVE SEMIVOLATILE POLLUTANTS

Collections were done with mild heating of the restrictor to avoid plugging from ice formation. The methylene chloride samples were also collected by placing the collection solvent in a 5°C temperature block rather than heating to avoid ice formation. Results are adapted from ref. 11.

Analyte	SFE collection efficiency			
	Hexane	Methanol	CH_2Cl_2	CH_2Cl_2 (5°C)
Phenol	43 ± 2	55 ± 12	77 ± 2	98 ± 1
1,2-Dichlorobenzene	46 ± 5	58 ± 13	78 ± 5	100 ± 2
2-Nitrophenol	57 ± 5	61 ± 8	80 ± 5	99 ± 1
Pyrene	80 ± 5	58 ± 7	90 ± 3	99 ± 5
Benzo[*ghi*]perylene	71 ± 2	67 ± 11	93 ± 2	99 ± 4

determination of the quantitative abilities of the collection device must be determined using appropriate spike recovery studies prior to further development of the SFE method. Fortunately, even with relatively simple (and inexpensive) collection methods using a few ml of liquid solvent, quantitative collection (>90%) of analytes as volatile as monoterpenes, n-octane, naphthalene, and phenol are relatively simple to achieve [11–13]. When more volatile analytes are of interest, the use of sorbent trapping or on-line SFE–GC techniques should be investigated [1,2,7,8].

Effect of extraction flow rate and cell design. Once quantitative collection conditions have been developed for the analytes of interest, the effects of the "step 2" (sweeping the analytes out of the cell) experimental parameters can be evaluated. Factors that could potentially control the rate at which an extracted analyte is swept through the sample cell include the volume of the cell (and associated dead volume not occupied by the sample), cell orientation, and flow-rate of the supercritical fluid.

First, the extraction cell should be selected to minimize the dead volume of the system, since in general, this will allow larger samples to be extracted with lower extraction fluid flow-rates (thereby reducing the amount of fluid needed and simplifying the collection of more volatile analytes). However, the exact cell size needed for various samples is often not available. In such cases, filling the cell with an inert material (*e.g.*, clean sand) at the extraction fluid inlet end to reduce the void volume of the cell may be useful. Alternatively, simple considerations of flow patterns during SFE indicate that an extracted analyte should experience the least dead volume possible to minimize the time required for its removal from the cell. Therefore, if insufficient sample is available to fill the extraction cell, the dead volume experienced by the analyte will be minimized by holding the extraction cell in a vertical position, and flowing the extraction fluid from the top to the bottom.

The effect of cell (and extraction fluid flow) orientation on the removal of an easily extracted alkane (*n*-tridecane) from a 2-g sample of sand placed in a 10-ml extraction cell is shown in Fig. 1. All extractions were performed in duplicate with a flow-rate

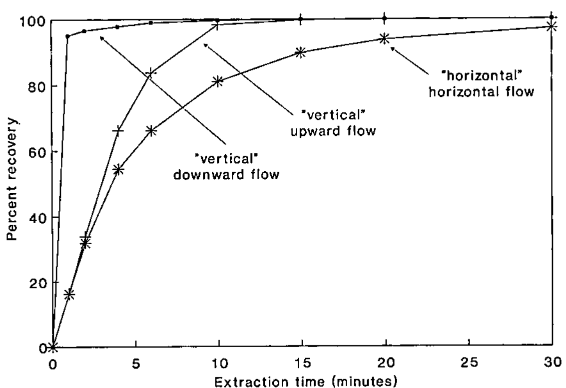

Fig. 1. Effect of cell orientation and extraction fluid flow direction on the extraction rate of *n*-tridecane from a 2-g sample of sand placed in a 10-ml extraction cell.

ly and the fluid was pumped from the top to the bottom, essentially all (>95%) of the tridecane is recovered after only *ca.* 3 ml (2 min into the extraction) of fluid has passed through the cell (vertical "down-flow" in Fig. 1). Since the void volume above the sample was *ca.* 8.5 ml, this rapid recovery demonstrates that the linear velocity of the fluid was sufficient to prevent significant mixing of the extracted tridecane with the CO_2 present in the cell above the sample. In contrast, when the extraction was performed with the fluid flow from bottom to top, the recovery of the tridecane is retarded by *ca.* 10 min, presumably because the extracted tridecane mixes in the *ca.* 8.5 ml of fluid present above the sample (vertical "up-flow" in Fig. 1). Similarly, the poorer flow patterns that exist when the cell is placed in the horizontal position also result in much slower recovery of the tridecane than when a vertical "down-flow" configuration is used (Fig. 1). While the dead volume considerations shown in Fig. 1 may seem obvious and trivial, it is interesting to note that the majority of commercial SFE instruments available to date utilize either vertical "up-flow" or horizontal cell orientations rather than the vertical "down-flow" orientation shown to be superior for partially-filled cells.

The example shown in Fig. 1 demonstrates the potential range of effects of cell orientation, flow direction, and cell dead volume on the recovery of

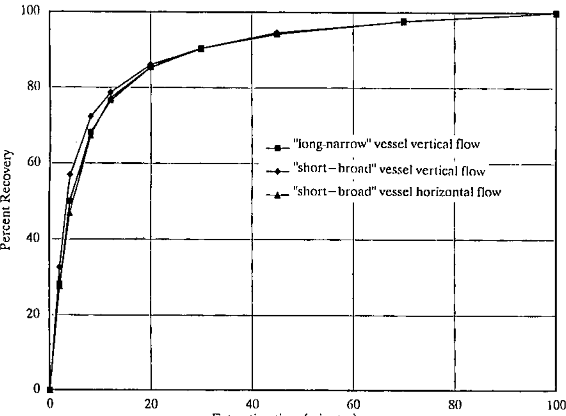

Fig. 2. Effect of cell orientation and cell dimensions on the extraction rate of fluoranthene from a 3-g sample of railroad bed soil. The "long-narrow" (132 mm × 5 mm I.D.) and "short-broad" (33 mm × 10 mm I.D.) vessels each had a volume of 2.5 ml. Extraction efficiency (100%) was based on the final amount extracted after 100 min.

ditions used. However, when extraction cells are completely filled with the sample, the effect of cell orientation (and shape) is reduced as shown in Fig. 2 by the extraction of fluoranthene from railroad bed soil using a "short-broad" and a "long-narrow" extraction cell that have identical volumes [11]. While small differences in extraction rates from sorbent resins using different cell dimensions have

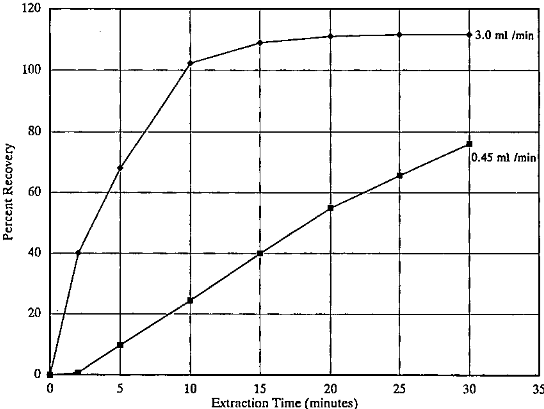

Fig. 3. Effect of SFE flow-rate on the extraction of used motor oil from a 5-g sample of soil. Extractions were performed with 340 atm CO_2 at 80°C as per the TPH method recently approved by the US Environmental Protection Agency [3,6]. Extraction efficiency (100%) was based on 4 h of Soxhlet extraction using trichlorotrifluoroethane.

been reported [14], our experience with more than one hundred non-homogeneous environmental samples (*e.g.*, sludges, soils and sediments, exhaust and air particulates) have nearly always shown a kinetic limitation in the partitioning step (discussed in more detail below) that far outweighs any small effects that might result from extraction cells having different dimensions (but the same volumes). Thus, the vast majority of real-world samples that we have investigated show little, if any, detectable effect on the extraction rate of the native analytes based on cell shape or orientation when sample cells are kept full (Fig. 2).

The effect of fluid flow-rate on SFE extraction rates can either be nearly negligible, or can be very important depending on the process that controls the overall rate of extraction from a particular real-world sample. Assuming that the cell orientation and dead volume considerations are properly addressed as discussed above, two limiting cases for the effect of the fluid flow-rate can be imagined. First, the extraction flow-rate may be directly related to the rate at which analytes are recovered from the sample, or second, the fluid flow-rate will have no significant effect on the SFE extraction rate (as long as the fluid flow is sufficient to transport extracted analytes out of the cell). Fig. 3 shows the effect of supercritical CO_2 flow-rate on the extraction rate of a sample of the first type, *i.e.*, spilled motor oil hydrocarbons from soil (as determined by infrared spectrometry [6]). As shown in Fig. 3, the rate at which the hydrocarbons are extracted is closely related to the flow-rate of the supercritical CO_2, with higher extraction flow-rates (3.0 ml/min *vs.* 0.45 ml/min) yielding faster recoveries. In contrast, the extraction of PAHs from a railroad bed soil and PCBs from a river sediment show virtually no dependence on the flow-rate of supercritical CO_2 as shown in Fig. 4. Little if any differences in the extraction rate was observed for flow-rates of 0.3 to 0.9 ml/min for fluoranthene from the soil (Fig. 4, top). When the flow-rate was dropped to *ca.* 0.15 ml/min, the recovery rate was slower, but since the void volume of this sample was *ca.* 1.5 ml, the slower apparent recovery rate was simply a result of inefficient sweeping of the cell. However, when the flow-rate was sufficient to sweep the dead volume of the cell every few min (*i.e.*, 0.3 ml/min or greater), further increases in the extraction fluid flow-rate

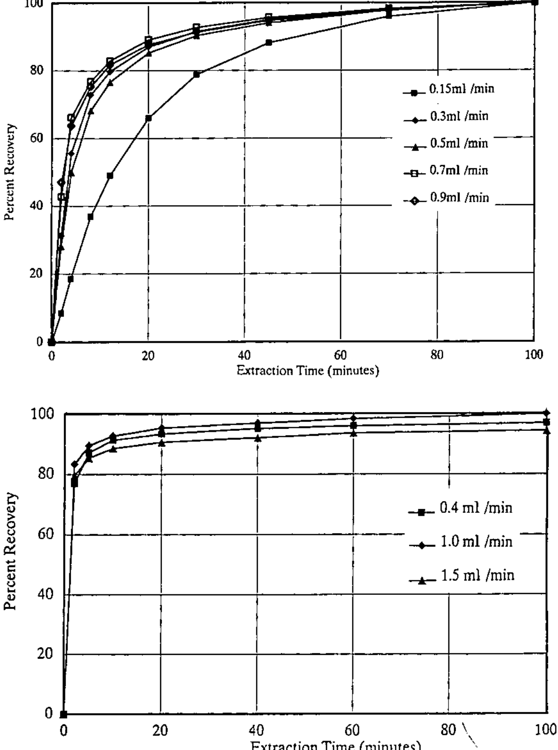

Fig. 4. Effect of SFE flow-rate on the extraction rate of fluoranthene from a 3-g sample of railroad bed soil (top) and of 2,3′,4,4′,5-pentachlorobiphenyl from river sediment (bottom). The soil sample was extracted with 400 atm CO_2 at 50°C. Extraction efficiency (100%) was based on the final amount extracted after 100 min. The recovery of the 2,3′,4,4′,5-pentachlorobiphenyl was based on the highest recovery achieved using 100 min of extraction with CO_2 at 200°C, which was slightly higher (113%) than the recovery of the same species reported by NIST based on two sequential 16-h Soxhlet extractions [20].

yielded no increase in the extraction rate. Similarly, no significant difference was observed in the extraction rate of 2,3′,4,4′,5-pentachlorobiphenyl from the sediment when the flow-rates were 0.4–1.5 ml/min (Fig. 4, bottom).

The results shown in Figs. 3 and 4 clearly demonstrate that different mechanisms control the extraction rates of these two samples. Although the mechanisms that control SFE recoveries of analytes from heterogeneous environmental samples are not nearly well enough understood to fully explain the different behavior of these two samples, our experience with a wide variety of samples indicates that some useful generalizations can be made. Samples that show a high degree of dependence on the extraction fluid flow-rate (as shown in Fig. 3) generally have very high concentrations of analytes which appear to limit the extraction rate because of solubility limitations in the extraction fluid. For example, the motor oil-contaminated soil had a total hydrocarbon concentration of *ca.* 80 mg/g (determined by both SFE and conventional Soxhlet extraction for 4 h using trichlorotrifluoroethane). Since the concentration of these analytes was very high, it is likely that the bulk of the hydrocarbons were not exposed to active sites on the soil matrix and were therefore more "available" for extraction. Therefore, the extraction problem was primarily one of solvating the hydrocarbons, and the extraction rate was increased by exposing the sample to larger volumes of the supercritical CO_2 extraction solvent per unit time.

In contrast, the railroad bed soil sample shown in Fig. 4 contained a total PAH concentration of *ca.* 100 μg/g (major species ranged from phenanthrene to PAHs with molecular masses of 252), so the majority of the individual PAH molecules could interact with active sites on the soil matrix. Since the flow-rate of the extraction fluid had virtually no effect on the extraction rates of the PAHs (as long as the sample void volume was swept every few min), the extraction rate appears to be limited by the kinetics of the partitioning process between the soil matrix and the extraction fluid rather than being limited by the ability of the CO_2 to solvate the PAHs. In our experience, this type of behavior (*i.e.*, extraction rates that are relatively independent of fluid flow-rate) generally applies to heterogeneous environmental samples as long as the concentration of the pollutants is not so high that interaction of the majority of individual pollutant molecules with the matrix active sites is prevented simply by having too high of pollutant concentrations on the sample.

In a practical sense, the extraction rate behavior exhibited by a particular sample at different flow-rates is useful to determine minimum flow-rates that will yield efficient recoveries. Flow rate studies are also useful to determine the reasonable upper sample size that can be extracted (*e.g.*, larger samples have larger associated void volumes in interstitial spaces, and therefore require higher flow-rates simply to sweep the sample void volume every few

min). Extractions that are controlled primarily by the partitioning kinetics (rather than having large amounts of available extraction fluid for the solvation step) can potentially be efficiently extracted in the static mode (that is, with no continual flow of the extraction fluid) provided that a short dynamic extraction is performed after the static extraction step simply to flush the extracted analytes out of the extraction cell. When extraction rates are controlled mostly by the fluid flow-rate, the use of extraction cell volumes (or any measurement of the total volume of fluid passed through the sample) is a useful parameter to describe an SFE method. However, for the majority of the heterogeneous environmental samples we have investigated, the extraction rate does not depend significantly on the fluid flow-rate, and the total volume of extraction fluid passed through a sample has little relevance to extraction efficiency since the contact time of the sample and the fluid is more important than the amount of extraction fluid that is used.

Finally, the results of the flow-rate studies provide insight as to whether the efficient extraction of a particular sample depends more on the partition constant between the fluid and the matrix active sites (*i.e.*, the thermodynamics of the solvation process), or on increasing the rate at which partitioning occurs between the matrix and the extraction fluid. Such information is useful in developing quantitative SFE extraction conditions as discussed below.

Analyte partitioning from matrix into extraction fluid. The least understood step that controls the SFE efficiencies obtained from heterogeneous environmental solids is the partitioning of the pollutant molecules from the active sites in the sample matrix into the supercritical fluid (step 1). Because of the large number of possible interactions that might occur between the pollutant molecules and an environmental matrix, a fundamental understanding of these partitioning processes has been impossible to attain. However, a preliminary (although admittedly naive) description of the processes that control SFE rates can be useful in developing quantitative SFE methods for complex environmental samples. The consideration of three general factors; analyte solubility, kinetic limitations, and analyte–matrix–extraction fluid interactions is useful for attempting to understand the extraction process in support of the development of quantitative SFE conditions.

The first (and most obvious) requirement of an SFE condition is the ability of the extraction fluid to solvate the target analytes. While suitable solubility data in supercritical fluids is not available for the majority of environmentally-interesting analytes, a recent review contains a sufficient number of related solubilities to allow some generalizations to be made [15]. First, organic pollutants that are sufficiently polar and non-volatile that they cannot be analyzed with conventional capillary GC generally do not have sufficient solubility to be efficiently extracted with pure CO_2 under conventional SFE conditions [*e.g.*, 300–600 atm (1 atm = 101 325 Pa), 45 to 80°C]. (A notable exception to this general rule is fat components such as triglycerides.) For example, the use of pure CO_2 to extract ionic species such as the surfactant linear alkylbenzenesulfonate (LAS) shows little if any recoveries because of their low solubility. However, as their solubility in the supercritical CO_2 is increased by the addition of an organic modifier or ion pairing reagents, high extraction efficiencies can be obtained [16,17].

Just as polar and high-molecular-mass analytes generally do not have sufficient solubility to dissolve in pure CO_2, species that are amenable to GC analysis generally do have sufficient solubility to make their extraction using pure CO_2 seem likely. For example, the solubilities of several common pollutants are shown in Table II. Based on the solubility data, it should be possible to quantitatively

TABLE II

ESTIMATED SOLUBILITIES OF REPRESENTATIVE ORGANICS IN SUPERCRITICAL CO_2 AT 400 atm AND 50°C

Solubilities were estimated based on the tabulations given in ref. 15.

Species	Solubility (mg/ml)
Docosane	320
Phenol	170
p-Chlorophenol	140
Hexachloroethane	230
Diphenylamine	31
Naphthalene	160
Phenanthrene	13
Pyrene	3
Dibenzothiophene	11

extract a 1-g sample contaminated with 13 000 μg/g (*ca.* 1%, w/w) of phenanthrene using only 1 ml of supercritical CO_2 at 400 atm and 50°C. Since the relevant environmental concentrations of such pollutants are typically much lower (*e.g.*, ng/g to μg/g), one might expect that pure CO_2 extractions of such species would be highly efficient. While many of the early SFE investigations were based on the assumption that attaining high solubility in the supercritical fluid should be sufficient to obtain high extraction efficiencies from environmental samples, the unfortunate truth is that high solubility in the extraction fluid is generally not a sufficient condition to yield high extraction efficiencies [1,5,9,18–21], and such results clearly demonstrate that additional factors for real-world samples must be considered.

In addition to the obvious need for adequate solubility, a successful extraction condition must overcome the interactions between the analyte and the matrix to affect a favorable partitioning into the supercritical fluid (loosely termed the "thermodynamic problem" for this discussion). The extraction condition must also cause this partitioning to occur rapidly (on the time scale of the extraction experiment) for high recoveries to occur in a reasonable time (loosely termed the "kinetic problem"). Obviously, these factors depend on the nature of the interactions between the analytes and matrix components. Unfortunately, the nature of analyte–matrix interactions between pollutants and heterogeneous environmental samples is not well understood, and the potential for different types of interactions seems nearly endless as composition of individual matrix components is considered. For example, the pollutants in a soil sample may be associated with a variety of inorganic (*e.g.*, alumina, silica) and/or organic (*e.g.*, humic and fulvic) active sites, each with different binding strengths. (In contrast, the number of possible interactions between analytes and sorbent resins is relatively limited.) In addition, the extraction of the pollutants may be inhibited by physical barriers including being located in interstitial micropores in the soil particles (or between clay plates), being covered by (or associated with) bulk organic material, or by being coated with droplets of water that may need to first be extracted before the analyte is accessible for extraction. The differences in potential interactions multiply when matrices in addition to soil are considered (*e.g.*, sludges and exhaust particulates), however, in every case both the "thermodynamic" and "kinetic" problems need to be solved to develop a successful extraction method.

The effect of extraction flow-rate on the extraction rate can be used to investigate whether the major limitation to achieving rapid extractions is primarily a "thermodynamic" (*i.e.*, the distribution of the analyte between the supercritical fluid and the sample matrix at equilibrium) or "kinetic" (i.e, the time required to approach that equilibrium) problem. For samples that show a dramatic increase in extraction rate when the extraction fluid flow-rate is increased (Fig. 3), the kinetics of the extraction process appear to be fast, and the extraction will be improved by increasing the proportion (partition constant) of the analytes in the extraction fluid or by simply exposing the sample to more fluid. In contrast, if there is no large effect of fluid flow-rate on the extraction rates, it appears that the kinetics of the desorption process is slow, and these slow kinetics limit the overall extraction rate more than the equilibrium distribution of the analyte between the matrix and the extraction fluid [18,19,21–23]. Finally, it must be remembered that many different interactions of a pollutant species with a sample matrix are possible, and the lack of understanding of the processes that control analyte–matrix interactions and therefore control SFE extraction mechanisms makes *a priori* prediction of quantitative extraction conditions impossible. However, sufficient work has been reported to allow a somewhat logical approach to developing an SFE method to be suggested as discussed below.

Validating a quantitative SFE method

In addition to the large number of different analyte–matrix combinations and interactions, the development of any extraction method for real-world environmental samples is severely limited by the fact that it is simply not possible to know the exact concentration of any target pollutant on any sample. Since samples with known concentrations of native (not spiked) pollutants are not possible to obtain, the development and validation of a quantitative extraction method for environmental samples is generally based on one of the three following approaches, each depending on assumptions that may or may not be valid:

(i) Determining the recovery of known concentrations of spiked compounds from the sample (or similar) matrix. This approach assumes that spiked analytes behave like native analytes during the extraction, and also assumes that the spike is not lost between spiking and extraction from processes such as volatilization.

(ii) Comparison of the recoveries of native analytes with those achieved using conventionally-accepted extraction methods (including the use of standard reference materials). This approach assumes that the conventional method is quantitatively efficient.

(iii) Perform multiple sequential extractions of the same sample. This approach assumes that the final extraction performed removes all of the native analytes and that no additional analytes are associated with the sample by stronger interactions than the analytes that were already extracted.

While all three approaches have been used to develop (and to attempt to validate) SFE and other extraction methods, the complexity of environmental samples and their potential interactions with pollutant molecules makes heavy reliance on any one validation technique unwise. (Obviously, the limitations of extraction validation methods apply to any extraction method, and are not associated only with SFE.) Perhaps the least reliable technique for validating the quantitative abilities of an extraction method is the use of spike recoveries [21], simply because the spiked analytes are not exposed to (*e.g.*, contaminated soils) or formed with (*e.g.*, soot samples) the same matrix active sites as are the native pollutants. The potential errors in validating an SFE method based on spike recovery studies are shown in Fig. 5 by the relative extraction rates of native naphthalene and spiked [$^2H_8$]naphthalene from three different samples: urban air particulate matter, a petroleum waste sludge, and soil from a railroad bed. Each sample was spiked with the [$^2H_8$]naphthalene at the same approximate concentration as the native naphthalene, and the samples were extracted for 30 min with pure CO_2 at 400 atm and 60°C. As shown in Fig. 5, quantitative recovery (>90%) of the spiked [$^2H_8$]naphthalene was achieved after only 5 min of extraction, while the recovery of the native naphthalene was *ca.* 55%, 18%, and 5%, for the soil, air particulate, and waste sludge samples, respectively. If a commonly-

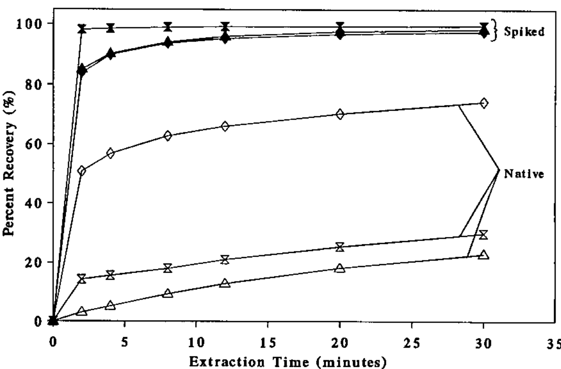

Fig. 5. Extraction rates of spike [$^2H_8$]naphthalene and native naphthalene from petroleum waste sludge, urban air particulates, and railroad bed soil. Percent recoveries were based on the total amounts extracted by sequential extractions with pure CO_2 (shown in the figure) at 400 atm and 60°C followed by CO_2–10% methanol (same conditions) and finally by 14 h of sonication in methylene chloride.

applied criterium for a successful extraction method was used (*e.g.*, that >90% of the target spike was recovered), the results shown in Fig. 5 demonstrate that the same extraction time and conditions would fail to extract between 45% and 95% of the native naphthalene from the real-world samples. Even after 30 min of extraction with pure CO_2, the recoveries of the native naphthalene were only 74 ± 8%, 30 ± 5%, and 23 ± 6%, respectively, compared to >98% for the recovery of the [$^2H_8$]naphthalene spike from all three samples. The results shown in Fig. 5 also clearly demonstrate that, as discussed above, solubility in the supercritical fluid is not a sufficient extraction condition since the solubility of naphthalene is *ca.* 160 mg/ml, while the concentration of naphthalene was only *ca.* 1 μg/g, 3 μg/g, and 100 μg/g for the air particulate, soil, and sludge samples, respectively (and since *ca.* 25 ml of supercritical CO_2 was used for each extraction). It should also be noted that aging the spiked samples for 14 h compared to extracting freshly spiked samples had no effect on the extraction rates shown in Figs. 5 and 6.

While the results shown in Fig. 5 clearly demonstrate that the use of spike recoveries is not valid for determining quantitative extraction conditions, some spiked analytes do behave in a manner that is

similar to that displayed by the native analytes. For example, the SFE rates of the higher-molecular-mass PAH, chrysene, are more similar to those of the spiked [$^2H_{12}$]chrysene when extracted from the same three samples as shown in Fig. 6, although significant differences still exist between the extraction rates of the spiked and native chrysene for the air particulate and sludge samples. Since the differences shown between spiked and native PAHs must be a result of differences in the strength of the analyte–matrix interactions experienced by the individual spiked and native PAH molecules, it would seem logical that spike recovery studies may be more relevant for highly contaminated samples with relatively few significant analyte–matrix interactions (*e.g.*, the motor oil-contaminated soil sample shown in Fig. 3). However, the results shown in Figs. 5 and 6 clearly demonstrate that spike recovery studies are best used to evaluate the collection efficiencies of an extraction method, and should not be used to determine quantitative extraction conditions.

The second suggested method for validating quantitative extraction conditions is to compare the

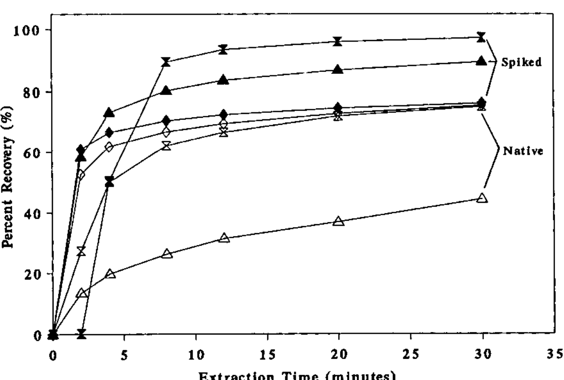

Fig. 6. Extraction rates of spike [$^2H_{12}$]chrysene and native chrysene from petroleum waste sludge, urban air particulates, and railroad bed soil. Percent recoveries were based on the total amounts extracted by sequential extractions with pure CO_2 (shown in the figure) at 400 atm and 60°C followed by CO_2–10% methanol (same conditions) and finally by 14 h of sonication in methylene chloride. The slight lag in the recovery of the [$^2H_{12}$]chrysene from the urban air particulates was a result of chromatographic retention of the spike compound on the urban air particulate sample during SFE since the spike (placed on the top of the sample) had to be eluted through the sample before recovery.

results of the proposed SFE method with those of conventional and well-accepted extraction methods such as extraction with a liquid solvent in a Soxhlet apparatus. This approach includes the use of certified reference standards, such as those available from NIST, that contain native pollutants for which concentrations have been certified based on exhaustive Soxhlet extraction and multiple analysis methods. (The use of certified reference materials to validate SFE methods for the extraction of PAHs and nitro-PAHs from urban air particulate matter, diesel exhaust particulates, and marine sediment, and PCBs from river sediment have been the subject of earlier reports, see refs. 5,20,24–26.) Perhaps the greatest advantage of certified reference materials is that investigators involved in SFE methods development can have a single "benchmark" data set for which to compare results between methods and laboratories. Similarly, when reference materials are not available, comparison of SFE results with standard liquid solvent extractions (Soxhlet or sonication) provides a somewhat consistent method to evaluate SFE results between methods and laboratories. However, it should be noted that conventional liquid solvent extractions may not yield quantitative extraction of native analytes, and therefore a highly efficient SFE extraction may yield higher recoveries than Soxhlet or sonication extraction. For example, the recoveries obtained for PAHs using SFE with $CHClF_2$ as the extraction fluid (30 min) were substantially higher from a petroleum waste sludge sample than those obtained using 18 h of sonication with methylene chloride (*e.g.*, recoveries of phenanthrene and benz[*a*]anthracene by SFE were *ca.* 120% and 150%, respectively, of those obtained from the sonication extraction [5].

The final approach to validating the quantitative abilities of an extraction method, performing multiple extractions of a single sample, can be quite misleading or very useful depending on how it is performed. Early work in SFE (including work performed in this laboratory) often used a second sequential SFE extraction performed under conditions identical to the first extraction in order to estimate the overall extraction efficiency. The assumption was that if (for example) the second extract contained substantially lower concentrations of the analytes than the first extraction, then the extraction must be nearly quantitative. While this as-

sumption may be true if all of the individual molecules of a particular analyte extracted at the same rate, this assumption is clearly not valid for the majority of environmental samples we have investigated. For example, Fig. 7 shows the extraction rate (with pure CO_2 at 400 atm and 50°C) of PCB congeners 2,3′,4,4′-tetrachlorobiphenyl and 2,3′,4,4′,5-pentachlorobiphenyl from river sediment (NIST SRM 1939). The extraction curves show initially fast extractions, followed by increasingly slow rates of extraction as has been previously described by a kinetic model based on diffusion kinetics [22,23]. (It should be noted that diffusion of the analyte in the sample matrix is not likely to be the major limiting step for the extraction of heterogenous environmental samples since grinding the samples does not generally increase the extraction rate.) However, the model provides a useful mathematical description of the extraction rates observed for these samples. If, for example, two sequential extractions of PCB-contaminated sediment were performed for 40 and 60 min, respectively, (*i.e.*, 0–40 min and 40–100 min in Fig. 7), no significant concentrations would be detected in the second extract, which could be interpreted that the first 40-min SFE extraction was quantitatively efficient. However, this is clearly not true, since the recovery of two PCB congeners was only *ca.* 50 and 70% at 40 min (based on the values certified by NIST based on Soxhlet extraction).

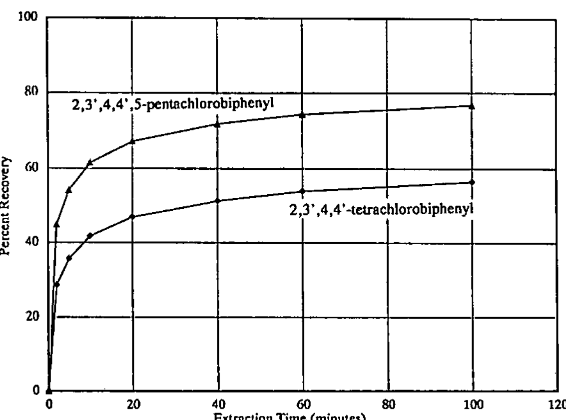

Fig. 7. Extraction rates of 2,3′,4,4′,5-pentachlorobiphenyl (top) and 2,3′,4,4′-tetrachlorobiphenyl (bottom) from river sediment using 400 atm pure CO_2 at 50°C. Percent recoveries are based on those reported by NIST based on two sequential 16-h Soxhlet extractions.

Quantitative recovery of the PCBs from the same sample has been achieved using methanol-modified CO_2, $CHClF_2$, and high-temperature extraction with pure CO_2 [5,20]. Note that, with the exception of the high concentration motor oil-contaminated soil (Fig. 3), all of the samples discussed in this paper show similar extraction curves as those of the PCB congeners shown in Fig. 7 (*i.e.*, an initial fast extraction followed by a slow rise), which further demonstrates that the determination of quantitative recovery based on multiple sequential extractions with the same extraction condition is not valid.

In contrast, the use of multiple extractions of a single sample with a different (and presumably stronger) extraction condition can be a very useful way to validate the quantitative ability of an extraction method for real-world samples. This could include extracting the residue from an SFE extraction by Soxhlet or sonication in an appropriate liquid solvent, by extracting the residue with a "stronger" SFE condition (*e.g.*, by adding a modifier), or by a combination of the two approaches. For example, the total concentration of the PAHs in the petroleum waste sludge, urban air particulates, and the railroad bed soil discussed earlier (100% recovery for Figs. 5 and 6) were determined by sequential extraction with 30 min of pure CO_2 (400 atm, 60°C), 30 min with CO_2 modified with 10% methanol (400 atm, 60°C), and finally by 14 h of sonication of the SFE residue in methylene chloride [21]. With such an approach, if no significant concentrations of the target analytes are seen in the conventional liquid solvent extract performed on the residue, it seems reasonable to conclude that the SFE extraction was quantitatively efficient.

Developing a quantitative SFE method

The understanding of SFE mechanisms for the extraction of organic pollutants from heterogeneous environmental samples is simply not well enough developed to propose a single approach to SFE methods. However, careful consideration of the various mechanical and physicochemical aspects of SFE that are discussed above has led to a general approach that has proven very useful for the development of quantitative SFE methods in our laboratory. The following discussion attempts to list a sequential method of developing a quantitative SFE method based on the scheme shown in Fig.

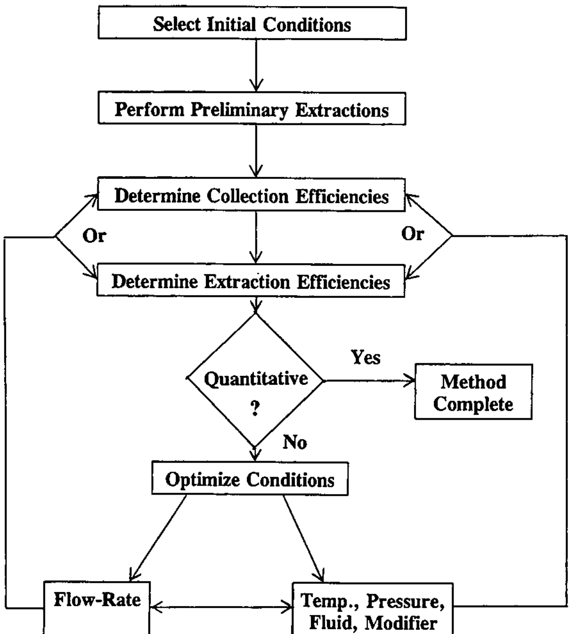

Fig. 8. Proposed interactive scheme for the development of quantitative SFE methods for complex environmental samples. Discussion of the individual steps is given in the text.

8, however, it must be recognized that such development is an interactive process and some of the steps may need to be repeated during the optimization process.

Selection of initial extraction conditions. The first methods development task is to determine the initial extraction conditions based on considerations of the polarity of the target analytes (and solubility data, if available), the matrix composition (*e.g.*, water content, organic content, mineral composition, particle size), and any literature reports of successful SFE methods for similar samples. If no relevant solubility or extraction results are available for the target analytes, initial conditions may be chosen based on the polarity of the analyte, (*e.g.*, using the general rule described above that pure CO_2 will generally solvate GC-able analytes at "normal" extraction conditions such as 400 atm and 50°C). If the analytes are fairly polar (or ionic) or have high-molecular-masses, the addition of an organic modifier (or for ionic compounds, an ion pairing reagent [16]) may be necessary to obtain sufficient solubility of the analyte in the extraction fluid. The use of a more polar fluid such as $CHClF_2$ may also be useful as discussed below, however, little data exists in the literature to help select such fluids. If no basis exists (other than the polarity of the target analytes) for selecting initial conditions, reasonable starting points (for non-polar analytes) would be extraction with 0.5 to 2 ml/min of pure CO_2 at the upper pressure limit of the SFE system (typically 340 to 650 atm, depending on the manufacturer) and a moderate temperature (50–100°C), and (for polar analytes) extraction with similar conditions with CO_2 modified with 5–10 vol.% of an organic modifier that is itself a good solvent for the target analyte, and has properties that do not interfere with the subsequent analysis (*e.g.*, the selection of modifiers with high boiling points interferes with GC analysis); if the analysis is performed by spectrometry, the modifier must not absorb at the detection wavelength. The addition of the modifier will also raise the critical temperature of the mixture, so the extractions should be performed at slightly higher temperatures (*e.g.*, 70°C for modified CO_2).

Preliminary extractions of representative samples. The choice of initial extraction conditions also should include consideration of "mechanical" problems related to the sample matrix which may occur during the extraction since the solution to these problems may also affect the collection efficiencies. For example, samples with high concentrations of water and/or extractable matrix components will likely require that linear restrictors be heated and/or that drying or dispersing agents be added to the sample to avoid plugging of the restrictor during the SFE step. Preliminary extractions of representative samples should be performed to investigate if such problems are significant. These preliminary extractions are also useful to select a "reasonable" sample size based on sensitivity requirements, sample availability (*e.g.*, 1 g is a very large sample of air particulate matter, but is a small soil sample), and sample homogeneity. (Because of the flow considerations discussed above, SFE is most easily applied to smaller samples, and samples of a few grams or less is a reasonable starting point.) It is particularly useful to perform preliminary extractions of a limited number of samples that represent the range of analyte, water, and co-extractable matrix concentrations that might be expected in future samples so that the analyst is familiar with the

types of matrix problems to be encountered. Because of the shape of the extraction rate curves generally encountered with heterogeneous environmental samples, preliminary extraction times of 30 min are useful since longer extraction times (using the same SFE conditions) generally do not yield substantially higher recoveries.

Determination of collection efficiencies. Once the preliminary extraction conditions have been determined, the collection efficiencies of target analytes which have been spiked onto a relatively non-active matrix (*e.g.*, sand) at concentrations expected to be encountered in real-world samples should be determined. Quantitative recovery of the spiked analytes will demonstrate that: (i) the collection system is efficient, (ii) sufficient flow is available to overcome the void volume effects in the cell, and (iii) the analytes are soluble enough to be dissolved in the supercritical fluid (however, this does *not* necessarily demonstrate that the fluid can extract the analytes from real samples as discussed above).

In general, the quantitative collection of analytes less volatile than *n*-octane or naphthalene is not difficult in a few ml of the proper liquid solvent [11–13], as long as care is taken in performing the collection step (although it is possible to have low collection efficiencies of non-volatile analytes when the liquid solvent collection system is poorly designed as in ref. 27). However, the collection in a few ml of solvent of more volatile compounds such as benzene and toluene is difficult (*ca.* 45% and 75% efficiency, respectively [28], and the quantitative collection of such volatile analytes may require the use of sorbent traps or on-line approaches such as SFE–GC.

If low recoveries of the spike are observed, the reason can often be determined by observing the spike recovery efficiency of a homologous series of related analytes. For example, if an extraction is being developed for PAHs, recoveries could be determined for a spike containing a range of PAHs including naphthalene (M_r 128) and representative PAHs with molecular masses of 178, 202, 228, 252, and 276. If the spike recoveries of the more volatile components are low, and the recoveries of the less volatile compounds are high, volatilization losses are indicated, and the trapping system must be improved as discussed in refs. 10–13 (losses from volatilization during the spiking step must also be considered). Conversely, if the recoveries of the volatile components are high, and the recoveries of the less volatile components are low, then the extraction condition (*e.g.*, solvation strength of the supercritical fluid) is not sufficient for the larger (less soluble) PAHs (although losses by deposition in the depressurization system may also be responsible). Regardless of the trapping method used, quantitative spike recoveries must be demonstrated before further development of the SFE method is warranted.

Determine extraction efficiency. Once high spike recoveries have been achieved, the first determination of the SFE efficiency of the target analytes from real-world samples can be performed. At this stage in the development, one hopes for good luck, *i.e.*, that the conditions that were satisfactory for spike recoveries will also yield quantitative recovery of the native analytes. Since the concentration of the native analytes can not be known in real-world samples, the definition of "quantitative" must first be determined. For reasons discussed earlier "quantitative" should not be based on spike recovery data. Therefore, the decision must be made whether to base "quantitative" recovery using the SFE method on the recovery of conventional extraction (*e.g.*, Soxhlet or sonication) results, or the use of multiple sequential extractions using different extraction techniques. If, based on the definition of "quantitative," the present SFE conditions yield satisfactory recoveries, the method can be considered complete. However, if the recoveries are not "quantitative," additional development of the method will be required (Fig. 8).

Optimizing SFE conditions. Since the spike recovery studies have already demonstrated that the target analytes are sufficiently soluble to be extracted with the initial SFE conditions, low recovery of the analytes must be based on the inability of the initial SFE conditions to efficiently overcome matrix–analyte interactions. Alternatively, the sample could contain too high concentrations of the target (and non-target) analytes that could saturate the extraction fluid. The flow-rate studies described above are a simple method to determine which of these mechanisms is predominant. If the use of higher flow-rates results in substantial increases in recovery of the native analytes, it is likely that simply exposing the sample to more fluid by increasing the extraction flow-rate (which may make analyte collection

more difficult) or increasing the extraction time will yield quantitative recoveries. Simple changes to increase the solubility of the analytes such as raising the extraction pressure (*i.e.*, fluid density) should also be considered.

However, if increasing the extraction flow-rate (or extraction time) does not yield substantial increases in the recoveries of the native target analytes (as shown in Fig. 4), the initial SFE conditions are not sufficient to efficiently overcome analyte–matrix interactions (*i.e.*, interactions not experienced by the spiked analytes). Assuming that the upper pressure of the extraction system is already being exploited, three useful parameters are available to increase the extraction rates, *i.e.*, use of different (more polar) fluids than CO_2, the addition of organic modifiers to CO_2, and increasing the extraction temperature.

Depending on the SFE system used, the effect of increasing the extraction temperature with pure CO_2 extractions can be very simple to evaluate (a simple and inexpensive approach for performing SFE at temperatures up to 200°C is described in ref. 20). Even though the solubility of the target analyte may actually decrease at higher temperatures (and constant pressure) because of lower CO_2 density, extraction at 200°C has been shown to be extremely effective in obtaining quantitative recoveries of PCBs from sediment and PAHs from air particulate matter, indicating that the kinetics of the partitioning process are improved [20]. For example, when extractions were performed for 40 min with pure CO_2 at 350 atm, increasing the extraction temperature from 50 to 200°C yielded *ca.* one-and-one-half to two-fold increases in the recovery of PCBs, and two- to six-fold increases in extraction efficiencies of the PAHs. While results of SFE with pure CO_2 at "normal" temperature (50°C) were not quantitative based on the concentrations certified by NIST (based on 32–48 h of Soxhlet extraction), extraction at 200°C yielded recoveries that generally met or even exceeded the certified concentrations [20].

Increased recoveries may also be achieved by using different SFE fluids. Unfortunately, no fluids have the attractive characteristics attributed to CO_2 (low toxicity, low reactivity, and low environmental impact), although N_2O and $CHClF_2$ may be worth investigating when recoveries with pure CO_2 are low. N_2O yields higher recoveries than CO_2 for some samples (*e.g.*, PAHs from marine sediment, chlorinated dioxins from fly ash [25,29]), however N_2O did not yield increased recoveries from some samples such as PAHs from waste sludge or PCBs from river sediment [5]. In addition, N_2O can react with easily oxidized organics, and may present a safety hazard for routine applications [30]. $CHClF_2$ (freon-22) has been the most efficient pure SFE fluid that we have encountered, and has been shown to yield excellent recoveries of nitro-PAHs, PAHs, PCBs, and even some ionic species from a variety of matrices [5,24]. Despite its excellent characteristics as an extraction fluid, $CHClF_2$ does cause fused-silica restrictors to break easily and is less desirable because of ozone destruction caused by freons (although $CHClF_2$ has a relatively low ozone-depletion potential [31]).

If higher temperature extractions or the use of alternative fluids are impractical or ineffective, the addition of organic modifiers to CO_2 is the next logical step. Although methanol has been the most often used modifier, many other potentially useful modifiers (including ion pairing reagents [16,17]) should be evaluated. Unfortunately, little information is available to aid the choice of modifiers (and their concentrations), and until the action of modifiers (and related analyte–matrix interactions) is better understood, the optimal selection of modifiers to extract complex environmental samples should be based on a survey of suitable candidates (as well as consideration of the modifier's effect on collection recoveries and on the subsequent method used to analyze the extract).

Three basic approaches to adding modifiers can be used, *i.e.* purchasing a pre-mixed cylinder of the modifier in CO_2, purchasing an SFE system (*e.g.*, dual pump) capable of modifier addition, or simply adding the modifier to the extraction cell with the sample. Unfortunately, surveying the abilities of several modifiers with the first two approaches can be quite time-consuming and expensive. While adding the modifier to the sample in the extraction cell has the disadvantage that the modifier is not continually introduced during dynamic (continual flow) SFE, its inherent simplicity and low cost suggests that the initial choice of modifier identity and concentration be based on this method. An appropriate survey of modifiers can easily and rapidly be performed using a single pump by adding an appropri-

TABLE III

ENHANCEMENT IN PAH RECOVERIES FROM URBAN AIR PARTICULATES USING DIFFERENT MODIFIERS IN CO_2

Values given are the quantities of each PAH extracted with a 5-min static extraction with the modifier listed followed by a 10-min dynamic extraction with pure CO_2 divided by the quantities of each PAH extracted from a fresh sample using a 15-min dynamic extraction with pure CO_2.

Modifier (%, v/v)	Enhancement in recovery *vs.* pure CO_2		
	Fluoranthene	Chrysene	Benzo[*ghi*]perylene
Methanol (10)	1.0	1.1	1.3
Methanol (1)	0.9	1.0	1.1
Toluene (10)	1.1	1.4	4.8
Toluene (1)	1.0	0.8	1.1
Aniline (10)	2.4	1.8	2.0
Aniline (1)	2.3	1.1	1.4

ate volume of the modifier to the extraction cell with the sample, pressurizing and performing a static extraction (no flow out the cell) for 5–30 min, then recovering the analytes with a dynamic extraction step with pure CO_2 for 10 to 30 min.

The results of such a modifier survey for the extraction of PAHs from urban air particulate matter are shown in Table III. Each extraction was performed on 400-mg samples placed in a 2.5-ml extraction cell. Each modifier was added at 1 or 10% of the cell volume, the cell was pressurized with 400 atm CO_2 (80°C), and the static extraction was performed for 5 min followed by a dynamic extraction for 10 min with pure CO_2. As shown in Table III, the enhancement of the PAH recoveries (calculated as the ratio of the individual PAHs extracted with the modifier compared to the amount extracted in 15 min with pure CO_2) with the different modifiers varied by both the polarity of the modifier, its concentration, and the individual PAH. For example, methanol was the poorest modifier for all of the PAHs, and only yielded slight improvement in recovery for benzo[*ghi*]perylene at the 10% concentration. Toluene yielded no significant enhancement for fluoranthene, however 10% toluene was by far the best modifier for the benzo[*ghi*]perylene. In contrast, 10% aniline yielded the same approximate enhancement (*ca.* two) for all of the PAHs. (It must be noted that these were only 5-min extractions in presence of the modifier so that differences between modifiers would be accentuated. A normal SFE would involve a longer contact of the modifier with the sample, and it is likely that both toluene and aniline modifiers would yield good recoveries of the PAHs.)

The addition of modifier directly to the sample may not yield as high of extraction efficiencies as dynamic extractions (using a dual pump system or pre-mixed cylinders) since the modifier is present only during the static extraction step. When the modifier acts by increasing the solubility of the analyte in the extraction fluid, a constant addition of modifier should yield higher extraction efficiencies. However, if the modifier acts primarily by facilitating the removal of the analytes from the matrix active sites (and not by increasing its solubility in the extraction fluid), the simple addition of the modifier to the sample (with a static extraction step followed by a dynamic extraction step with pure CO_2) may be sufficient and eliminate the need to purchase a dual pump system or pre-mixed fluids.

CONCLUSIONS

The development of quantitative SFE conditions is facilitated by an interactive process of developing quantitative collection conditions for the extracted analytes, determining the extraction kinetics (including effect of the extraction fluid flow-rate), and finally by determining the extraction condition that will cause efficient and rapid partitioning of the analytes from the matrix active sites into the extraction fluid. Regardless of the approach used to increase SFE recoveries, any significant changes in the extraction condition should be followed by a new spike recovery study to determine collection efficiencies (*e.g.*, higher temperature extractions may reduce the collection efficiencies of cryogenic and liquid solvent traps, the addition of an organic modifier may reduce the collection efficiency of a sorbent trap). While repeated determinations of spike recoveries may seem laborious, it has been our experience that careful attention to collection efficiencies during methods development can save great amounts of overall effort, since poor recoveries are often incorrectly attributed to the extraction process rather than the collection process (and

therefore the methods development efforts are focused on the wrong step in the SFE scheme). The speed at which the extractions can be performed (*e.g.* a typical extraction time is 30 min, and more than one extraction can be performed at a time) makes the development work faster than might be expected. Indeed, the SFE experiments can often be performed more rapidly than the analysis of the extracts (*e.g.*, a GC analysis normally requires 30 to 60 min) particularly when many fractions are collected from a single extraction as described for the extraction kinetic studies. In addition, the use of this general approach has helped our laboratory develop and validate quantitative SFE conditions for a variety of analytes ranging from volatile non-polar species to non-volatile ionic species from a large range of complex environmental matrices. In every case, SFE extraction conditions have been developed that yield high efficiencies with extraction times of 40 min or less.

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Review

Use of full-spectrum absorbance and emission detectors in environmental analyses

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ABSTRACT

Several classes of environmentally important organic molecules can be both qualitatively and quantitatively analyzed through the use of HPLC with a full-spectrum detector. Specific attention will be paid to the polycyclic aromatic hydrocarbons (PAHs), since this compound class is not only important environmentally, but also provides an ideal example of the capabilities of this combination of analytical tools. The basic principles of these types of full-spectrum detectors, what makes this approach so useful in PAH analyses, and some environmental applications will be discussed.

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1. INTRODUCTION

High-performance liquid chromatography is commonly used in environmental analyses. The limitations of the detectors used in HPLC have, however, prevented its universal use. No detector for HPLC sensitive enough to detect all compounds, analogous to the flame-ionization detector in gas chromatography, is currently available [1]. The universal detectors, such as the refractive index, light scattering, and thermal conductivity detectors, do not have the sensitivity needed for environmental work. Detectors, such as monochromatic UV absorbance and fluorescence detectors, are very sensitive. These devices are too selective. When these detectors are used, many compounds are not

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observed because of the varying optimal wavelengths for different compounds. The recent development of full-spectrum UV absorbance and fluorescence detectors does somewhat overcome this limitation because all wavelengths are observed. Additionally, full-spectrum detectors provide information that helps identify the peaks.

Full-spectrum absorbance detectors first became commercially available in the early 1980s, and now a wide variety of different designs and features are available. These detectors are widely used, with applications in the analysis of petroleum and its refined products [2,3], pharmaceuticals [4], fullerenes [5], and many other areas, as well as in environmental areas. A general discussion of the strengths and disadvantages of full-spectrum detectors when applied to environmental problems will be made in this review. There are currently no commercially available full-spectrum fluorescence detectors, so this discussion will cover work performed on custom, in-house designed and made instruments. Some advantages and problems associated with this type of fluorescence detection will also be described.

2. FULL-SPECTRUM ABSORBANCE DETECTORS

Previously published reviews describe some of the more general features of full-spectrum absorbance detectors [1,6,7]. Full-spectrum detectors can be divided into two types, either fast-scanning or photodiode array-based optics. Photodiode array-based full-spectrum UV absorbance detectors are fundamentally different from either fast-scanning or monochromatic detectors. In the latter, a source produces broadband light which passes through a monochromator, yielding a beam of a single wavelength. This then passes through a flow cell. Only intensity at one wavelength is measured at a time. Fast-scanning detectors, as the name implies, rapidly change the wavelength passing through the flow cell. Their output is a spectrum "on the fly". In monochromatic detectors, sample absorbance at a sole wavelength is measured, yielding the familiar chromatograms.

2.1. Detector design: fast-scanning and photodiode-array detectors

The fast-scanning detectors operate using conventional optics. Design and electronics changes allow for very fast slewing rates for the diffraction gratings, acquisition times for the detector elements, storage of data, etc. The output of a fast-scanning detector can be effected by factors such as flow-rate and peak widths (and, therefore, column efficiency). The scanning rate must be sufficiently high so that changes in concentration as a peak passes through the detector flow cell do not result in distortions in individual spectra.

The second type of full-spectrum detector, the photodiode-array detector (PAD), passes the total light through the flow cell and then disperses it (usually with a diffraction grating). The dispersed light is measured by an array of photo-sensitive semiconductor material (the actual array of photodiodes). Alternatively this device is known as a diode-array detector (DAD).

Comparison of PADs to fast-scanning detectors shows they are usually less sensitive, 0.1 milliabsorbance unit (mA.U.) peak detection limit *versus* 0.02 mA.U. for fast-scanning detectors. They also have a shorter dynamic range, 0.5 mA.U. to 2 A.U. *versus* 0.1 mA.U. to 3 A.U. This is primarily due to the use of photo-multipliers in fast-scanning devices [6], which are more light-sensitive than photodiodes.

There is a spatial limit on the number of photodiodes that can fit in the sensing array. When the dispersed light is focused on the array, there is a tradeoff between spectral resolution, spectral range, and sensitivity. The balance between resolution and range results in two types of PADs, either higher-resolution instruments with an upper limit around 375 nm or lower resolution ones with a limit of 600 nm or more. For most classes of compounds, neither of the limits on resolution or range imposed by the array size are major problems. Most compounds absorb below 300 nm and have very little spectral fine structure.

For certain compound classes, however, balancing resolution and wavelength range can have limitations. Many polycyclic aromatic hydrocarbons (PAHs) of environmental concern, for example benzo[*a*]pyrene, perylene, indeno[1,2,3-*cd*]pyrene, and dibenzo[*b,def*]chrysene, have several bands above 375 nm [8,9]. Using an instrument with limited spectral range makes identification and quantitation difficult since only one part of the spectrum is used. Also as wavelength increases, fewer com-

pounds absorb. A limited wavelength range lowers selectivity of detection.

The absorbance spectra of most organic compounds have very few bands. These are usually broad, with little fine structure [10]. Normally a detector with higher resolution does not gather more useful information than one with lower resolution. Exceptions include differentiating unsubstituted and methyl- and other alkyl-substituted molecules, and the PAHs (see below).

2.2. Data analysis

Full-spectrum detectors, by monitoring all the wavelengths throughout a range, can also yield the traditional type of chromatogram and, additionally, either a contour map or three-dimensional (3-D) display showing all the data. An example of a 3-D chromatogram is shown in Fig. 1 [2]. It must be stated that this mode of data presentation is generally only used for esthetic impact. Much experience is needed in order to readily grasp the information in a 3-D format.

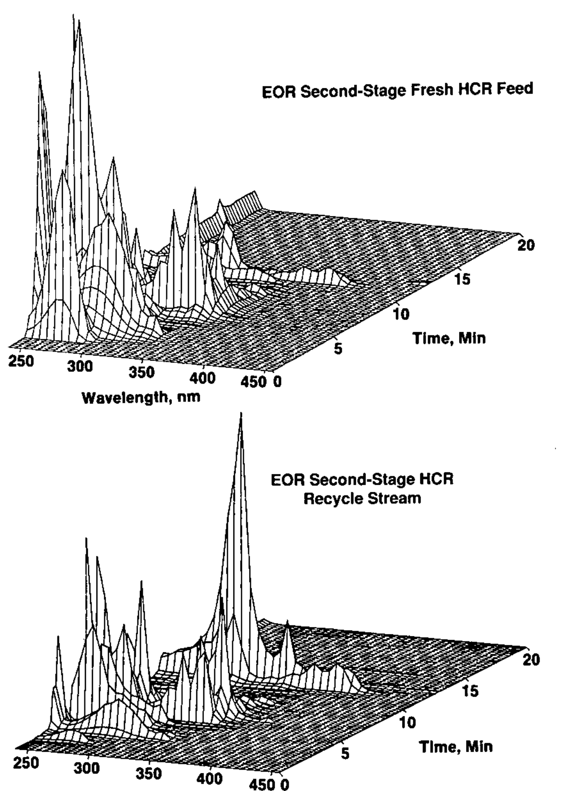


Fig. 1. Three-dimensional chromatograms of PAHs in refined petroleum products. From ref. 2.

Due to the much greater amount of data collected during a PAD run, as compared to single-wavelength detection, the amount of data storage is a key feature in PADs. If the detector is operated so that all spectra are stored at set intervals (as opposed to the mode where only certain spectra are stored, for example, at peak maxima), a run can easily take several megabytes of data storage. The chromatograms shown in Fig. 1 took over 1.5 megabytes of disk storage. PADs must have features which make storage of data and its manipulation and analysis easy.

Co-elution of peaks is not as severe a problem with PADs as with conventional detectors. If only two components co-elute, and the sampling rate of the PAD is set to be fast enough to discern a difference in retention time, spectral deconvolution techniques can be used [11–13]. With these, each component's contribution to the total series of spectra in the peak can be calculated by solving a series of algebraic equations. This allows both components to be identified and quantified. Analysis of peak data in this fashion also is used to determine purity of a peak. Deconvolution is a common method of data evaluation among PADs, since all spectral data points are collected simultaneously. There are potential problems in deconvolution of data from fast-scanning detectors. If any spectral skewing occurs due to the sequential collection of each wavelength in a spectrum, deconvolution is impossible.

Certain PADs can exhibit non-linear responses if wide bandwidths are used [14]. The non-linearity is a function of the bandpass and the shape of the analyte absorbance band, and arises because the algorithms used to reconstruct chromatograms from the original spectral data do not adequately account for the different molar absorptivity values of a compound at different wavelengths. The use of wider spectral ranges has been suggested to make the PAD a "universal" detector [15], which would greatly increase non-linearity.

The full-spectrum mode also makes peak identification possible. Commercial instruments commonly have the capability of matching unknown spectra to those of a previously collected library [16–18].

These comparisons yield "goodness of fit" or "purity of the peak" to show the reliability of the matches. As in the case of raw data collection, when building spectral libraries data storage is an important factor. For work with compound classes such as the PAHs (see below), a library of over a hundred spectra would be necessary [19].

3. FULL-SPECTRUM FLUORESCENCE DETECTORS

Full-spectrum fluorescence detectors are also divided along the lines of fast-scanning and diode array-based instruments. One major, fundamental difference exists between full-spectrum absorbance and fluorescence detectors [20–24]. There are currently no commercial instruments designed specifically to do full-spectrum fluorescence detection, so the only efforts have been with either modified fluorometers or in-house built systems. Their widespread use has, therefore, not occurred.

For fast-scanning optical systems, the low background inherent to right-angle viewing results in the usual very low detection limits common in conventional fluorescence spectrometry. For PAD-based systems, the lack of sensitivity to low light levels and the relatively high background signal require design changes. The light sources in most of these systems are lasers or special flashlamps which have extremely high intensities [22,23]. Cooled or intensified detector elements have been used [25,26].

Other work has relied on the higher concentrations inherent to peaks in micro-column HPLC, although signal averaging of spectra was also used to increase sensitivity [20,21]. These are acute problems since the intensity of fluorescence emission for most compounds is very weak. These limitations are true for most compound classes, but the PAHs, with their high quantum yields, do not usually require these steps.

One of the earliest reports of full-spectrum fluorescence detection was the use of an optical multichannel analyzer, a device similar to a television camera [27]. The optical element was a silicon-intensified target (SIT) imager.

The additional dimension of fluorescence lifetime has created an interesting variation on full-spectrum fluorescence detection [28]. This was made possible by the use of a novel modulated excitation source and PAD. More definitive identification of peaks in a complex sample and spectral separation of co-eluting analytes were obtained.

One limitation that full-spectrum fluorescence detectors have, even when they do become commercially available, that absorbance detectors do not is that emission spectra of some common PAHs change drastically with solvent [26,29]. The differences in the strengths of solvent interactions yield changes in the number of emission bands and their relative intensities. Pyrene and several other PAHs have been used as probes of solvent polarity because of this effect (less polar solvents only interact generally through Van der Waals forces and so have weak interaction with the PAHs, while more polar solvents interact by dipole–dipole forces which interact strongly with the π electrons in the aromatic rings). The spectra of benzo[*e*]pyrene and benzo[*ghi*]perylene in *n*-hexane and ethanol are shown in Fig. 2, reproduced from the GRAND spectral database [30]. These gross changes make identification in complex mixtures or when using solvent gradients difficult. Dual spectral libraries in a polar and a non-polar solvent have been suggested as solutions [30]. This would suggest a normal- and reversed-phase library when full-spectrum fluorescence is used.

There are no current comprehensive fluorescence spectral references that are aimed at compounds of environmental interest. One recent set covers the various types of spectra of PAHs, and similar hetero-atom containing molecules [9]. The PAHs are

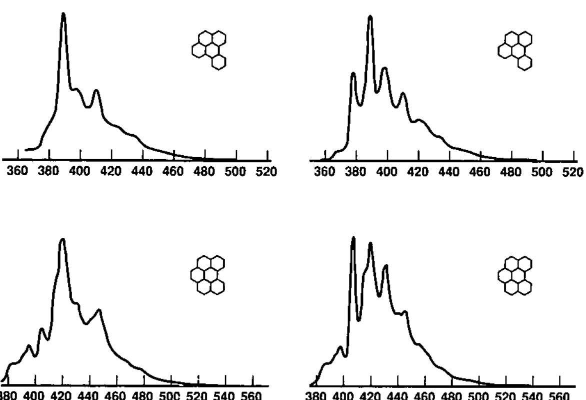


Fig. 2. Fluorescence spectra of benzo[*e*]pyrene and benzo[*ghi*]perylene. Spectral solvents: left, *n*-hexane; right, ethanol. From ref. 30. Scale in nm.

the most widely covered compound class in the literature, but spectra are widely scattered throughout many references.

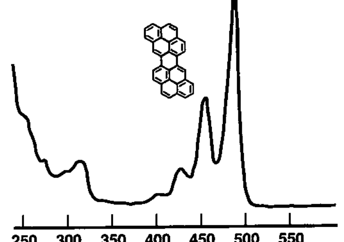
As is the case with the full-spectrum absorbance detectors, data acquisition and storage are major concerns. In one system used for micro-column HPLC [21,22], only 375 spectra could be collected during a run. The narrowness of the peaks and their close elution times required hard wiring of the array output, rather than acquisition through a software-driven system, in order to rapidly collect the spectra.

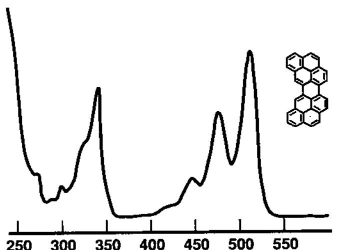
4. ULTRAVIOLET–VISIBLE ABSORPTION SPECTROMETRY OF POLYCYCLIC AROMATIC HYDROCARBONS

The PAHs are very environmentally important molecules. The PAHs are produced by a wide variety of sources and are almost ubiquitous [31,32]. Several are potent carcinogens. They are also a unique class of compounds when UV–visible absorbance and fluorescence spectrometries are considered. The fluorescence excitation and UV absorbance spectra are almost always identical since they arise from the same electronic transitions [33]. The simplicity of structure yields very characteristic spectra. Each molecule is composed only of carbon and hydrogen atoms arranged in aromatic rings, and has a particular size and shape. For each aromatic ring arrangement, the numbers of bands, their locations, and their relative intensities form a unique fingerprint [34].

The most comprehensive spectral reference is still Clar's pair of books, published almost thirty years ago [8]. It contains hundreds of PAH absorbance spectra. Other collections of spectra have also been published [9,35], but many useful spectra of more recently synthesized PAHs are scattered throughout the literature [36–40]. Still, the amount of information in a PAH absorbance spectrum is sufficient enough that identification can be made using published spectra, when authentic standards are unavailable [41,42].

A set of UV absorbance spectra of several eight-ring isomers, molecular mass (M_r) 400, is shown in Figs. 3 and 4 as an example. Pairs of very structurally similar isomers were included to show the uniqueness of the absorbance spectra. The bands in these spectra are typical. The use of UV spectra, in





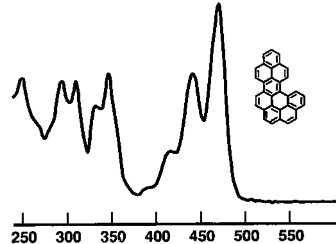
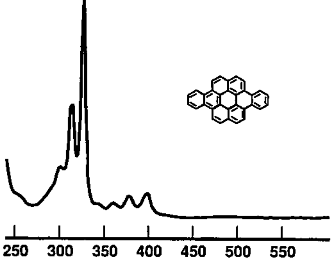
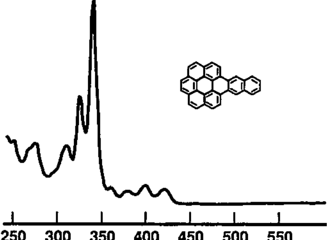


Fig. 3. UV absorbance spectra of M_r 400 PAHs. Scale in nm.

combination with HPLC methods aimed at separating isomers, results in quantitative results with definitive identification [43,44]. This is very important for environmental PAH analyses since the degree of biological impact varies greatly among an isomer set.

The pattern of bands in an alkyl-substituted PAH is very similar to that in the unsubstituted compound [35]. Red-shifts of a few nanometers occur for each linkage to the aromatic core of the molecule. Slightly larger shifts than expected from only two alkyl linkages are seen if the substitution is a saturated ring (typically 5–10 nm per ring). Fig. 5 shows spectra for several substituted pyrenes. The patterns of absorbance bands are extremely similar. Each spectrum has shifted incrementally. Note the





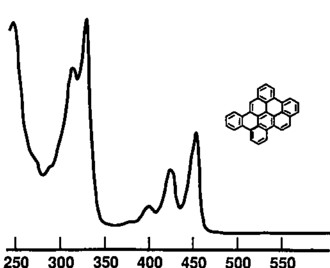


Fig. 4. UV absorbance spectra of M_r 400 PAHs. Scale in nm.

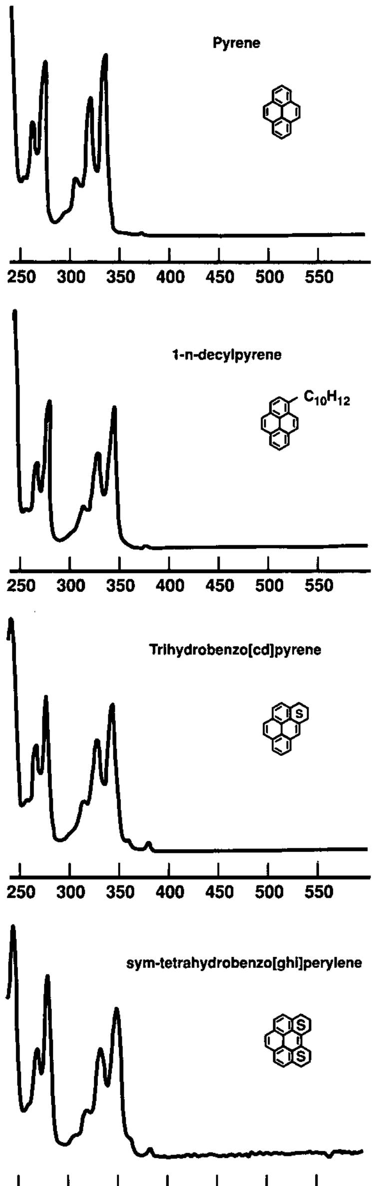


Fig. 5. UV absorbance spectra of pyrene and three alkylpyrenes. Scale in nm.

relatively large shift for the pyrene with two-ring substitution.

As can be seen in the figures, the spectral resolution necessary to perform spectral matching is only about 5 nm, much larger than that in commercial instruments. In fact, the nominal resolutions (spectral range divided by the number of diodes) of 1 nm or less commonly available in many commercial instruments are adequate. Even when the observation of small wavelength shifts are needed, such as with those in the alkyl PAHs, 1 or 2 nm resolution is sufficient.

The electronic transitions in the PAHs are π to π^* and so the molar absorptivities are usually 10^5 or greater [8,9]. This results in very high sensitivity for PAD methods. Spectra with 1 mA.U. intensity can be used to identify a peak. This corresponds to only a few nanograms of each PAH analyte per injection. The high quantum efficiencies of PAHs result in fluorescence detection limits usually a thousand

times lower than those of absorbance techniques [9].

5. ENVIRONMENTAL APPLICATIONS

The combination of HPLC with a PAD has been used to determine PAHs in carbon black and other soots [19–21], diesel exhaust [42], sediments in biomass reactors [43], and marine sediments [44]. However, the typically complex nature of samples obtained either directly from the environment (soil extracts, industrial wastewaters) or as extract from plants, fruits, or other biological systems represents a significant analytical challenge. While HPLC can provide significant resolution capabilities for these mixtures, the PAD contributes by allowing direct identification of a particular component, identification of a secondary metabolite, or provides information about peak homogeneity [45,46]. The promise [47] of enhanced analysis capabilities through the PAD usage has begun to be fulfilled. In 1987, the first review [48] of environmental analysis by HPLC and the PAD was published. The five references described therein foreshadowed the wide span of applications we are now beginning to see appear.

5.1. Analysis of herbicides and degradation products

With the exception of pharmaceutical applications, the determination of various herbicides (and algicides) and degradation products in water samples is the largest area of application at this time. Analysis for selected chlorophenoxy acid (dicamba and dinoseb) [49] and chlorotriazine-based herbicides [50] from water using various sample pretreatments are reported, as well as the linkage of HPLC–PAD with thermospray mass spectrometry for the detection [51] of atrazine, cyanazine, simazine, and other chlorotriazines. Several reports have also been published of analysis protocols for plant-protective agents and metabolites [52,53] and phenoxy-acid herbicides and bentazone (54–56].

5.2. Analysis of pesticides, insecticides, and metabolites

HPLC with a PAD has been applied to a broad variety of pesticides in drinking and surface water. Multi-residue analyses of both pesticides [57] and herbicides in drinking and surface water after solid-phase extraction [58] has been reported, as well as comparative studies between several multi-residue methods for eight pesticides common to regional agriculture (Spain) in citrus fruits [59]. Simultaneous determinations of 22 nitrogen-containing pesticides in drinking, ground, and surface water [60] and the extraction, clean-up, and determination of carbamate pesticides [61] in soil samples by HPLC–PAD are also reported.

5.3. Miscellaneous environmental applications

Searching the complex matrix of industrial wastewater for particular pollutants [62], identifying selected organic pollutants in river water [63], detecting phthalate esters in river waters [64], distinguishing various nitrophenols in rain-water [65], and detecting nitroaromatics [66] in surface water from the site of a former ammunition plant are all applications made either possible or much easier by photodiode-array detection of the related HPLC separation. Applications of environmental interest are not limited to water, plants, and soils. An example of using the PAD to confirm carbonyl structures in atmospheric chemistry studies has been reported [67], as well as the detection of more than 60 steroidal compounds in forensic analysis [68] of illegal preparations employed in cattle-breeding.

Lastly, HPLC–PAD has also found use in environmental applications of a more biological nature. A general method using HPLC–PAD for the analysis of mycotoxins and other fungal secondary metabolites has been reported [69], and the homogeneity of major fractions of aquatic fulvic acids from the Suwannee River as well as the presence of several repeated or similar structures within the fractions were demonstrated by the PAD [70].

6. CONCLUSIONS

Full-spectrum detectors give the chromatographer much more information than previously possible. By yielding both quantitative and qualitative information, their application to environmental problems is very powerful. This is especially true for analyses of PAHs, a classes of compounds with a uniquely high degree of spectral information.

7. ACKNOWLEDGEMENTS

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Review

Determination of polycyclic aromatic hydrocarbons by liquid chromatography

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ABSTRACT

Reversed-phase liquid chromatography (LC) using fluorescence detection is a powerful analytical technique for the measurement of polycyclic aromatic hydrocarbons (PAHs) in environmental samples. The National Institute of Standards and Technology (NIST) has been involved in the development of LC methods for the measurement of PAHs since the mid-1970's particularly for the development of standard reference materials (SRMs) for PAH measurements in environmental samples. The NIST experience in the use of LC for the determination of PAHs in environmental samples is summarized in this paper including: selection of the appropriate column, approaches to analyzing complex PAH mixtures, and the accurate quantitation of PAHs in environmental samples.

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1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants resulting from emissions from a variety of sources including: industrial combustion and discharge of fossil fuels, residential heating (both fossil fuels and wood burning), and motor vehicle exhaust. Because of their mutagenic and carcinogenic properties, PAHs have been measured in a variety of environmental

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matrices including air, water, soil (sediment), and tissue samples. PAHs are usually present in environmental samples as extremely complex mixtures; these mixtures contain many isomeric structures and alkylated isomers which vary greatly in relative concentrations of the individual components and in carcinogenic and/or mutagenic properties.

Since its inception in the early 1970's, high-performance liquid chromatography (LC) has been used for the separation of PAHs. In 1971, Schmit *et al.* [1] first described the separation of PAHs using a chemically bonded octadecylsilane (C_{18}) stationary phase. Since Schmit's report, reversed-phase (RP) LC on chemically bonded C_{18} phases has become the most popular LC mode for the separation of PAHs [2–5]. The popularity of RP-LC for PAH separations is due, in part, to the excellent selectivity of this technique for the separation of PAH isomers. The complex mixtures of PAHs encountered in environmental samples contain numerous isomeric structures. Even when using high resolution open tubular column gas chromatography (GC), a number of isomeric PAHs are still difficult to separate on conventional methylpolysiloxane stationary phases, *e.g.*, chrysene and triphenylene; benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene; and dibenz[*a*,*c*]anthracene and dibenz[*a*,*h*]anthracene. Ultraviolet (UV) absorption and fluorescence spectroscopy provide extremely sensitive and, more important, selective detection for PAHs in LC. Finally, LC provides a useful fractionation technique for the isolation of PAHs for subsequent analysis by other chromatographic and spectroscopic techniques. Because of the excellent separation and detection selectivity of RP-LC, this technique has been specified as the method of choice by the U.S. Environmental Protection Agency (EPA) for the analyses of aqueous effluents for the determination of PAHs [6]. The structures of the 16 PAHs on the EPA priority pollutant list are shown in Fig. 1.

At the National Institute of Standards and Technology (NIST), we have been involved in the development and use of LC methods for the measurement of PAHs since the mid-1970's. These efforts have been part of both environmental monitoring programs and the development of standard reference materials (SRMs) for the measurement PAHs in environmental samples. This paper is a review article summarizing the NIST experience in the use of LC for the determination of PAHs in environmental samples including: selection of the appropriate column (*i.e.*, selectivity), approaches to analyzing complex PAH mixtures (*e.g.*, isolation of PAHs, selective detection and multidimensional LC), and accurate quantitation of PAHs in environmental samples particularly as applied to the measurement of PAHs in SRMs.

2. SELECTION OF THE APPROPRIATE LC COLUMN

2.1. *Differences in selectivity*

RP-LC on C_{18} stationary phases has been shown to provide excellent separations of PAHs. However, not all C_{18} stationary phases provide the same selectivity (*i.e.*, relative separation) for PAHs. In the early 1980's several studies [7–12] compared different commercial C_{18} columns from various manufacturers for the separation of PAHs with particular emphasis on the separation of the 16 PAHs identified by the EPA. These studies found that even though all of the different columns were "generically" C_{18} phases, some provided significantly enhanced selectivity for the separation of the 16 PAHs on EPA's priority pollutant list. During these early studies, it became evident that such investigations were somewhat limited because the exact details concerning the silica substrate and the bonded-phase syntheses were difficult to obtain from the LC column manufacturers. As a result of this limitation, investigations were initiated at NIST to understand more fully the influence of factors such as bonded-phase type, silica substrate characteristics, alkyl chain length, and C_{18} ligand density on selectivity of PAH separations in RP-LC. The results of these investigations have been published in several papers [13–19] and summarized in several review articles [20–22].

One of the most important findings of these investigations was that the separation of the 16 priority pollutant PAHs was greatly influenced by the type of synthesis used to prepare the bonded C_{18} phase. Bonded phases that are prepared using silane modification procedures can be classified as either monomeric or polymeric phases depending on the reagents and reaction conditions used for the bonded phase synthesis. The vast majority of C_{18} phases

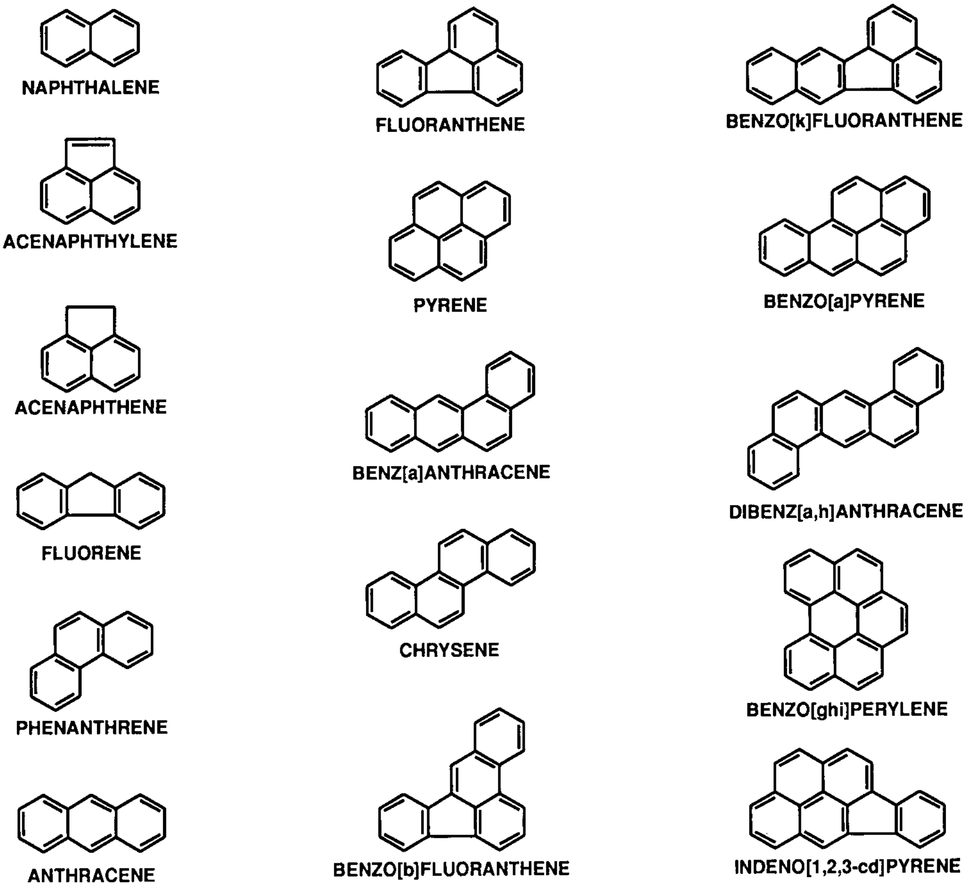


Fig. 1. Structures of the 16 PAHs identified as priority pollutants by the U.S. Environmental Protection Agency and contained in standard reference material (SRM) 1647.

are prepared by reaction of monofunctional silanes (*e.g.*, monochlorosilanes) with silica to form "monomeric" bond linkages. Polymeric phases are prepared using trifunctional silanes in the presence of water which results in cross-linking to form silane polymers on the silica surface. The resulting phase is conceptually not as well-defined as a monomeric phase; however, the chromatographic selectivity characteristics of polymeric C_{18} phases for PAH separations are distinct from those of monomeric C_{18} phases. The difference in the separation of the 16 priority pollutant PAHs on a monomeric and a polymeric C_{18} phase is illustrated in Fig. 2. Separation of all 16 PAHs is achieved on the polymeric C_{18} phase. However, on the monomeric C_{18} phase, the four-ring isomers chrysene and benz[*a*]anthracene are unresolved, and the six-ring isomers benzo[*ghi*]-perylene and indeno[1,2,3-*cd*]pyrene, the five-ring isomers benzo[*k*]fluoranthene and benzo[*b*]fluoranthene, and fluorene and acenaphthene are only partially resolved.

2.2. Classification of phase selectivity

A simle empirical test has been developed to assess the selectivity of C_{18} stationary phases for the separation of PAHs [18,21]. The test is based on the relative retention of three carefully selected PAH solutes as shown in Fig. 3. The retention of benzo[*a*]-pyrene (BaP), relative to 1,2:3,4:5,6:7,8-tetrabenzo-naphthalene (TBN, alternate name dibenzo[*g*,*p*]-chrysene) and phenanthro[3,4-*c*]phenanthrene (Ph-

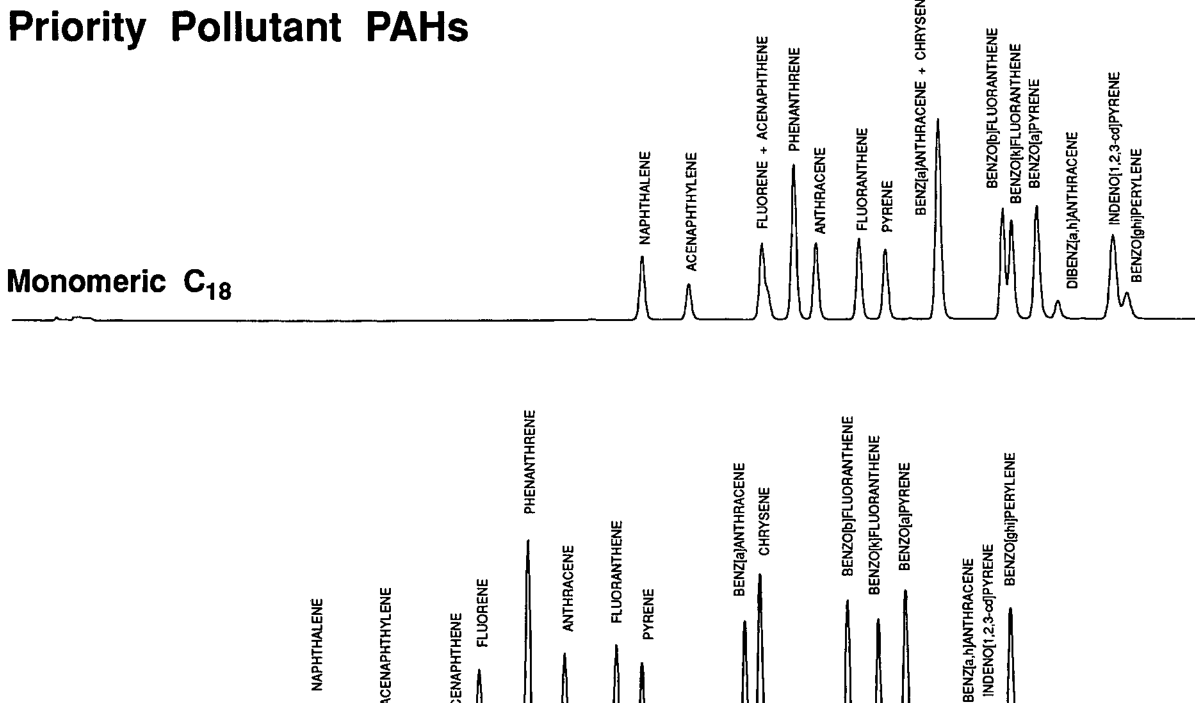


Fig. 2. Comparison of the separation of the 16 priority pollutant PAHs on a monomeric (Zorbax ODS) and a polymeric (Vydac 201TP) C_{18} stationary phase. Chromatographic conditions: mobile phase isocratic at 50% acetonitrile in water for 4 min, then linear gradient to 100% acetonitrile in 15 min at 2 ml/min; UV detection at 254 nm.

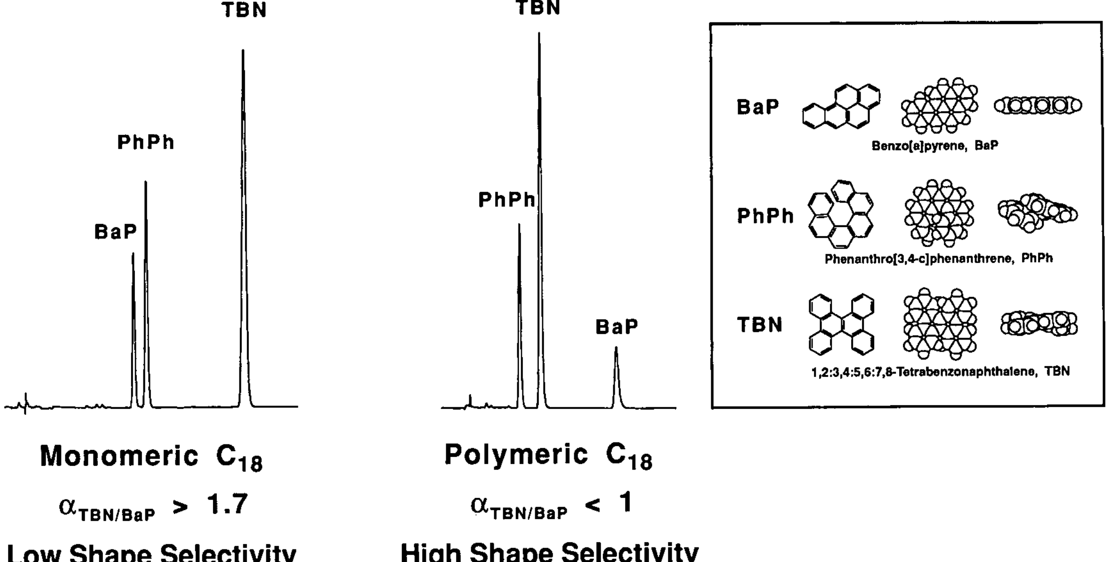


Fig. 3. Separation of SRM 869, Column Selectivity Test Mixture for Liquid Chromatography, on a polymeric and a monomeric C_{18} stationary phase. Structures of the three components in the mixture are illustrated in the box. Chromatographic conditions: mobile phase isocratic at 85% acetonitrile in water at 2 ml/min; UV detection at 254 nm.

Ph), provides a sensitive measure of the "polymeric" or "monomeric" character of the stationary phase. As shown in Fig. 3, the elution order of these three solutes on phases prepared with monomeric surface modification is BaP < PhPh < TBN, whereas phases prepared with polymeric surface modification give the elution order of PhPh < TBN < BaP. This test mixture is available from NIST as SRM 869, Column Selectivity Test Mixture for Liquid Chromatography (see discussion below for SRMs).

A quantitative measure of the phase selectivity can be calculated to allow relative comparisons among different C_{18} phases. The selectivity factor $\alpha_{TBN/BaP}$ (defined as k'_{TBN}/k'_{BaP}) has been shown to correlate with the retention behavior of PAHs and the bonded phase type [18,21]. A classification scheme has been adopted based on the measurement of $\alpha_{TBN/BaP}$ values for experimental and commercial C_{18} phases. Values of $\alpha_{TBN/BaP} \leqslant 1$ indicate polymeric C_{18} phases, and values of $\alpha_{TBN/BaP} \geqslant 1.7$ indicate monomeric C_{18} phases. For values $1 < \alpha_{TBN/BaP} < 1.7$, the bonded phase synthesis is less certain and may indicate a densely loaded monomeric phase or light polymerization with di- or trifunctional reagents. A listing of over 40 commercial C_{18} columns, grouped according to this classification scheme, is provided in Table 1. For the commercial columns the $\alpha_{TBN/BaP}$ values range from 0.56 to 2.18 with the majority of the columns classified as monomeric phases. Values of $\alpha_{TBN/BaP}$ as low as 0.38 have been obtained on heavily loaded experimental polymeric C_{18} phases.

The separation of the 16 priority pollutant PAHs on four C_{18} columns with different $\alpha_{TBN/BaP}$ values is shown in Fig. 4. Generally, only those columns with $\alpha_{TBN/BaP}$ values between *ca.* 0.6 and *ca.* 0.9 will provide complete resolution of the 16 EPA priority pollutants [21]. Separations of all 16 can also be achieved on columns with a $\alpha_{TBN/BaP} \leqslant 0.4$ but the elution order of dibenz[*a,h*]anthracene and benzo[*ghi*]perylene is reversed on the heavily loaded polymeric phase (see Fig. 4). Separation of all 16 components is generally not possible for $\alpha_{TBN/BaP} >$ 0.9. As indicated in Table 1, only a small number of columns have the appropriate selectivity for the separation of the 16 PAHs. Several columns listed in Table 1 that are classified as having polymeric-like selectivity are specifically marketed by the manufacturer for the separation of the 16 priority pollutant PAHs (*e.g.*, Hypersil Green PAH, Spherisorb PAH,

TABLE 1

SELECTIVITY CLASSIFICATION ($\alpha_{TBN/BaP}$) FOR VARIOUS COMMERCIAL C_{18} COLUMNS

Column	$\alpha_{TBN/BaP}$
Polymeric phases	
Bakerbond C_{18} Wide-Pore	0.56
Hypersil Green PAH	0.58
Phenomenex Envirosep PP	0.58
Chromspher PAH	0.59
BioRad RP 318	0.59
Supelcosil LC-PAH	0.63
Vydac 201TP	0.74
Spherisorb PAH	0.82
Erbasil C_{18} H	0.91
Intermediate phases	
ES Industries BF-C_{18}	1.04
LiChrospher 100 RP-18	1.11
Bakerbond C_{18}	1.27
Erbasil C_{18} M	1.28
LiChrospher 60 RP-select B	1.36
Partisil 5 ODS-2	1.40
Partisil 5 ODS	1.48
Spherisorb ODS-1	1.50
Zorbax RX C_{18}	1.50
Brownlee ODS 5A	1.51
Sepralyte C_{18}	1.61
Spherisorb ODS-2	1.68
Monomeric phases	
Erbasil C_{18} L	1.76
Pecospher 5 Cr C_{18}	1.76
Partisphere C_{18}	1.79
Zorbax ODS	1.80
Serva C_{18}	1.84
Partisil 5 ODS-3	1.93
Hypersil ODS (HP)	1.94
Microsorb C_{18}	1.95
J&W Accuphase ODS 2	1.96
Novapak C_{18}	1.97
Ultrasphere ODS	1.98
Capcell C_{18} SG120Å	1.99
Supelcosil LC-18	2.00
IBM ODS	2.00
Brownlee Spheri 5 RP-18	2.02
ODS Hypersil	2.04
Cosmosil C_{18}-P	2.04
Ultracarb 5 C_{18} (20%)	2.05
J&W Accuphase ODS	2.07
YMC 120 Å "A"	2.08
Ultracarb 5 C_{18} (30%)	2.10
Adsorbosphere C_{18} HS	2.10
Supelcosil LC-18-DB	2.18

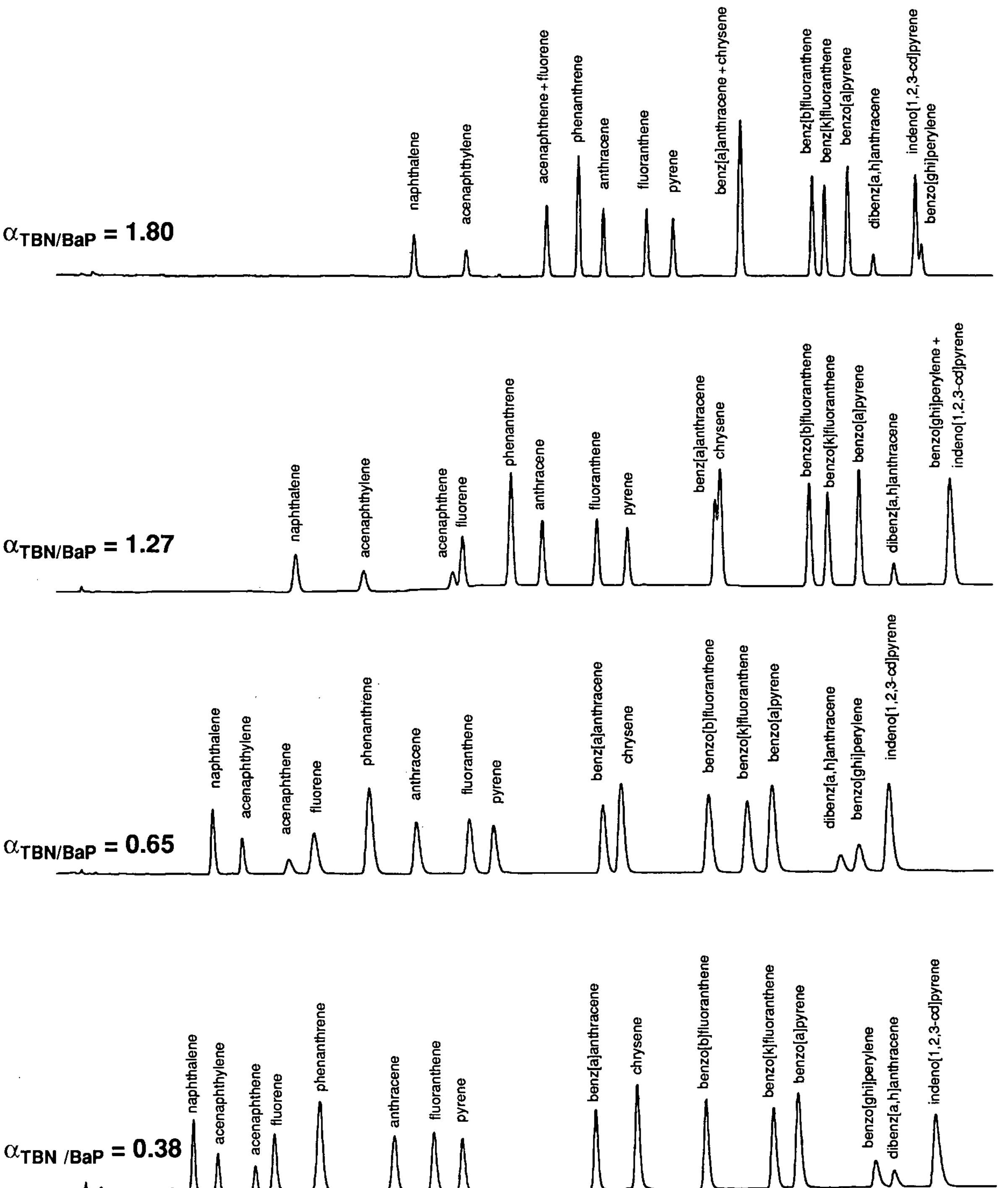


Fig. 4. Separation of SRM 1647 on four different C_{18} columns with different $\alpha_{TBN/BaP}$ values. Chromatographic conditions: columns, Zorbax ODS ($\alpha_{TBN/BaP}$ = 1.80), Bakerbond C_{18} (120 Å pore size) ($\alpha_{TBN/BaP}$ = 1.27), Vydac 201TP ($\alpha_{TBN/BaP}$ = 0.65), and Vydac 201TP (experimental high load) ($\alpha_{TBN/BaP}$ = 0.38); mobile phase linear gradient from 40% acetonitrile in water to 100% acetonitrile in 30 min at 2 ml/min; UV detection at 254 nm (from ref. 21).

Chromsphe PAH, and Supelcosil-LC PAH)*. However, several of these columns are actually the same bonded phase material repackaged by a different supplier (*e.g.*, Supelcosil-LC PAH and BioRad RP 318 are repackaged Vydac 201TP material). Most manufacturers of polymeric C_{18} phases typically select production batches of material that have the selectivity characteristics necessary to separate the 16 priority pollutant PAHs. However, the $\alpha_{TBN/BaP}$ value for polymeric C_{18} phases from different production lots from the same manufacturer may vary from 0.5 to 0.9; thus, the user may find unexpected selectivity differences that will require slight modifications of the LC method.

In addition to studies at NIST, SRM 869 has found use in a number of studies to characterize stationary phase selectivity [23,24], and several column manufacturers routinely use this mixture to monitor the quality control of the production of their C_{18} phases [25–27]. However, at present no LC column manufacturers routinely report the $\alpha_{TBN/BaP}$ value for each column production lot of stationary phase material. Analysts involved in the determination of PAHs by LC should be aware of the different selectivity characteristics of C_{18} phases and should determine the $\alpha_{TBN/BaP}$ value for each polymeric C_{18} phase used in their laboratory to access its selectivity characteristics prior to use. Polymeric phases with different selectivity characteristics are often useful for specific PAH separation applications. For example, Wise *et al.* [16,28,29] reported the use of a heavily loaded polymeric C_{18} phase ($\alpha_{TBN/BaP} = 0.46$) for the separation PAH isomers of molecular mass 278 and 302.

2.3. Selectivity in RP-LC of PAHs

Although the separation of the 16 EPA priority pollutant PAHs was the goal of some of the early studies on selectivity, the polymeric C_{18} phases were found to exhibit unique selectivity for the separation of PAH isomers. As mentioned previously, the separation of isomers is vital for the determination of PAHs since environmental PAH mixtures contain numerous isomeric structures. Several studies have examined the enhanced selectivity of polymeric *vs.* monomeric C_{18} phases for the separation of PAH isomers in general [11] and specifically for isomers of molecular mass 278 [16,29] and 302 [28,29] and methyl-substituted isomers [11,19,30]. Since PAH isomers differ only in the relative positioning of the aromatic rings or the substitution position of a methyl group, *i.e.*, the shape of the PAH, the enhanced selectivity of PAH separations is often referred to as the shape recognition ability of the stationary phase. The relationship between the PAH solute shape and retention in RP-LC on a polymeric C_{18} phase was first reported by Wise *et al.* [11]. In this study Wise *et al.* [11] defined the shape of the PAH as the length-to-breath ratio (L/B) of the box drawn about the molecule such that the maximum L/B value is produced. Retention of PAH isomers was observed to increase with increasing L/B values, *i.e.*, the more "rod-like" solutes eluted later than the more compact solutes as illustrated in Fig. 5 for the separation of the 278 molecular mass PAH isomers. In a later study, the similarity of LC retention on polymeric C_{18} phases and GC retention on liquid crystalline stationary phases was demonstrated for the same groups of PAH isomers [31]. The planarity of PAH solutes also affects their relative retention on C_{18} phases particularly on polymeric phases. In fact, the differences in the relative retention of planar *vs.* non-planar PAHs is the basis for the selectivity test mixture (SRM 869) discussed above. As shown in Fig. 3, BaP is a planar structure whereas PhPh and TBN are non-planar structures. On polymeric C_{18} phases non-planar PAHs elute early relative to planar PAHs [16,19]. To help visualize the combined effects of PAH shape (L/B) and non-planarity on retention on polymeric C_{18} phases, an empirical model was developed known as the "slot model" [16]. The retention of PAHs in reversed-phase LC on C_{18} phases is discussed in detail in a recent review [22].

Detailed investigations have been reported on the factors that affect PAH selectivity, as measured by the $\alpha_{TBN/BaP}$ value, including phase type [14], substrate pore size [15], alkyl phase length [17], phase coverage/density [13,16], and temperature [32]. These

* Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

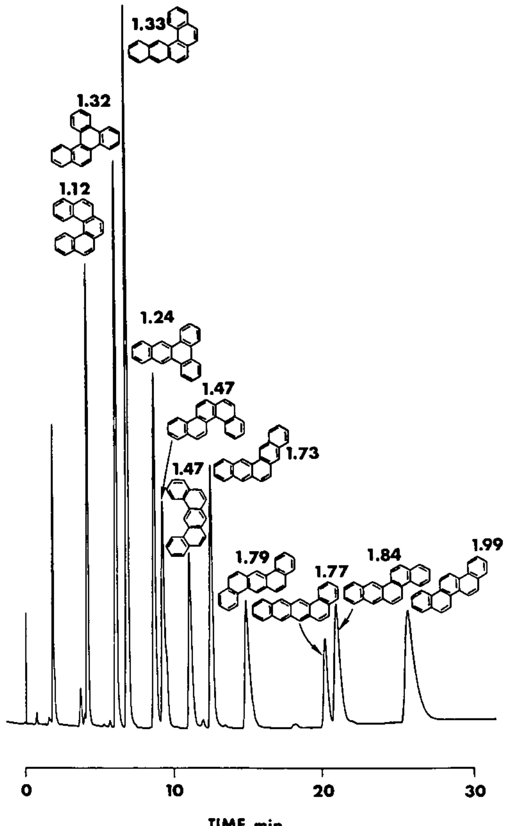


Fig. 5. Separation of eleven PAH isomers of molecular mass 278 on a polymeric C_{18} phase. Numbers above each peak correspond to length-to-breadth values for each isomer. Chromatographic conditions: Vydac 201TP column (experimental high loading); mobile phase linear gradient from 85% acetonitrile in water to 100% acetonitrile in 15 min at 1.5 ml/min; UV detection at 254 nm (from ref. 16).

results have been summarized recently in a review [21]. In general, selectivity for the separation of PAHs increases with decreasing $\alpha_{TBN/BaP}$ values. Furthermore, separations often can be reproduced under different conditions (*i.e.*, different combinations of phase type, alkyl chain length, and column temperature) as long as the $\alpha_{TBN/BaP}$ value is held constant. In practice, parameters such as phase type, ligand density, or alkyl chain length can only be varied by changing the column. However, column temperature is an important parameter that can be used to readily modify selectivity for PAH separations. Generally in LC analyses temperature is considered only for potentially increasing the column efficiency by increasing the temperature. The effect of temperature on phase selectivity (as monitored by the $\alpha_{TBN/BaP}$ value) for temperatures from −20°C to 100°C is illustrated in Fig. 6 for both monomeric and polymeric C_{18} phases. Changes in shape selectivity are relatively uniform for the polymeric C_{18} phase, whereas for the monomeric phase the selectivity is nearly constant above 25°C with significant changes at subambient temperatures. The selectivity *vs.* temperature plots in Fig. 6 offer useful information for the separation of PAHs by RP-LC. First, separations of PAHs using polymeric C_{18} phases are very sensitive to temperature variations which may result in changes in selectivity and not just variations in absolute retention time as would generally be observed with changes in temperature. As a result temperature control for PAH separations on a polymeric C_{18} phase is more important than for other LC analyses. Secondly, polymeric C_{18} phases generally should not be used at elevated temperature since any increases in column efficiency are greatly outweighed by a significant reduction in selectivity. Finally, a monomeric C_{18} phase will have "polymeric-like" selectivity at temperatures near 0°C and below. The separation of all 16 of the priority pollutant PAHs on a monomeric C_{18} has been demonstrated at −8°C [32]. Subambient temperatures can also be used with polymeric C_{18} phases to achieve enhanced selectivity characteristics that are not possible with available polymeric phases at ambient temperatures. This enhanced capability was demonstrated for the separation of all six methylchrysene isomers, which previously had not been separated by LC [32].

3. APPROACHES FOR THE DETERMINATION OF PAHs

Even though separation of the 16 priority pollutant PAHs is easily achieved by RP-LC on the appropriate C_{18} column (see Fig. 2), natural mixtures of PAHs from environmental sample extracts are extremely complex due to numerous isomeric structures including alkyl-substituted isomers. Thus, the analysis of these PAH mixtures requires the use of selective detection and/or the use of "multidimensional" LC procedures to accurately measure individual PAHs.

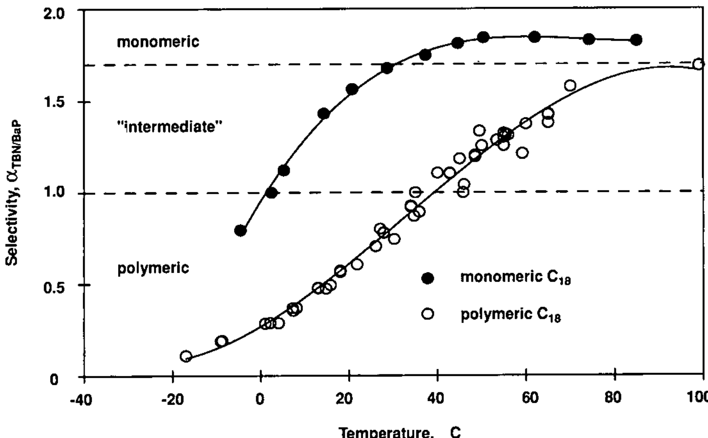


Fig. 6. Shape selectivity plotted as a function of temperature for a monomeric (Zorbax ODS) and a polymeric (Vydac 201TP) C_{18} phase (from ref. 32).

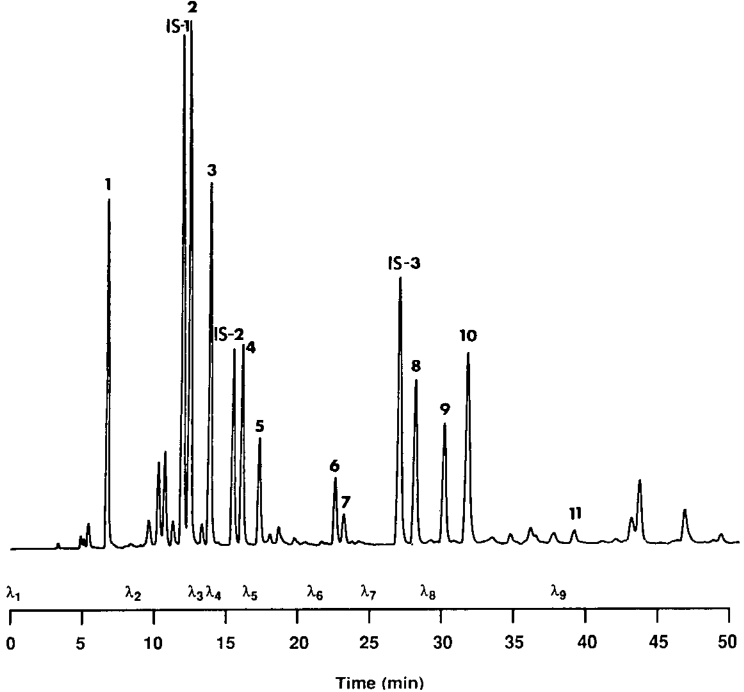


Fig. 7. RP-LC analysis of a coal tar sample (SRM 1597) using wavelength programmed fluorescence detection. Fluorescence conditions (excitation λ/emission λ in nm): λ_1 (280/340), λ_2 (249/380), λ_3 (250/442), λ_4 (285/450), λ_5 (333/390), λ_6 (285/385), λ_7 (406/440), λ_8 (296/405), and λ_9 (300/500). Peak identifications: 1 = naphthalene, IS-1 = [$^2H_{10}$]phenanthrene, 2 = phenanthrene, 3 = anthracene, IS-2 = [$^2H_{10}$]fluoranthene, 4 = fluoranthene, 5 = pyrene, 6 = benz[*a*]anthracene, 7 = chrysene, IS-3 = [$^2H_{12}$]perylene, 8 = perylene, 9 = benzo[*k*]fluoranthene, 10 = benzo[*a*]pyrene, and 11 = indeno[1,2,3,-*cd*]pyrene. Chromatographic conditions: Vydac 201TP column; mobile phase linear gradient from 50% acetonitrile in water to 100% acetonitrile in 50 min at 1.5 ml/min (from ref. 37).

3.1. Selective detection

UV absorption and fluorescence detection are the most widely used LC detectors for the measurement of PAHs. UV detection provides a nearly "universal" detector for PAHs; however, for quantitation in complex environmental PAH mixtures, the fluorescence detector offers far more sensitivity and, more importantly, selectivity than UV detection. By selection of the appropriate excitation and emission wavelengths, a high degree of specificity can be achieved. The analysis of a coal tar extract using RP-LC with fluorescence detection is illustrated in Fig. 7. The excitation and emisson wavelengths were changed during the chromatographic run (*i.e.*, wavelength programming) to achieve optimal sensitivity and/or selectivity for individual PAHs. The excitation and emission wavelengths currently used at NIST for the analysis of environmental samples are summarized in Table 2. Fluorescence excitation and emission spectra for a number of PAH standards have been published [33–35] and these spectra can be used as the basis for selection of optimal wavelength conditions for the PAHs measured. RP-LC with fluorescence wavelength programmed detection has been used at NIST to measure PAHs in several sample types including oil [36], coal tar [37], air and diesel particulate samples [38], marine sediment [39], and mussel tissue [40]. Three perdeuterated PAHs ([$^2H_{10}$]phenanthrene, [$^2H_{10}$]fluoranthene, and [$^2H_{12}$]perylene) were used as the internal standards for quantification of the PAHs in the coal tar sample shown in Fig. 7. Perdeuterated PAHs are excellent internals standards for RP-LC separations of PAHs since they elute immediately prior to the non-deuterated PAH (generally with baseline resolution) and they have nearly the same fluorescence characteristics as the non-deuterated PAHs [36,38]. Recently the method illustrated in Fig. 7 has been modified to include [$^2H_8$]naphthalene and [$^2H_{12}$]-benzo[*a*]pyrene for a total of five internals standards from two to five aromatic rings as shown in Table 2.

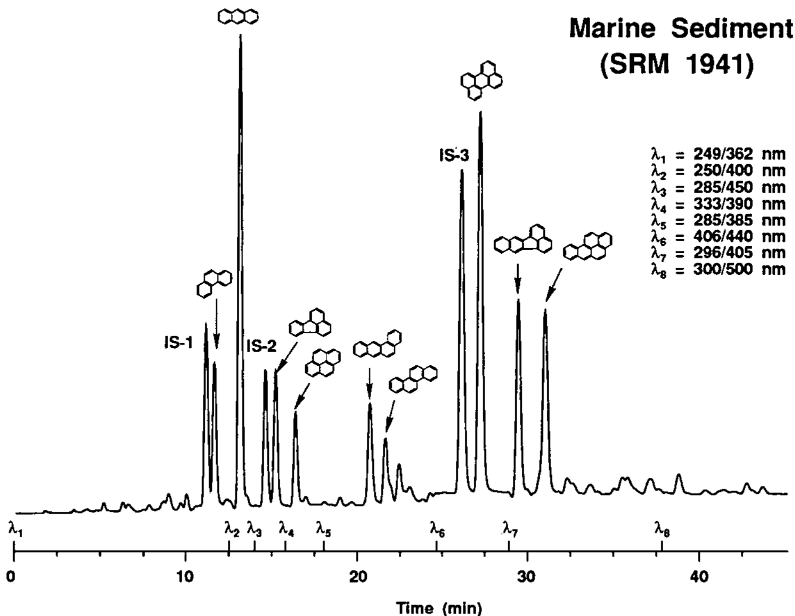
TABLE 2

FLUORESCENCE WAVELENGTH PROGRAMMING CONDITIONS FOR THE LC DETERMINATION OF SELECTED PAHs

Wavelength change	Excitation wavelength (nm)	Emission wavelength (nm)	PAHs determined
1	280	340	[$^2H_8$]Naphthalene (IS)[a]
			Naphthalene
2	249	362	[$^2H_{10}$]Phenanthrene (IS)
			Phenanthrene
3	250	400	Anthracene
4	285	450	[$^2H_{10}$]Fluoranthene (IS)
			Fluoranthene
5	333	390	Pyrene
6	285	385	Benz[*a*]anthracene
7	260	360	Chrysene
8[b]	406	440	[$^2H_{12}$]Perylene (IS)
			Perylene
8a	295	425	Benzo[*b*]fluoranthene
9	296	405	Benzo[*k*]fluoranthene
			[$^2H_{12}$]Benzo[*a*]pyrene (IS)
			Benzo[*a*]pyrene
			Dibenz[*a,h*]anthracene
			Benzo[*ghi*]perylene
10	300	500	Indeno[1,2,3,-*cd*]pyrene

[a] IS = internal standard.

[b] Wavelength conditions for 8 and 8a are used during separate chromatographic runs due to coelution of perylene and benzo[*b*]fluoranthene.



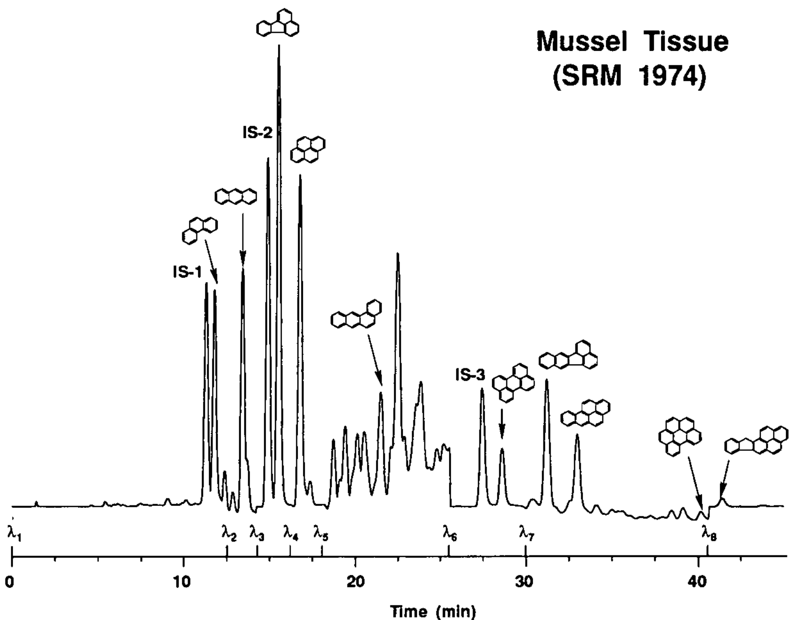


Fig. 8. RP-LC analysis of marine sediment (SRM 1941) and mussel tissue (SRM 1974) using wavelength programmed fluorescence detection. Chromatographic conditions: Vydac 201TP column; mobile phase linear gradient from 50% acetonitrile in water to 100% acetonitrile in 50 min at 1.5 ml/min.

For the coal tar sample in Fig. 7, a wavelength change was not performed between benz[*a*]anthracene and chrysene. However, by using a column with the appropriate selectivity, sufficient separation can be achieved to allow a wavelength change. Generally, temperature control must be maintained to achieve the retention time reproducibility required to make wavelength changes between closely eluting chromatographic peaks.

Even though the coal tar extract shown in Fig. 7 is a complex mixture of PAHs, it could be analyzed directly by LC without any additional cleanup or isolation of the PAH fraction. However, extracts of environmental samples such as sediment and tissue generally require additional cleanup or isolation steps to obtain accurate results for the measurement of individual PAHs. However, because of the selectivity of fluorescence detection, environmental extracts can often be analyzed by LC with less sample cleanup than would be required for GC analyses [38]. The analyses of marine sediment and tissue samples using RP-LC with wavelength programmed fluorescence detection are shown in Fig. 8 [39,40]. These sample extracts were cleaned up using a normal-phase solid phase extraction (SPE) procedure to remove the polar compounds (see below).

3.2. Multidimensional LC approach

A second approach to the measurement of PAHs in complex mixtures is the use of multidimensional chromatographic procedures. At NIST we have routinely used a multidimensional LC approach to isolate the PAH fraction or groups of PAH isomers prior to analysis by RP-LC with fluorescence or UV detection or by gas chromatographic techniques. This multidimensional procedure consists of a normal-phase LC separation of the environmental extract or PAH mixture on an aminopropylsilane stationary phase. In 1979 Wise *et al.* [41] demonstrated that the normal-phase elution of PAHs from the aminopropylsilane column was based on the number of aromatic carbon atoms in the PAH, *i.e.*, isomeric PAHs elute as a group and alkyl-substituted PAHs elute very near the unsubstituted parent PAHs. Thus, the aminopropylsilane column can be used to isolate a fraction containing only isomeric PAHs and alkyl-substituted isomers. The normal-phase LC retention characteristics for over 80 PAHs on the aminopropylsilane column have been reported [7,40]. This normal-phase LC procedure has been used as the first step in multidimensional chromatographic procedures for (i) cleanup and isolation of the total PAH fraction from environmental samples, (ii) isolation of selected isomeric PAHs in complex mixtures, and (iii) detailed characterization of PAH mixtures.

3.2.1. Cleanup and isolation of total PAH fraction

The normal-phase LC cleanup and isolation of the total PAH fraction is routinely used at NIST as the sample preparation procedure prior to analysis by GC with flame ionization detection (FID) and mass spectrometric (MS) detection or by RP-LC with fluorescence detection. For GC–FID analysis complete isolation of the PAH fraction is required (*i.e.*, using an LC separation) to eliminate the aliphatic hydrocarbon and polar interferences. The same cleanup/isolation procedure is generally applied for GC–MS analyses, even though MS provides sufficient detection specificity to allow minimal cleanup, to minimize loss of chromatographic efficiency when using on-column injection techniques for open tubular GC columns. However, a simple SPE procedure using the same aminopropylsilane phase can be used to remove the compounds more polar than PAHs. The SPE procedure does not remove the aliphatic hydrocarbons from the PAH fraction; however, these compounds do not interfere in the RP-LC with fluorescence detection procedure or in the selective GC–MS procedure because of the selectivity of the detector. This approach was used for the cleanup of the sediment and mussel tissue samples shown in Fig. 8.

3.2.2. Isolation of selected isomeric PAHs

The second application of the multidimensional LC procedure is for the isolation and measurement of selected isomeric PAHs in complex samples. This approach is useful in two ways: (i) to isolate an isomeric fraction from a complex mixture such as an oil matrix to measure the major PAH components and (ii) to isolate an isomeric PAH fraction to enrich the concentration of minor PAH isomers not easily measured in the total PAH fraction. The first approach is illustrated in Fig. 9 for the determination of pyrene and fluoranthene in a shale oil sample. The pyrene–fluoranthene isomer fraction was iso-

lated from the shale oil by injection of the diluted shale oil onto the aminopropylsilane column and collecting the fraction corresponding to the elution of pyrene and fluoranthene. This fraction was then analyzed by RP-LC with UV and fluorescence detection. The necessity of the fluorescence detection to achieve accurate quantitation of the pyrene and fluoranthene is illustrated by the comparison of the chromatograms with UV and fluorescence detection (Fig. 9). This application also illustrates the suitability of the perdeuterated PAHs as internal standards for the complete multidimensional LC procedure, *i.e.*, on the aminopropylsilane column the perdeuterated PAHs elute slightly after the

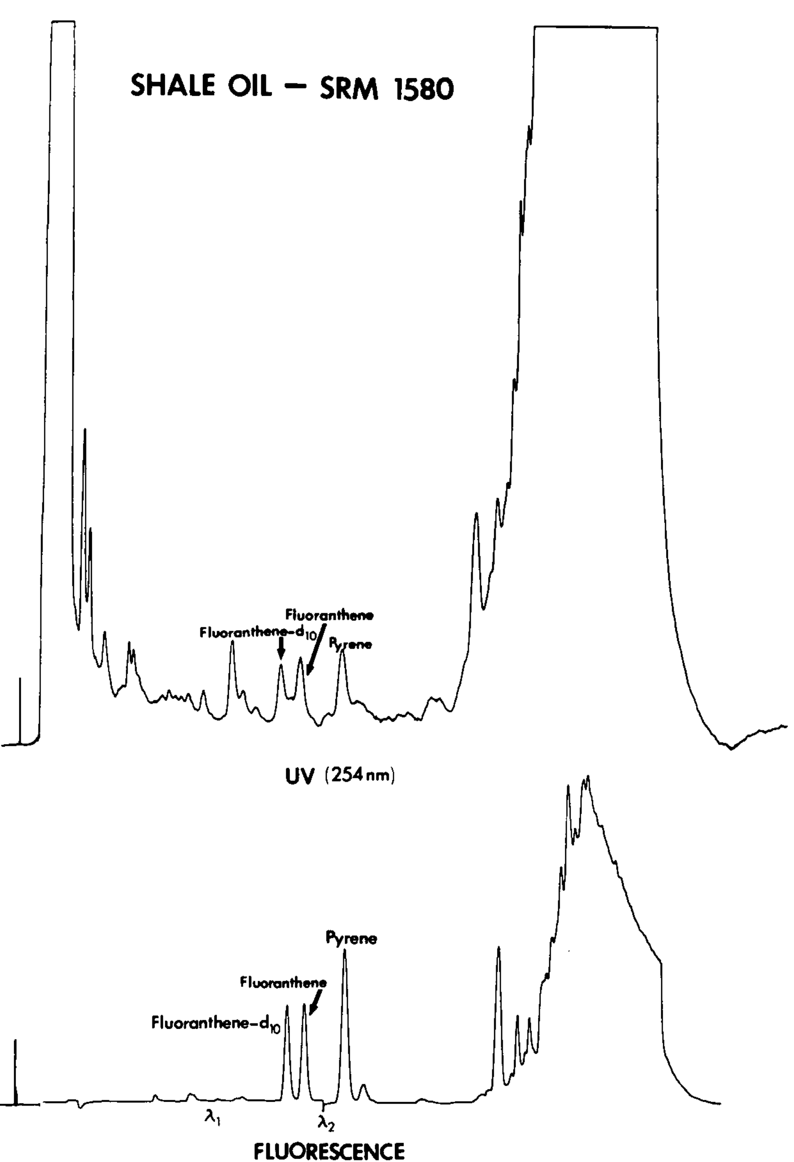


Fig. 9. RP-LC analysis of the fluoranthene–pyrene fraction (16 aromatic carbon atoms) isolated from a shale oil sample (SRM 1580). Fluorescence conditions (excitation λ/emission λ in nm): λ_1 (285/450) and λ_2 (335/385). Chromatographic conditions: Vydac 201TP column; mobile phase isocratic at 45% acetonitrile in water until fluoranthene eluted, then linear gradient to 100% acetonitrile in 5 min at 1.5 ml/min (from ref. 36).

non-deuterated PAHs but still within the isomeric group. This multidimensional LC approach was used to measure PAHs in the shale oil and petroleum crude oil SRMs [36,42,43] (see discussion below) including a novel on-line approach [44].

Another application of the multidimensional LC procedure for the isolation and measurement of isomeric PAHs is to enrich the concentration of minor PAHs components that are not easily measured in the total PAH fraction. This approach is illustrated by the determination of triphenylene and benzo[*ghi*]perylene in the coal tar and marine sediment samples shown in Figs. 7 and 8. Using the LC–fluorescence approach for the analysis of the coal tar and PAH fraction from the marine sediment extracts, it was not possible to obtain accurate data for triphenylene or benzo[*ghi*]perylene because of low detection sensitivity and/or selectivity (see Figs. 7 and 8). To obtain accurate LC measurements for these two compounds, the normal-phase LC fractionation procedure was employed to isolate separate fractions for the four-ring PAH isomers (18 aromatic carbons: triphenylene, chrysene, and benz[*a*]anthracene) and the six-ring isomers (22 aromatic carbons: benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, and anthanthrene). Prior to sample extraction. perdeuterated PAHs were added as internal standards to represent each isomer group, *e.g.*, [$^{2}H_{12}$]triphenylene and [$^{2}H_{12}$]benz[*a*]anthracene were added for the four-ring isomer fraction and [$^{2}H_{14}$]benzo[*ghi*]perylene was added for the six-ring fraction. The normal-phase LC separation of an air particulate extract is shown in Fig. 10. The four-ring and six-ring PAH fractions were collected, concentrated, and analyzed by RP-LC–fluorescence as illustrated in Fig. 11 for a sediment sample. Chrysene and triphenylene are generally not quantified individually by GC analysis since they coelute on conventional stationary phases, and their concentrations are reported as a combined value. Using the multidimensional LC procedure, results for triphenylene and chrysene were determined in the sediment sample as shown in Table 3. The two LC results in Table 3 for chrysene (Direct and Fraction) are in good agreement (425 ± 42 and 473 ± 5 ng/g). The GC–FID and GC–MS results for chrysene–triphenylene are 577 ± 12 and 702 ± 16, respectively, (mean of 639 ng/g) compared to the sum of LC results for triphenylene (192 ng/g) and chrysene (mean of 449 ng/g) which is 641 ng/g. This same approach was also used to measure chrysene and triphenylene in air particulate material [38] and a coal tar extract [37].

The measurement of benzo[*ghi*]perylene (and often indeno[1,2,3-*cd*]pyrene) in the total PAH fraction is generally difficult (even though their concentrations are similar to other major PAHs), due to low fluorescence sensitivity and selectivity for these PAHs. By using the normal-phase LC fractionation procedure, these two isomers are enriched sufficiently to provide good measurements. The RP-LC–fluorescence analysis of the six-ring PAH fraction from the marine sediment SRM is shown in Fig. 11. The results of these analyses are summarized and compared in Table 3. Using this approach, the LC results for benzo[*ghi*]perylene (504 ± 7) were in good agreement with the GC–FID results (478 ± 14 ng/g) and within about 12% of the GC–MS measurements (567 ± 26 ng/g). For indeno[1,2,3-*cd*]pyrene all four results agreed within 3%, *i.e.*, 572 ± 28 ng/g (GC–FID), 573 ± 20 ng/g (LC Direct), 575 ± 8 ng/g (LC Fraction), and 559 ± 19 ng/g (GC–MS). Anthanthrene, which has not been mea-

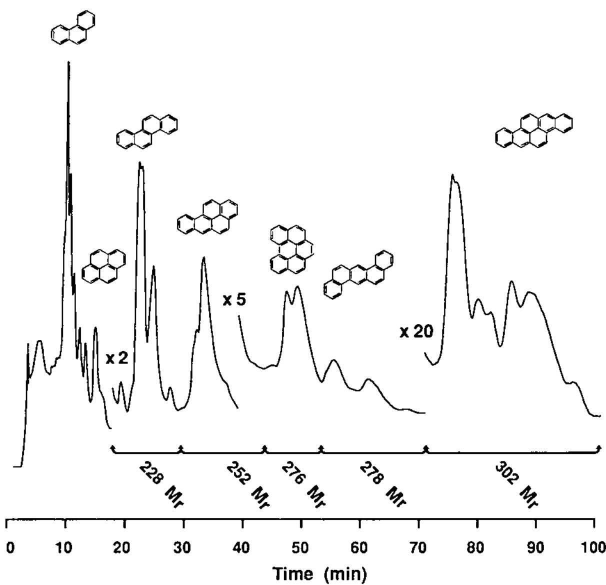


Fig. 10. Normal-phase LC fractionation of air particulate extract (SRM 1648) to isolate the isomeric PAH groups. Chromatographic conditions: μBondapak NH_2 semipreparative column; mobile phase of 2% methylene chloride in hexane at 5 ml/min; UV detection at 254 nm.

TABLE 3

SUMMARY OF ANALYTICAL RESULTS (ng/g DRY WEIGHT) FOR THE DETERMINATION OF PAHs IN SRM 1941, ORGANICS IN MARINE SEDIMENT

Compound	GC–FID	GC–MS	LC–FL (Direct)	LC–FL (Fraction)	Certified value[a]
Phenanthrene	597 (4)[b]	603 (10)	531 (12)		577 ± 59
Anthracene	202 (6)	228 (12)	174 (8)		202 ± 42
Fluoranthene	1116 (20)	1401 (41)	1135 (10)		1220 ± 240
Pyrene	1008 (16)	1238 (18)	989 (34)		1080 ± 200
Benz[*a*]anthracene	538 (12)	599 (14)	516 (7)	521 (11)[d]	550 ± 78
Chrysene	577 (12)[c]	702 (16)[c]	425 (42)	473 (5)[d]	
Triphenylene				192 (3)[d]	
Benzo[*b*]fluoranthene	635 (17)	864 (28)	839 (14)	843	780 ± 190
Benzo[*j*]fluoranthene	351 (14)				
Benzo[*k*]fluoranthene	439 (19)	857 (25)[f]	456 (6)[e] 441 (8)[e]	443 (16)	444 ± 49
Benzo[*e*]pyrene	472 (25)	672 (24)			
Benzo[*a*]pyrene	566 (12)	754 (49)	674 (12)	690 (25)	670 ± 130
Perylene	415 (8)	437 (27)	411 (6)	426 (5)	442 ± 33
Benzo[*ghi*]perylene	478 (14)	566 (26)		504 (7)	516 ± 83
Indeno[1,2,3-*cd*]pyrene	572 (28)	559 (19)	573 (20)	575 (8)	569 ± 40

[a] The certified values are weighted means of results from two or more analytical techniques. Each uncertainty is obtained from a 95% prediction interval plus an allowance for systematic error among the methods used. The allowance for systematic error is equal to the greatest difference between the weighted mean (certified value) and the component means for the analytical methods used. In the absence of systematic error, the resulting uncertainty limits will cover the concentration of approximately 95% of samples of this SRM having a minimum sample size of approximately 5 g.

[b] Uncertainties (values in parentheses) for GC–FID, LC–FL, and GC–MS measurements are ± one standard deviation of a single measurement; for GC–FID measurements, twelve samples analyzed in triplicate; for LC measurements, three samples analyzed in triplicate; for GC–MS measurements, four samples analyzed in duplicate.

[c] Value is for chrysene and triphenylene.

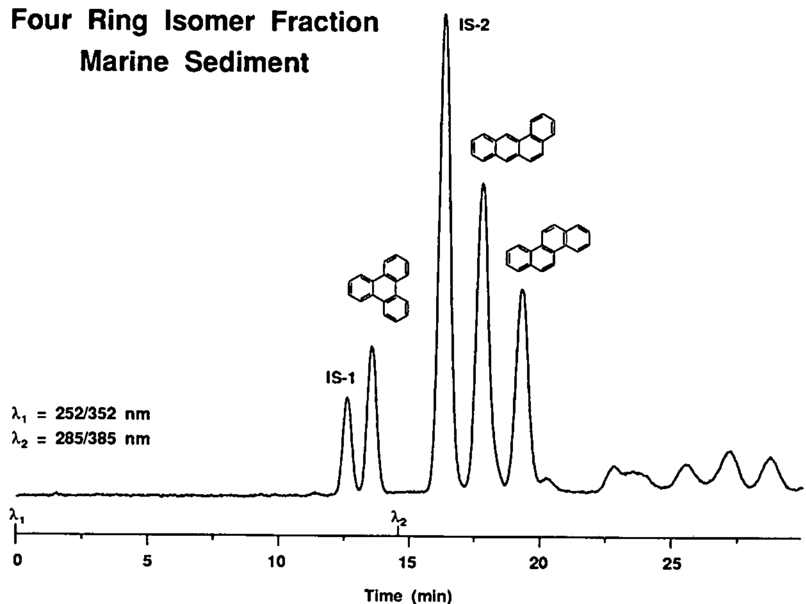
[d] Determined using [$^{2}H_{12}$]triphenylene as internal standard.

[e] Benzo[*k*]fluoranthene was determined at different times, *i.e.*, during initial analyses of total PAH fraction and during benzo[*b*]fluoranthene analyses.

[f] Value is for benzo[*k*]fluoranthene and benzo[*j*]fluoranthene.

sured previously at NIST in total PAH fractions, is readily quantified in the six-ring fraction and has been measured in a new marine sediment SRM using this approach.

The measurement of dibenz[*a,h*]anthracene and other five-ring catacondensed PAH isomers (molecular mass 278) is another example of the application of the multidimensional LC technique to measure minor level PAHs. Dibenz[*a,h*]anthracene, which is listed as one of the EPA priority pollutant PAHs, is often quantified based on either GC or LC measurements. However, for GC measurements on a stationary typically used for PAH analyses (*e.g.*, 5% phenyl-substituted methylpolysiloxane), dibenz[*a,c*]anthracene coelutes with dibenz[*a,h*]anthracene. RP-LC measurements of dibenz[*a,h*]anthracene are highly questionable since the resolution of isomers is dependent on the column used (see discussion above), and the fluorescence selectivity for dibenz[*a,h*]anthracene is generally not sufficient to preclude the possibility of interference from coeluting PAHs in a complex mixture [43]. To quantify dibenz[*a,h*]anthracene and five other 278 molecular mass isomers in several environmental matrix SRMs, the normal-phase LC procedure was used to isolate the dibenz[*a,h*]anthracene isomer fraction as shown in Fig. 10. The dibenz[*a,h*]anthracene fraction was then analyzed by RP-LC–fluorescence as shown in



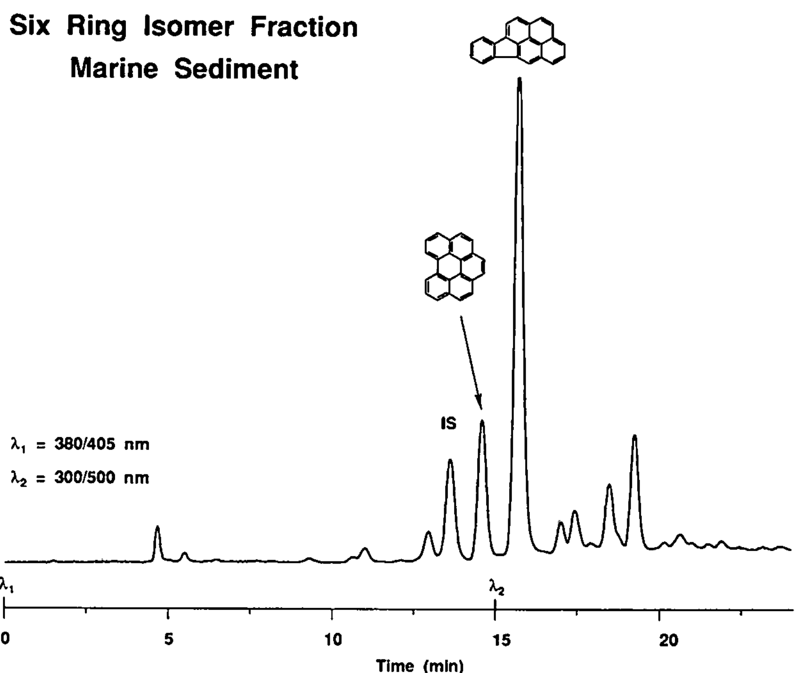


Fig. 11. RP-LC analysis of the 228 and 276 molecular mass fractions isolated from the marine sediment (SRM 1941) extract. Chromatographic conditions: Vydac 201TP column; mobile phase (four ring fraction): isocratic at 60% acetonitrile in water for 15 min, then linear gradient to 100% acetonitrile in 5 min at 1.5 ml/min; mobile phase (six ring fraction): isocratic at 80% acetonitrile in water for 5 min, then linear gradient to 100% acetonitrile in 15 min at 1.5 ml/min; fluorescence detection.

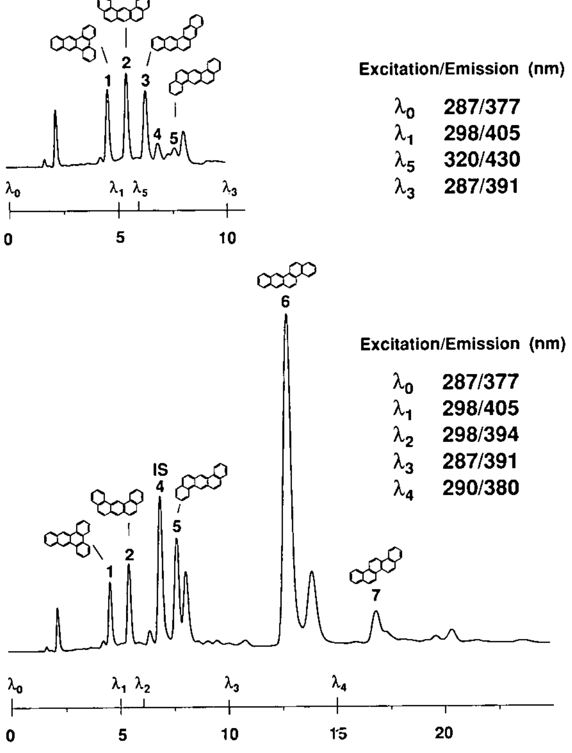


Fig. 12. RP-LC analysis of 278 molecular mass fraction isolated from a coal tar extract (SRM 1597). Peak identifications: 1 = dibenz[*a,c*]anthracene, 2 = dibenz[*a,j*]anthracene, 3 = pentaphene, 4 IS (internal standard) = [$^2H_{14}$]dibenz[*a,h*]anthracene, 5 = dibenz[*a,h*]anthracene, 6 = benzo[*b*]chrysene, 7 = picene. Chromatographic conditions: Vydac 201TP column ($\alpha_{TBN/BaP}$ = 0.46); mobile phase isocratic at 90% acetonitrile in water for 10 min and then a linear gradient to 100% acetonitrile in 2 min at 1.5 ml/min; column temperature of 32°C (from ref. 29).

Fig. 12 for the coal tar extract (SRM 1597). The same multidimensional LC approach was used to measure individual isomers of molecular mass 302 (dibenzopyrene–fluoranthenes); the RP-LC analysis of the dibenzopyrene–fluoranthene fraction is shown in Fig. 13. Using this multidimensional procedure, the concentrations of six PAH isomers of molecular mass 278 and nine PAH isomers of molecular mass 302 were determined in four natural matrix SRMs [29].

3.2.3. Detailed characterization of PAH mixtures

The third application of the normal-phase LC procedure is to isolate isomer fractions for analysis by GC–MS and RP-LC–fluorescence to provide detailed characterization of the PAH mixture. This approach has been used to provide qualitative information on over 180 PAHs in two air particulate material SRMs [45].

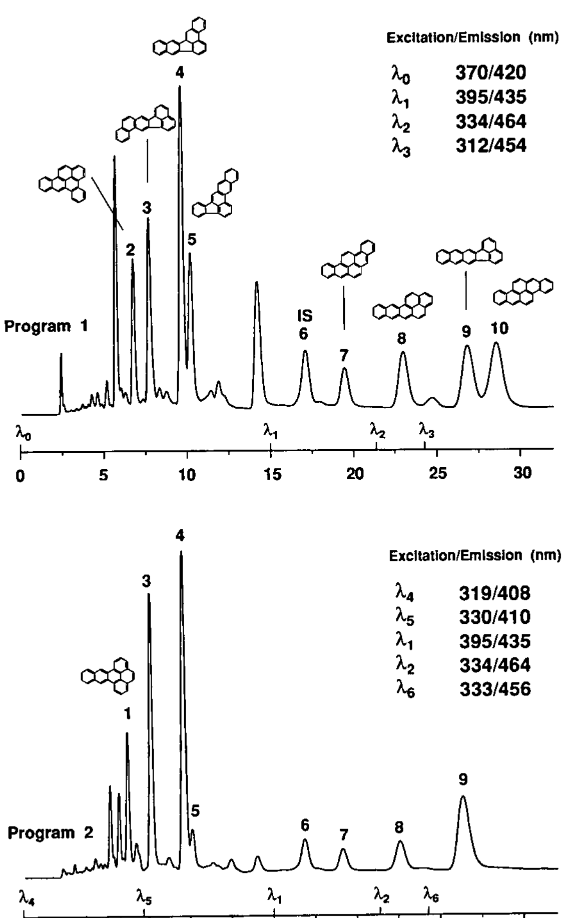


Fig. 13. RP-LC analysis of 302 molecular mass fraction isolated from coal tar extract (SRM 1597). Peak identifications: 1 = naphtho[2,3-*e*]pyrene, 2 = dibenzo[*a,e*]pyrene, 3 = naphtho[1,2-*k*]fluoranthene, 4 = dibenzo[*b,k*]fluoranthene, 5 = naphtho[2,3-*b*]fluoranthene, 6 IS (internal standard) = [$^2H_{14}$]dibenzo[*a,i*]pyrene, 7 = dibenzo[*a,i*]pyrene, 8 = naphtho[2,3-*a*]pyrene, 9 = naphtho-[2,3-*k*]fluoranthene, 10 = dibenzo[*a,h*]pyrene. Chromatographic conditions: Vydac 201TP column ($\alpha_{TBN/BaP}$ = 0.46); mobile phase isocratic at 100% acetonitrile at 1.5 ml/min; column temperature of 29°C (from ref. 29).

TABLE 4

NIST SRMs FOR THE DETERMINATION OF PAHs

PANH = polycyclic aromatic nitrogen heterocycles; PAQ = polycyclic aromatic quinones; PASH = polycyclic aromatic sulphur heterocycles.

SRM No.	Title	Date issued	Certified constituents	Non certified constituents	Literature references
Performance standard					
869	Column selectivity test mixture for liquid chromatography (PAHs)	1990			18, 21
Calibration solutions					
1491	Aromatic hydrocarbons in hexane–toluene	1989	PAHs (23)	PAHs (1)	
1644	Generator columns for PAHs	1981	PAHs (3)		50,51
1647c	Priority pollutant PAHs (in acetonitrile)	1993	PAHs (16)		
2260	Aromatic hydrocarbons in toluene (nominal concentration 60 μg/ml)	1991	PAHs (23)	PAHs (1)	
Natural matrix materials					
1580	Organics in shale oil	1980	PAHs (5); phenols (3) PANH (1)	Phenols (6) PANH (1)	36, 42, 43
1582	Petroleum crude oil	1984	PAHs (5); PASH (1)	PAHs (5) Phenols (2); PANH (1)	36, 43
1597	Complex mixture of PAHs from coal tar	1987	PAHs (12)	PAHs/PACs (18)	37
1648	Urban particulate matter	1978	Trace elements (9)	Trace elements (25) PAHs (13)	45
1649	Urban dust/organics	1982	PAHs (5)	PAHs (9)	40, 45
1650	Diesel particulate material	1985	PAHs (5); Nitro-PAHs (1)	PAHs (6); Nitro-PAHs (3); PAQ (1)	43
1939	Polychlorinated biphenyls (congeners) in river sediment	1990	PCBs (3)	PCBs (14); pesticides (5); PAHs (5)	49
1941	Organics in marine sediment	1989	PAHs (11)	PAHs (24); pesticides (7); PCBs (15); trace elements (32)	39
1974	Organics in mussel tissue (*Mytilus edulis*)	1990	PAHs (9)	PAHs (19); pesticides (9); PCBs (13); trace elements (36)	40
1975	Diesel particulate extract (in preparation)	1993	PAHs; nitro-PAHs		

4. SRMs FOR THE DETERMINATION OF PAHs

To accurately identify and quantify individual PAHs in complex environmental samples, the analyst must use analytical procedures that have been validated as to their accuracy. To assist in validating the accuracy of analytical methods for the determination of PAHs, NIST has developed a number of PAH related SRMs [46–49]. Reference materials are used primarily for the following purposes: (i) to calibrate the measurement system, (ii) to validate the reliability and precision of a new analytical method, and (iii) to provide quality control of routine analyses by analyzing the SRM at appropriate, regular time intervals. The NIST SRMs for PAH measurements are summarized in Table 4. These SRMs represent two different levels of analytical difficulty: (i) simple calibration solutions containing a number of PAH analytes and (ii) natural matrix materials. A third category, performance standards such as SRM 869, has been described above. The calibration solutions are useful for several purposes including: (i) calibration of chromatographic instrumentation for retention times and detector response factors for quantitation, (ii) spiking or fortifying samples, (iii) analyte recovery studies, and (iv) determining method response factors. The natural matrix materials, which are similar to the actual

environmental samples analyzed both in analyte concentrations and potential matrix interferences, can be used to validate the complete analytical procedure including extraction, isolation/cleanup procedures, and the final chromatographic separation and quantification. Thus, the natural matrix SRMs are generally more suitable for use in the validation of new analytical procedures and for routine quality control purposes.

The most popular of the organic environmental SRMs is SRM 1647, an acetonitrile solution of the 16 PAHs on the EPA's priority pollutant list (see Fig. 1). This SRM was prepared at the request of EPA in support of EPA Method 610, which specifies the use of RP-LC with fluorescence detection for the determination of PAHs [6]. SRM 1647 has found widespread use as a calibration solution to determine retention times and detector response factors in LC. Because of the popularity of SRM 1647, it has been reissued three times since first issued in 1981 and is now available as SRM 1647c. Since SRM 1647 is used primarily for calibration of RP-LC instrumentation and spiking of aqueous-based matrices (*i.e.*, because of the acetonitrile solvent), two similar solutions (SRMs 1491 and 2260) have been prepared in hexane and/or toluene to provide a solvent more compatible with GC and normal-phase LC analyses and for spiking into non-aqueous matrices. In addition to the 16 PAHs included in SRM 1647, SRMs 1491 and 2260 contain eight additional PAH analytes, which were included in these SRMs specifically to meet the needs of a national marine pollution monitoring program in the U.S. SRMs 1491 and 2260 contain the same 24 analytes but at concentrations that differ by approximately a factor of 10.

Since 1980, seven natural matrix SRMs have been issued with certified concentrations of PAHs and other polycyclic aromatic compounds (PACs): SRMs 1580, 1582, 1597, 1649, 1650, 1941, and 1974. SRM 1648, Urban Particulate Matter, was issued in 1978 and certified for inorganic constituents; however, data have been reported in the literature for PAH concentrations in this material [45]. Summaries of the concentrations of PAHs in these SRMs have been published [46–49]. These SRMs represent several matrix types, relative PAH concentrations, and sources of the PAH (*i.e.*, petrogenic or pyrolytic). SRMs 1580 and 1582 are representative of oil matrices with petrogenic PAHs (*i.e.*, formed from low-temperature processes) which have high levels of alkyl-substituted PAHs relative to the unsubstituted PAHs. These two materials have been described in more detail elsewhere [37,42]. SRMs 1648 and 1649 are two air particulate samples that were collected in the mid-1970's in St. Louis MO, USA and Washington, DC, USA, respectively; the PAHs mixtures on these materials are representative of pyrolytic sources. An extensive characterization and comparison of the PAH content of these two air particulate SRMs have been reported in the literature [45]. The diesel particulate sample (SRM 1650) is representative of heavy duty diesel emissions in the early 1980's. SRM 1597, which is a complex natural pyrolytic mixture of PAHs from a coke oven tar, has the most extensive quantitative characterization for PAHs.

The most recent SRM matrices for PAH measurements are sediment and mussel tissue. SRM 1941 is a marine sediment that was collected in the Baltimore Harbor (MD, USA) with PAH concentrations of 500–1300 ng/g [39]. SRM 1939, which is certified for polychlorinated biphenyl (PCB) congeners, is a river sediment collected from the Hudson River (NY, USA) and is representative of sediment with high levels of PCB congeners and chlorinated pesticides (100–7000 ng/g and 60–550 ng/g, respectively), but low levels of PAHs (50–200 ng/g) [48]. To meet the need for a natural matrix marine tissue reference material for organic contaminants, NIST issued SRM 1974, "Organics in Mussel Tissue (*Mytilus edulis*)" which was prepared from mussels collected in Boston Harbor (MA, USA) [40]. The mussel tissue was cryogenically homogenized and the SRM is provided as a frozen powder-like homogenate, thereby providing a matrix similar to the sample matrices typically encountered in marine tissue analyses. The PAH concentrations of these marine matrix SRMs have been summarized recently [47,48].

The natural matrix SRMs in Table 4 are also useful for methods development for PAHs that have not been certified or measured at NIST. In this instance SRMs are homogeneous natural environmental matrices that are readily available to other laboratories for comparison of analytical results. As laboratories involved in environmental pollution monatoring expand the number of PAHs measured due to emphasis on toxicity/mutagenicity, pollution

TABLE 5

SUMMARY OF ANALYTICAL RESULTS (ng/g DRY WEIGHT) FOR THE DETERMINATION OF PAHs IN SRM 1974, ORGANICS IN MUSSEL TISSUE (*Mytilus edulis*)

Compound	LC–FL[a]	GC–MS[a]	Certified[b]
Phenanthrene	44.6 (2.7)	45.3 (7.3)	45 ± 11
Anthracene	5.97 (0.52)	6.14 (0.72)	6.1 ± 1.7
Fluoranthene	289 (10)	255 (21)	272 ± 47
Pyrene	294 (10)	259 (12)	276 ± 30
Perylene	8.56 (0.35)	8.5 (1.7)	8.5 ± 2.4
Benzo[*b*]fluoranthene	55.9 (2.2)	48.7 (5.2)	52.3 ± 9.4
Benzo[*a*]pyrene	20.1 (2.3)	17.1 (2.2)	18.6 ± 3.8
Benzo[*ghi*]perylene	19.6 (1.4)	20.3 (2.3)	20.0 ± 2.3
Indeno[1,2,3-*cd*]pyrene	15.6 (1.4)	13.6 (1.4)	14.6 ± 2.7

[a] Uncertainties (values in parentheses) are one standard deviation of a single measurement treating all measurements as statistically independent and identically distributed; LC–FL results are from analyses of six samples and GC–MS results are from analyses of twelve samples.

[b] The certified values are equally weighted means of results from two analytical techniques. The uncertainty is obtained from a 95% prediction interval plus an allowance for systematic error between the methods used. In the absence of systematic error, the resulting uncertainty limits will cover the concentration of approximately 95% of samples of this SRM having a minimum sample size of 15 g (wet weight).

source identification, etc., publishd results of analyses of these SRMs by other laboratories will provide a valuable database for comparison within the scientific community.

4.1. Comparison of LC vs. GC–MS

At NIST the certification of environmental matrix SRMs is based on the use of two or more "independent" analytical methods. The required independent analytical procedures include different extraction and cleanup/isolation procedures as well as separation and detection techniques. For the measurement of PAHs in all of the natural matrix materials in Table 4, RP-LC using fluorescence detection was used as one of the analytical techniques. The LC approaches described above for the determination of PAHs were developed primarily for use in the certification of these materials. GC–MS has been used as the second technique for the certification analyses of the majority of these SRMs. As a result of the certification measurements for these SRMs, we have several sets of data comparing LC–fluorescence and GC–MS for the determination of PAHs. In 1990 we reported an extensive comparison of LC–fluorescence and GC–MS measurements for PAHs in three natural matrix SRMs (SRMs 1580, 1582, and 1650) and in extracts of the air particulate SRM (1648) and diesel particulate SRM (1650) [43]. A similar comparison can be made for the results of the analyses of the two most recent natural matrix SRMs, the marine sediment and the mussel tissue, as shown in Tables 3 and 5, respectively. The LC–fluorescence and GC–MS results for SRM 1941 and 1974 were generally in good agreement. For the most recent SRM measurements (see Table 5), differences in the mean values for the two techniques were 1–4% for phenanthrene, anthracene, perylene, and benzo[*ghi*]perylene; 12% for fluoranthene and pyrene; and 13–15% for benzo[*a*]pyrene, benzo[*b*]fluoranthene and indeno[1,2,3-*cd*]pyrene.

5. CONCLUSIONS

RP-LC with fluorescence detection is an excellent analytical technique for the measurement of PAHs in environmental matrices. In using RP-LC the analysts must be aware of selectivity differences among C_{18} columns from various manufacturers and make effective use of these differences for a specific separation need. Results obtained by using

LC–fluorescence are comparable to those obtained from GC–MS; however, LC–fluorescence has the advantage of being able to measure some PAH isomers that can not be quantified easily by GC–MS. A number of environmental matrix SRMs are available for use in validating analytical procedures for the determination of PAHs.

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Analysis of polycyclic aromatic hydrocarbons with an ion-trap mass detector and comparison with other gas chromatographic and high-performance liquid chromatographic techniques

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ABSTRACT

Some polycyclic aromatic hydrocarbons with a wide range of molecular masses were analysed by high-resolution gas chromatography using an ion-trap mass detector and a flame ionization detector. The sensitivity limits and the possibility of automatic identification through library search were evaluated. The results were compared with those obtained using other analytical techniques: mass spectrometry with a quadrupole analyser and high-performance liquid chromatography with a diode-array UV detector and fluorimetry. The relative sensitivity and the minimum amounts detectable with the various techniques were determined.

INTRODUCTION

Because of the known and potential mutagenic and carcinogenic hazards of polycyclic aromatic hydrocarbons (PAHs), a class of substances now ubiquitous in the human environment [1] because of their presence in combustion products and industrial waste, these compounds require accurate identification and quantification in many environmental samples. Sophisticated extraction and separation procedures have been applied for this purpose [2–9].

The analysis of the purified extracts can be carried out with gas and liquid chromatographic methods and with different detection devices, and obviously the ideal technique should permit perfect resolution of all of the PAHs from interfering substances, a very low detection limit, reproducibility of the retention data in order to help in identification and constancy of response to permit easy quantification without the need for frequent use of reference standards. Some of these goals are often impossible to achieve simultaneously (resolution and analysis speed; high sensitivity and correct identification), and the analyst should therefore apply different techniques offering the best result in a particular field or select the method that has the best performance in each case.

The main problem with the determination of PAHs is the complexity of the environmental matrices and the presence along with the PAHs of many interfering substances that, having physical and chemical behaviour similar to that of PAH, cannot be completely removed by repeated extraction and purification procedures. The identification and quantitation of PAHs therefore require the use of

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methods that simultaneously give high resolution and response selectivity.

High-performance liquid chromatography (HPLC) is therefore associated with diode-array detection systems, which permit simultaneously the identification and quantitation of the compounds, or with fluorescence detectors [10–13].

High-resolution gas chromatography (HRGC) with capillary columns is used in combination with flame ionization detection (FID) and photoionization detection (PID), multimode ionization detection (MMID) and quadrupole analyser mass spectrometry (QUAD) [14–17].

Various combinations of these techniques have also been proposed [18,19]. The highest sensitivity is given by the HPLC–fluorescence (HPLC–FL) method, which permits the determination of PAH amounts in the picogram range. The reported detection limits for the quadrupole mass spectrometer operated in single-ion monitoring (SIM) mode range between 0.1 and 0.5 ng [7]. However, neither the HPLC–FL nor the HRGC–MS–SIM technique permits identification and, for maximum sensitivity in quantitation, should be applied to previously identified compounds in order to select the proper excitation and emission wavelength (HPLC–FL) or the characteristic ion mass free from interferences (HRGC–MS–SIM). Other techniques, having lower sensitivity, should therefore be used for identification purposes.

The use of the ion-trap mass detector permits the entire mass spectra to be obtained with a sensitivity greater than that of quadrupole/SIM and therefore the application of identification algorithms to very small sample amounts. Ion-trap detection (ITD) was therefore used as the detection system in HRGC with bonded-phase open tubular columns, in parallel with FID, and the sensitivities obtained were compared with those obtained by quadrupole MS and by HPLC with constant- or variable-wavelength UV detection and with fluorimetric detection.

Our application of these methods to many environmental samples showed that the HRGC–ITD technique offers a sensitivity that for many compounds is higher than that obtained by HPLC–FL, with the further advantage of permitting the identification of the compounds by means of automatic library search. The simultaneous use of ITD and FID in many instances allows quantitation to be carried out without the need for standard samples of all of the detected PAHs.

EXPERIMENTAL

In order to investigate the behaviour of various detection systems over a wide range of molecular masses of PAHs, a mixture containing sixteen compounds included in the priority pollutants list of the US Environmental Protection Agency (EPA) was used as reference standard (Table I).

The GC–MS analyses were carried out using a Saturn II ion-trap spectrometer (Varian, Palo Alto, CA, USA) connected to a Model 3400 gas chromatograph also equipped with a flame ionization detector. The outlet of the column could be connected simultaneously to the two detectors, by means of a zero-volume "*y*" press-fit glass connector and deactivated capillary tubings. The length of the connecting tubes must be selected in order to act as a restrictor on the ITD side of the system, thus avoiding the backflushing of air and hydrogen from the base of the flame ionization detector to the

TABLE I

MOLECULAR MASS OF THE ANALYSED COMPOUNDS AND THEIR RETENTION TIMES (t_R) ON GC AND HPLC COLUMNS

For analysis parameters see Experimental section.

Compound	Molecular mass	t_R (min)	
		GC	HPLC
Naphthalene	128	13.51	8.24
Acenaphthylene	152	18.52	9.54
Acenaphthene	154	19.29	10.97
Fluorene	166	21.05	11.40
Phenanthrene	178	24.02	12.51
Anthracene	178	24.10	13.34
Fluoranthene	202	27.43	14.61
Pyrene	202	28.24	15.27
Benz[*a*]anthracene	228	32.13	17.92
Chrysene	228	32.21	18.25
Benzo[*b*]fluoranthene	252	35.43	20.65
Benzo[*k*]fluoranthene	252	35.48	21.43
Benzo[*a*]pyrene	252	36.53	22.21
Dibenz[*a,h*]anthracene	278	42.48	23.84
Benzo[*ghi*]perylene	276	44.03	24.72
Indeno[1,2,3-*cd*]pyrene	276	42.29	25.14

vacuum manifold of the ion-trap detector. The flow-rates to the two detectors should be adjusted in order to dispatch to each of them an amount of sample proportional to their sensitivity, thus permitting simultaneously identification by ITD and quantification through FID response (see below). For maximum sensitivity, all the sample must be sent to the ITD, by directly connecting the column to the vacuum system of the ion trap or by closing the FID side of the "*y*" arrangement. A DB5 capillary column was used (J&W Scientific, Folsom, CA, USA), 5% phenyl–95% methyl polysiloxane-bonded phase, 30 m × 0.32 mm I.D., film thickness 0.25 μm. The programmed temperature run used for the determination of sensitivity was: initial isotherm at 50°C for 5 min, programming rate 8°C/min up to 280°C. The injector (Varian 1075 split/splitless) was set at 250°C and the flame ionization detector at 300°C.

Other initial temperatures, programming rates and upper isotherm lengths were also tested. The conditions reported above were found to be suitable for routine analyses, as they allow a complete separation of all of the compounds in a reasonable time.

The acquisition parameters for ITD were: mass range 80–350 u, target 25 000, scan rate 1 s, acquisition time depending on the length of the programmed run, threshold one count, filament delay 240 s, mass defect 100 millimass/100 u, background mass 95 u.

Analyses were also made with the same column type on a Finnigan INCOS-50 quadrupole mass spectrometer connected to a Varian 3400 gas chromatograph, in order to compare the sensitivity and response ratio of ITD and quadrupole analysers.

HPLC analyses were carried out with a Varian Model 9095 liquid chromatograph equipped with Model 9065 diode-array UV detector and a Model 821 FP spectrofluorimeter. A Supelcosil LC-PAH column (Supelco, Bellefonte, PA, USA), 25 cm × 4.6 mm I.D., particle diameter 5 μm, was used. The mobile phase (acetonitrile–water) gradient was: 40% acetonitrile + 60% water for 2 min, increasing linearly up to 100% acetonitrile in 25 min; isocratic 100% acetonitrile up to 35 min. UV detection was performed both at the fixed wavelength of 254 nm and at various wavelengths corresponding to the maximum of the absorption peaks of the different compounds.

The spectrofluorimeter wavelength was programmed during the analysis: the initial values were $\lambda_{ex} = 280$ nm and $\lambda_{em} = 340$ nm; 14 min after the injection the parameters were changed to $\lambda_{ex} = 280$ nm and $\lambda_{em} = 410$ nm; after 25 min elapsed time the values were adjusted again to $\lambda_{ex} = 305$ nm and $\lambda_{em} = 500$ nm.

RESULTS AND DISCUSSION

Table I shows the retention times of the analysed compounds obtained with the temperature and solvent programmes described above during GC and HPLC analysis. The HPLC technique permitted both a better resolution and a shorter analysis time of the standard mixture used but, because in environmental samples the number of interfering compounds may be much greater than in our sample, the higher resolving power of the capillary GC technique is sometimes necessary to permit the analysis of authentic samples. The columns used and the conditions of analysis were not optimized to give the

TABLE II

SENSITIVITY (pg) OF ION TRAP DETECTION AND OF FLUORIMETRIC HPLC DETECTION

For ITD the minimum identifiable amount by means of library search is shown. Note. Fluorimeter settings: from naphthalene to anthracene: λ_{ex} 280 nm, λ_{em} 340 nm; from fluoranthene to benzo[*ghi*]perylene: λ_{ex} 280 nm, λ_{em} 410 nm; for indeno[1,2,3-*cd*]-pyrene: λ_{ex} 305 nm, λ_{em} 500 nm.

Compound	ITD	Fluorimetry
Naphthalene	20	60
Acenaphthylene	20	–
Acenaphthene	2	10
Fluorene	2	100
Phenanthrene	2	100
Anthracene	2	–
Fluoranthene	2	40
Pyrene	2	200
Benz[*a*]anthracene	20	20
Chrysene	20	–
Benzo[*b*]fluoranthene	20	20
Benzo[*k*]fluoranthene	20	20
Benzo[*a*]pyrene	20	20
Dibenz[*a,h*]anthracene	80	40
Benzo[*ghi*]perylene	40	40
Indeno[1,2,3-*cd*]pyrene	20	60

best resolution, but were selected only to give a separation good enough to allow the comparison of different detection systems. When more complex mixtures have to be resolved, other columns and analytical parameters should be used. The knowledge of the retention index of the compounds [20] can assist in the preidentification of many peaks. If different programming speeds have to be used to permit the best resolution to be obtained, computer prediction of the programmed temperature retention times by starting from two or three isothermal run permits tentative identification to be carried out in analysis conditions different from those used for the analysis of standard samples [21–24].

When interfering peaks not belonging to PAH compounds are present in the chromatogram obtained from the environmental samples, identification based on retention times is not adequate. Mass spectrometry with quadrupole detection operated in SIM mode offers a sensitivity similar to that of FID: minimum detectable amounts ranging from 0.1 to 0.5 ng depending on the molecular mass of the compound [7]. The amount necessary to obtain a full-scan spectrum of reasonable intensity is more than one order of magnitude greater, and therefore an amount of some nanograms should be injected for each compound on a HRGC–MS–QUAD system to obtain a spectrum good enough for identification [25–28].

In contrast, using ITD it is possible to obtain with full-scan spectrum a sensitivity higher than that obtained in MS–QUAD–SIM mode, and to apply therefore the automatic library search programmes available. Table II shows for ITD the minimum identifiable quantity, *i.e.*, the minimum injected quantity that produces correct library search identification within first five search hits. ITD also shows a fair linearity of about four orders of magnitude from the minimum identifiable quantity up to 2000 pg, with correlation values higher than 0.997 over a five-point calibration for all of the compounds in the PAH mixture [29–31]. The detection limits of the fluorimetric detector, experimentally measured using the conditions described above, are also reported and show that ITD permits identification at concentrations smaller than those obtained with the HPLC–FL method.

The sensitivity reported for the HPLC–FL method is not the highest possible with this technique, because, as shown in the Experimental section, only three combinations of excitation (λ_{ex}) and emission (λ_{em}) wavelengths were used during the elution of the compounds. It is possible to select for every compound the λ_{ex} and λ_{em} values yielding the highest sensitivity [32,33], by programming the fluorimetric detector and the signal integrator.

In order to obtain accurate results, however, the retention times must be perfectly reproducible and the peaks separated by a baseline segment long enough to permit all the automatic steps for wavelength change to be carried out.

Deactivation of integration, change of λ_{ex} and λ_{em}, equilibration of the signal at the new baseline level, activation of the integrator and monitoring of the new baseline value require two or three times the base width of the peaks, and therefore a resolution on 2.5 or greater is preferable.

This resolution value can only be obtained when few PAHs are analysed, and in the absence of interfering compounds. When the analysis of samples extracted from complex matrices is carried out, it is only possible to change the detector parameters a few times during the elution of the chromatogram. Various PAHs are therefore detected with the same wavelength combination: the first change of λ_{em} from 340 to 410 nm is made after the elution of anthracene; the second (λ_{ex} from 280 to 305 nm, λ_{em} from 410 to 500 nm) before the elution of indeno-[1,2,3-*cd*]pyrene.

This wavelength programme offers a suitable compromise for the analysis of real samples and was therefore used for the determination of the sensitivities reported in Table II.

UV detection yields a sensitivity lower than that of fluorimetry but, by using a diode-array detector, permits the spectrum of every peak to be recorded for identification purposes and, by selecting the wavelength of maximum absorbance, an increase in sensitivity.

Table III shows the minimum concentration detected using non-concentrated samples and different HPLC detection systems: constant-wavelength, variable-wavelength and fluorimetry. The minimum concentration required by the EPA 610 method [34] after concentration of the water sample at a ratio of 1:1000 is also shown. If the same concentration procedure is applied before HPLC analysis, the values in the table (mg/l) should be converted into

TABLE III

MINIMUM DETECTABLE CONCENTRATION USING NON-CONCENTRATED SAMPLES AND VARIOUS HPLC DETECTION SYSTEMS

The sensitivity of the EPA 610 method is based on a water sample after concentration at a ratio of 1:1000.

Compound	Constant wavelength (254 nm) (mg/l)	Variable wavelength		Fluorimetry (mg/l)	EPA 610 method (μg/l)
		mg/l	nm		
Naphthalene	0.5	0.025	215	0.003	1.8
Acenaphthylene	0.5	0.05	224	–	2.3
Acenaphthene	2.0	0.025	224	0.0005	1.8
Fluorene	0.1	0.1	258	0.001	0.21
Phenanthrene	0.05	0.05	249	0.005	0.64
Anthracene	0.025	0.025	249	–	0.66
Fluoranthene	0.2	0.05	234	0.002	0.21
Pyrene	0.2	0.05	239	0.01	0.27
Benz[*a*]anthracene	0.1	0.05	287	0.001	0.013
Chrysene	0.05	0.05	263	–	0.15
Benzo[*b*]fluoranthene	0.1	0.1	254	0.001	0.018
Benzo[*k*]fluoranthene	0.2	0.1	234–239	0.0005	0.017
Benzo[*a*]pyrene	0.5	0.5	254	0.001	0.023
Dibenz[*a,h*]anthracene	0.4	0.05	297	0.002	0.030
Benzo[*ghi*]perylene	0.2	0.2	254	0.002	0.076
Indeno[1,2,3-*cd*]pyrene	0.1	0.1	249	0.003	0.043

the μg/l range. Neither constant-wavelength nor variable-wavelength UV detection can achieve the sensitivity required by the EPA 610 method for some of the late-eluting compounds. Fluorimetric detection, on the other hand, allows the required sensitivity to be obtained with a smaller concentration ratio, less than 100 for the heaviest compounds and about ten-fold for the highest. This method can therefore be used for the analysis of environmental samples when the complexity of the matrix is not so high as to give too many interfering peaks. If the chromatogram is very complex, the HPLC method is not selective enough to permit the separation of all the compounds, and was therefore used as a screening method to measure the overall concentration of PAH and as a preparative and prefractionating method to reduce the complexity of the mixture by separating the components into different HPLC fractions to submit to further HRGC analysis [6,35], and to identify and quantitate by ITD.

As seen above, the INCOS presearch and identification software of the Varian Saturn ion-trap mass spectrometer permits the identification to be successfully carried out with the amounts of PAHs shown in Table II, by comparison with library data. Authentic standard samples of all of the PAHs, often difficult to obtain with sufficient purity, are therefore not necessary for identification. However, Table IV shows that the relative response of ITD to the various PAHs (calibration carried out with the molecular masses listed in Table I) varies within one order of magnitude, and pure standard samples should be necessary for quantitation. Quadrupole mass analysis shows a smaller dependence of response on the compound, but, as seen above, its sensitivity is lower than that of ITD.

If the amount of each PAH in the sample, if necessary after suitable concentration procedures, is high enough to be detected with FID, the near-identical response of this detector to all the compounds having the same general structure allows the use of authentic standard samples of all the PAHs to be avoided [14,17,36].

TABLE IV

WEIGHT RESPONSE FACTORS (RELATIVE TO FLUORANTHENE) OF PAHs TO VARIOUS DETECTION SYSTEMS IN GAS CHROMATOGRAPHY

Compound	ITD	Quadrupole	FID
Naphthalene	1.44	–	1.01
Acenaphthylene	1.61	–	1.01
Acenaphthene	1.09	–	1.04
Fluorene	1.09	0.86	1.04
Phenanthrene	1.59	0.92	1.00
Anthracene	1.52	0.88	0.99
Fluoranthene	1.00	1.00	1.00
Pyrene	1.29	1.07	0.99
Benz[*a*]anthracene	0.66	1.24	0.99
Chrysene	0.70	–	1.01
Benzo[*b*]fluoranthene	0.18	1.33	0.99
Benzo[*k*]fluoranthene	0.14	1.43	1.00
Benzo[*a*]pyrene	0.12	1.32	1.03
Dibenz[*a,h*]anthracene	0.11	–	0.98
Benzo[*ghi*]perylene	0.14	1.36	1.02
Indeno[1,2,3-*cd*]pyrene	0.17	–	1.01

If the flame ionization detector is mounted in parallel with the ion-trap detector at the end of the capillary column by using flow restriction on the ion-trap detector side in order to split the larger amount of the sample to the flame ionization detector, identification through library search can then carried out on the small (but large enough to give a suitable spectrum) amount of sample going to the ion-trap detector, while quantitative analysis is achieved by using the simultaneous FID chromatogram.

Owing to the similar response of FID to the various PAHs (Table IV), the use of non-corrected areas permits an accuracy of $\pm 5\%$ to be obtained, good enough for many applications in environmental analysis, where the main sources of uncertainty are sample pick-up and conservation, extraction and enrichment procedures, different recovery of various compounds, interferences, etc.

CONCLUSIONS

The analysis carried out under standard and reproducible chromatographic conditions on various PAHs permitted the sensitivity of the various detection systems used in HRGC and HPLC to be compared. Instruments of the latest generation commercially available were used, and therefore the relative sensitivities were determined on a common basis, partially confirming previously literature data, and, in some instances, showing a substantial increase in sensitivity.

Using an ion-trap detector sensitivities higher than those previously obtained by MS–QUAD–SIM and HPLC–FL methods were achieved and, at the same time, the compounds could be identified from their mass spectra by using standard library search programmes.

The uniform sensitivity of FID to PAHs with a wide range of molecular masses allows the quantitation to be carried out in routine analyses without frequent use of authentic samples of all the detected compounds, if the amount of sample is great enough to permit the use of this detection system.

In the same concentration range, the connection of ITD and FID in a parallel mode in order to obtain simultaneous chromatograms permits qualitative identification and quantitative determination to be carried out at the same time.

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Fully automated multi-residue method for trace level monitoring of polar pesticides by liquid chromatography

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ABSTRACT

A fully automated liquid chromatographic method using on-line trace enrichment, gradient elution and diode-array detection for the trace level determination of polar pesticides in surface water is described. The automated system uses specially developed software in the form of "user macros", allowing the on-line control of both the automated cartridge exchange unit for sample preparation and the liquid chromatograph with diode-array detector by means of the Pascal Workstation computer of that liquid chromatographic system. The collected data are automatedly evaluated, *i.e.*, pollutants present in the sample at a concentration level above an input treshold level are identified/determined and a report is printed. Parameters such as the sampling interval of the spectra, temperature of the analytical column compartment, wavelength/bandwidth ratios and data handling were optimized. The validation results for 27 pesticides are presented. At an analyte concentration of 1 μg/l the relative standard deviations of the retention times and peak areas in different types of water are in the range 0.2–1.5% and 1–15%, respectively. All calibration graphs are linear in the range 0.1–7 μg/l.

INTRODUCTION

In spite of their adverse effects on the environment, the total amount of pesticides used for agricultural and industrial purposes is still increasing. As a result, pesticides are nowadays present in all compartments of the environment, which explains the great interest in early-warning and monitoring systems for these compounds. The monitoring of these analytes causes substantial analytical problems since, because of recent legislation in many countries, sensitive methods are required [1,2]. For example, in surface water samples, organic micropollutants typically have to be determined at the low- to sub-μg/l level [3,4].

Analysis at these levels requires a concentration step. Solid-phase isolation has proved to be a good alternative to liquid–liquid extractions because of its simplicity, robustness and potential for automation [5–9]. In addition, most modern pesticides are fairly soluble in water and so are less amenable to extraction with organic solvents. As a result, liquid chromatography (LC) in combination with on-line trace-enrichment techniques, using small cartridges [5–7] or membrane extraction discs [7], are frequently preferred over gas chromatographic techniques. Detection is performed by diode-array (DA) detection, providing simultaneously structural information and quantitative data.

The development of a preliminary automated LC system for polar pesticides in various types of water was presented in a previous paper [7]. The aim of this extended study was to investigate a number of parameters to allow the identification and determination of a large group of polar pesticides at sub-μg/l levels in surface water. From the automation point of view the primary purpose was to develop a robust, user-friendly system that is easy to operate. This resulted in a fully automated LC method called SAMOS (System for Automated Measurement of Organic Micropollutants in Surface Water).

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EXPERIMENTAL

Solvents and chemicals

Stainless-steel analytical columns (150 mm × 4.6 mm I.D.) were laboratory packed with 5-μm C_{18} RoSil (RSL, Eke, Belgium) particles using acetonitrile as the slurry liquid. Further, a 150 mm × 4.6 mm I.D. Supelcosil LC-18-DB (Supelco, Bellefonte, PA, USA) column packed with C_{18} DB material (3-μm particles) and a 250 mm × 4.6 mm I.D. Supelcosil LC-18-DB column packed with C_{18} DB material (5-μm particles) were used. HPLC gradient-grade acetonitrile, methanol and water were obtained from J. T. Baker (Deventer, Netherlands). Ultra-pure water was prepared by ultrafiltration with a Milli-Q system (Millipore, Bedford, MA, USA). Disodium hydrogenphosphate, sodium dihydrogenphosphate and orthophosphoric acid (85%) were obtained from J. T. Baker. The various pesticides were supplied by Riedel-de Haën (Seelze, Germany), Promochem (Wesel, Germany), Dr. S. Ehrenstorfer (Augsburg, Germany) and Hoechst (Frankfurt, Germany). As stated by the manufacturers, they were all at least 95% pure. The pesticides used in the study are listed in Table I.

Surface water samples were collected at Lobith and at the 335-km point (River Rhine, Netherlands and Germany, respectively), Eysden and Keizersveer (River Meuse, Netherlands), Toulouse (River Garonne, France), Westminster Bridge, London (River Thames, UK) and the Rivers Uster and Mönchaltdorf (Switzerland).

Instrumentation

The LC analyses were performed with an HP 1090 liquid chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a PV5 ternary solvent-delivery system (SDS), an injection

TABLE I

PESTICIDES [a] USED AND THEIR WAVELENGTHS OF MAXIMUM ABSORBANCE

No.	Name	λ_{max} (nm)
1	Aniline	203
2	Carbendazim	201
3	1-(3-Chloro-4-hydroxyphenyl)-3,3-dimethylurea (CHPDMU)	207
4	Metamitron	201
5	Chloridazon	229
6	Dimethoate	201
7	Monomethyl metoxuron	207
8	Aldicarb	201
9	Bromacil	211
10	Cyanazine	221
11	2-Nitrophenol	213
12	Chlorotoluron	211
13	Atrazine	223
14	Diuron	213
15	Metobromuron	203
16	Metazachlor	201
17	Propazine	223
18	Warfarin	205
19	3,3'-Dichlorobenzidine (DCB)	213
20	Barban	207
21	Alachlor	201
22	Nitralin	229
23	Dinoseb	270
24	Dinoterb	271
25	Phoxim	281
26	Nitrofen	201
27	Trifluralin	207

[a] For structures with *Chemical Abstracts* Registry numbers and molar absorptivities, see refs. 5 and 8, respectively.

valve with a 25-μl loop and an HP 1040 DA detector equipped with a 10-mm flow cell. For single-wavelength monitoring the DA detector was set at 210 nm with a bandwidth of 10 nm. During recording of the absorbance spectra the optical slit of diode width was 4 nm and a sampling interval for recording of the spectra in the All Spectra mode of 1280 ms was used with a peak width of 0.2 min. Absorbance spectra were recorded from 200 to 400 nm. Data from the DA detector were collected and evaluated by the Pascal Workstation (PAWS) computer using Chemstation software.

Trace enrichment was carried out on 10 mm × 2.0 mm I.D. disposable trace-enrichment cartridges of the Prospekt (Spark Holland, Emmen, Netherlands) automated sample preparation unit. The commercially available cartridges (Spark Holland) were packed with PLRP-S, a styrene–divinylbenzene copolymer (Polymer Labs., Church Stretton, UK) with 20-μm particles and a 100 Å pore size, and used only once. Conditioning of the cartridges was performed with a solvent-delivery unit (SDU) from Spark Holland. Surface water was sampled with a Model 300 preparative pump (Gynkotek, Munich, Germany).

Although the SDU itself can be controlled by the Prospekt microprocessor, the Prospekt and the HP 1090 were connected through an auxiliary electronic connection (Fig. 1). The Prospekt and the Model 300 Gynkotek pump were also connected by using another auxiliary electronical connection of the Prospekt and the flow control input of the pump.

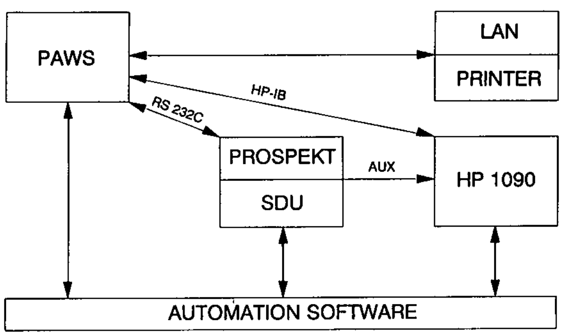

Fig. 1. Schematic diagram of the fully automated SAMOS system. The RS 232C and HP-IB are the communication interfaces and AUX is an auxiliary to connect the Prospekt and the SDU with the HP 1090; PAWS = Pascal Workstation; LAN = local area network. For further explanation, see text.

The PAWS computer controlled the HP 1090 by using both the standard HP-IB communication interface and the Chemstation software. On-line control of the Prospekt and SDU from the PAWS computer was performed via an RS 232C communication interface using additional software in the form of a laboratory-made "user macro" called SAMOSMAC.

Procedures

Fresh stock standard solutions (0.2 mg/ml) of the pesticides in methanol were prepared every 6 weeks. The working standard solutions of the test compounds were prepared by diluting the stock standard solutions daily with Milli-Q-purified, drinking or surface water to a concentration of 0.01–7 μg/l. All stock standard solutions were stored at 4°C in the dark. The stability of the analytes in the stock and working standard solutions, tested in a previous study [5], was sufficient to allow accurate measurements.

The gradient conditions were as follows: solvent B, acetonitrile–0.01 *M* phosphate buffer (pH 3) (90:10, v/v) and solvent A, acetonitrile–0.01 *M* phosphate buffer (pH 3) (5:95, v/v). The gradient profile was 100% A at 0 min, linearly to 100% B in 55 min and subsequently linearly to 100% A in 5 min. The flow-rate was 1.0 ml/min. Prior to use, all eluents were degassed with helium (15 min).

The trace-enrichment cartridges were conditioned with 2 ml of methanol (2 ml/min) followed by 2 ml of 0.001 *M* perchloric acid (1 ml/min), as an additional cleaning step, using the SDU. Thereafter, 100 ml of sample were enriched at a flow-rate of 4 ml/min [7]. Desorption was performed by coupling the cartridge on-line with the analytical column and starting the gradient (Fig. 2). For each analysis a new cartridge was used. In Table II the sample preparation programmes (SPP) are given. Before starting the first analysis, the PAWS computer was switched on, which resulted in a special soft-key set (on the screen) for handling of the macro SAMOSMAC. At a pre-programmed time, the deuterium lamp of the DA detector was switched on and the flow-rate of mobile phase B gradually increased from 0.05 to 1 ml/min in 3 min. Then the programmed sequence of analyses consisting of conditioning of the cartridge, enrichment of 100 ml of sample, a 60-min LC run, qualitative and quanti-

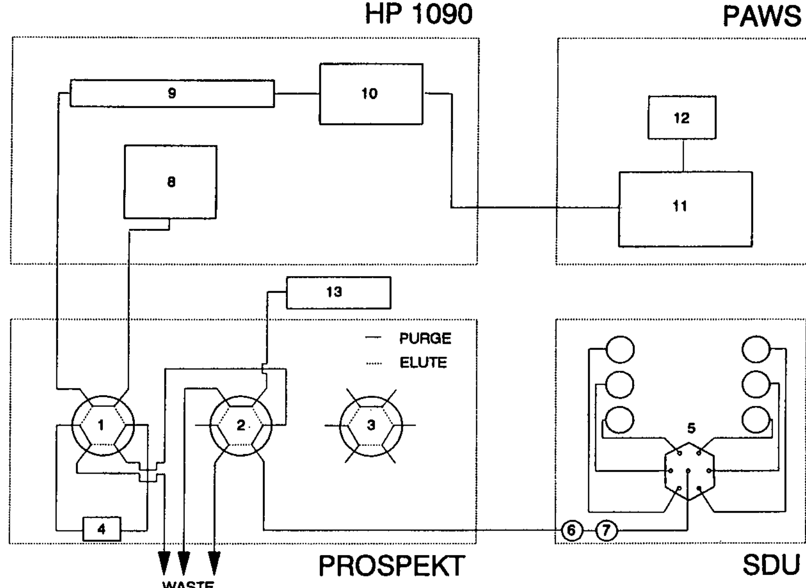

Fig. 2. Automated on-line trace enrichment–LC system for water samples. 1, 2, 3 = High-pressure valves of the Prospekt; 4 = trace-enrichment cartridge of the Prospekt; 5 = solenoid valve; 6 = pulse damper; 7 = purge pump; 8 = solvent delivery system of HP 1090 liquid chromatograph; 9 = analytical column; 10 = diode-array detector (DA); 11 = Pascal Workstation (PAWS) computer; 12 = printer; 13 = preparative pump for sample loading; SDU, solvent delivery unit.

tative evaluation of data obtained and printing of the report started. The system was equilibrated for 31 min during the first enrichment procedure and, in the following enrichment procedures, for 5 min between two successive analyses. No additional time was needed for computing of the data. At the end of a particular sequence of analyses the deuterium lamp was automatically switched off and the flow-rate was decreased gradually from 1.0 to 0.05 ml/min in 3 min.

RESULTS AND DISCUSSION

Compared with previous studies [7], the system configuration was changed by replacing the 1000 S DA detector and the Model 400 gradient systems (Applied Biosystems, Ramsey, NJ, USA) by the HP 1090 system because of the latter's advanced automation potential. If it is preferred to use another configuration, several of the parameters discussed below will have to be optimized again. The general strategy, however, will remain the same.

Sampling interval of spectra and wavelength/bandwidth ratio

In order to obtain sufficient data points for post-run calculations of the chromatograms (*e.g.*, subtraction, extraction of signals at different wavelength/bandwidth ratios), the spectra were taken continuously depending on the settings of the sampling interval. Therefore, the All Spectra option was used in all further experiments. The optical slit of diode width was set at 4 nm. With the average peak width of the test compounds of 0.1–0.4 min, it was possible to take 5–15 spectra of each eluting compound.

A significant increase in the noise level is expected to result from using relatively small sampling intervals. The results in Fig. 3 indicate that the optimum sampling interval should be at least 300–400 ms. In all further experiments a value of 1280 ms was chosen because a smaller sampling interval will require too much computer memory while a larger sampling interval will result in a loss of spectral resolution. The optical slit of diode width was set at 4 nm.

TABLE II

SAMPLE PREPARATION PROGRAMMES

Time (min:s)	Solvent	Valve 1	Valve 2	Flow-rate (ml/min)	Aux2	Aux6	Other
SPP of automated system using SDU for sampling[a]							
00:00		Purge	Elute				
00:01	01			2.0			CHC
02:00			Purge				
03:00	02		Elute				
05:00			Purge	1.0			
07:00	03		Elute	4.0			
08:00			Purge				
33:00				0.0			
33:30	04	Elute	Purge			On	
33:31	01			2.0			
34:31				0.0		Off	
65:00							End
SPP of automated system using additional preparative pump for sampling[a]							
00:00		Purge	Elute				
00:01	01			2.0			CHC
02:00	03		Purge	4.0	On		
03:00	02		Elute	2.0	Off		
05:00			Purge	1.0			
07:00	03		Elute	4.0	On		
32:00				0.0	Off		
32:30	04	Elute	Purge			On	
32:31	01			2.0			
33:31				0.0		Off	
65:00							End

[a] 01, methanol; 02, 0.001 *M* perchloric acid; 03, sample; 04, eluent; valves 1 and 2, positions according to Fig. 1; Aux 6, connection of Prospekt with HP 1090, positions on/off; Aux 2, connection of Prospekt with LC pump, positions on/of; CHC, change cartridge; End, end of programme.

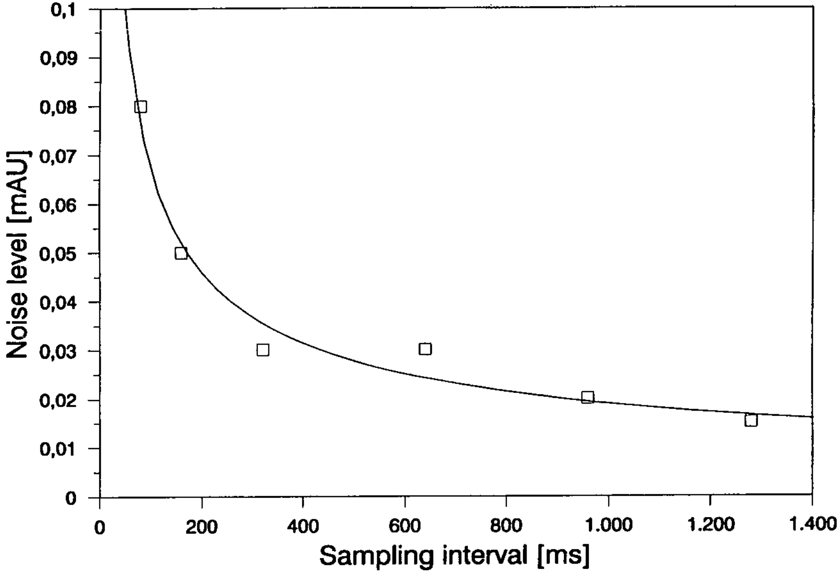

Fig. 3. Noise level as a function of the sampling interval. Optical slit of the diode width, 4 nm; detection wavelength, 210 nm; bandwidth, 10 nm.

In order to obtain a robust method, single-wavelength detection should be performed at an optimised bandwidth in order, to monitor simultaneously a large group of pesticides with high sensitivity. Particularly at the μg/l level, this is a critical choice. Most of the analytes have their maximum absorbance in the range of 200–210 nm (Table I). After testing a number of wavelengths in this range, using bandwidths between 5 and 20 nm, a compromise between the highest and the most selective responses was found with a wavelength of 210 nm and a bandwidth of 10 nm. These values were used in all further experiments.

Analytical column

In order to obtain the optimum sensitivity and selectivity, the choice of the analytical column that has to be used, in combination with a PLRP-S enrichment cartridge, is very important [7]. Two C_{18} columns, one packed with 3-μm particles (150 mm × 4.6 mm I.D.) and one packed with 5-μm particles (250 mm × 4.6 mm I.D.) were tested. In spite of the excellent performance of both columns, the longer C_{18} column was used for all further studies because of its lower back-pressure.

The test mixture of 27 pesticides was analysed at ambient temperature and 40, 45 and 50°C. The best results, with respect to peak shapes and back-pressure, were obtained at 45°C.

Validation of the system

The system was validated for a group of 27 pesticides (Table I). The repeatabilities of the retention times and peak areas were measured using River Rhine water (Table III). The relative standard deviations (R.S.D.s) of the retention times were in the range 0.2–1.5% and those of the peak areas were 1–15%. High R.S.D.s were observed only for analytes eluting between 12 and 25 min. This is partly due to matrix interferences and partly to breakthrough of the more basic compounds on the PLRP-S cartridge [7].

The correlation coefficients of nearly all of the calibration graphs for River Rhine water were over 0.99. The only exceptions were warfarin and phoxim, with correlation coefficients of 0.97. This is caused by small interferent that nearly co-elute with these analytes.

In Table III the identification limits of the individual pesticides in HPLC-grade, drinking and River Rhine waters are also given. The identification limit is the lowest concentration allowing identification of the compound according to its spectrum (see *Data handling*). Of the 27 pesticides, all but two possess identification limits $\leqslant$1.0 μg/l in surface water and $\leqslant$0.1 μg/l in HPLC-grade water.

Robustness of the system

The performance of the system was tested in two laboratories, one during a 5- and the other a 7-month period. Within these periods the systems were continuously switched on for 6–8 analyses per day and about once every week a sequence of 14–16 analyses was performed unattended overnight. This means that in total over 1000 samples were analysed, and during this time only two types of problems were encountered: (i) with each system the deuterium lamp had to be exchanged once, and (ii) when using non-filtered surface water for trace enrichment the SDU became clogged twice. Therefore, for the handling of unfiltered samples the SDU was replaced with a preparative pump (Fig. 2). The analytical columns did not deteriorate during the whole test period. The results demonstrated that the present (SAMOS) system is suitable for long-term unattended operation using non-buffered (*i.e.* pH 6–9) and non-filtered surface water samples.

Flexibility of the system

The flexibility of the system was tested for the 27 test compounds extended with an additional number of triazines, phenylurea herbicides and nitrophenols. The analytes were measured without changing any of the preset analytical parameters (see previous sections). In each trial, first a 25-μl loop injection was performed, followed by the enrichment of 100 ml of spiked Milli-Q-purified water. The results of, for example, the group of thirteen phenylurea herbicides, six of which were present in the original set of 27 test compounds and seven additional pesticides, show (Fig. 4) that all of the added analytes can be detected and identified at a level of 0.1–1 μg/l without any modifications to the system. The losses observed for the early-eluting compounds (3 and 44) are due to partial breakthrough [5].

Similar results were obtained for the triazines and nitrophenols. So far, in our department [5–7], about

TABLE III

REPEATABILITY OF RETENTION TIMES AND PEAK AREAS FOR 27 PESTICIDES IN RIVER RHINE WATER, IDENTIFICATION LIMITS IN HPLC-GRADE WATER, DRINKING WATER AND RIVER RHINE WATER AND LINEAR REGRESSION COEFFICIENTS [a]

No.	Name	RT_{RW} (R.S.D.)	PA_{RW} (R.S.D.)	IL_{HP}	IL_{DW}	IL_{RW}	R^2
1	Aniline	–	–	1.0	1.0	2.5	0.999
2	Carbendazim	12.4 (1.5)	270 (15)	0.03	0.1	0.25	0.995
3	CHPDMU	14.3 (1.2)	64 (8)	0.05	0.1	1.0	0.999
4	Metamitron	15.4 (0.9)	228 (5)	0.05	0.1	0.25	0.997
5	Chloridazon	16.2 (1.3)	243 (4)	0.03	0.1	0.25	0.993
6	Dimethoate	17.0 (1.3)	87 (8)	0.1	1.0	1.0	0.996
7	Monomethyl metoxuron	19.0 (1.1)	574 (5)	0.03	0.1	0.25	0.998
8	Aldicarb	21.2 (0.9)	146 (9)	0.05	0.25	1.0	0.997
9	Bromacil	22.4 (0.7)	175 (4)	0.1	0.25	0.5	0.992
10	Cyanazine	23.7 (0.6)	401 (6)	0.03	0.1	0.25	0.999
11	2-Nitrophenol	24.8 (0.7)	70 (12)	0.25	1.0	2.0	0.990
12	Chlorotoluron	27.0 (0.5)	760 (1)	0.03	0.1	0.25	0.999
13	Atrazine	28.0 (0.4)	489 (1)	0.03	0.05	0.05	0.995
14	Diuron	28.8 (0.5)	502 (4)	0.03	0.05	0.05	0.992
15	Metobromuron	29.8 (0.5)	314 (3)	0.05	0.1	0.25	0.998
16	Metazachlor	30.4 (0.8)	405 (3)	0.03	0.05	0.25	0.999
17	Propazine	32.4 (0.3)	356 (6)	0.01	0.25	0.25	0.991
18	Warfarin	33.1 (0.8)	567 (5)	0.03	0.25	0.25	0.976
19	3,3'-DCB	34.1 (0.6)	664 (6)	0.01	0.1	0.25	0.995
20	Barban	37.4 (0.3)	583 (1)	0.05	0.1	0.25	0.999
21	Alachlor	38.3 (0.4)	336 (4)	0.1	0.1	0.25	0.996
22	Nitralin	40.7 (0.4)	186 (2)	0.1	0.1	0.25	0.999
23	Dinoseb	41.3 (0.3)	114 (7)	0.1	0.5	1.0	0.999
24	Dinoterb	42.0 (0.3)	117 (8)	0.1	0.5	1.0	0.996
25	Phoxim	44.1 (0.3)	108 (8)	0.1	0.25	0.5	0.962
26	Nitrofen	46.5 (0.3)	484 (2)	0.03	0.1	0.25	0.999
27	Trifluralin	49.9 (0.2)	129 (8)	0.1	0.1	0.25	0.995

[a] RT_{RW} = retention time (min) in River Rhine water (n = 18–21); PA_{RW} = peak area (arbitrary units) in River Rhine water (n = 8); R.S.D. = relative standard deviation (%); IL_{HP} = identification limit in HPLC-grade water (μg/l); IL_{DW} = identification limit in drinking water (μg/l); IL_{RW} = identification limit in River Rhine water (μg/l); R^2 = linear regression coefficient in River Rhine water.

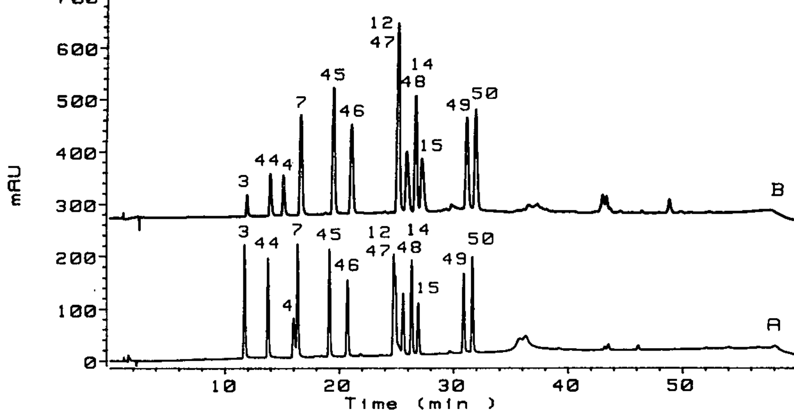

Fig. 4. LC–DAD traces of a mixture of thirteen phenylurea herbicides. (A) Loop injection of 25 μl (10 μg/ml); (B) preconcentration of 100 ml of spiked Milli-Q-purified water (5 μg/l). For peak numbers up to 27, see in Table I; 44 = desmethyl metoxuron; 45 = metoxuron; 46 = monuron; 47 = fluometuron; 48 = monolinuron; 49 = linuron; 50 = chlorobromuron. For further conditions, see text.

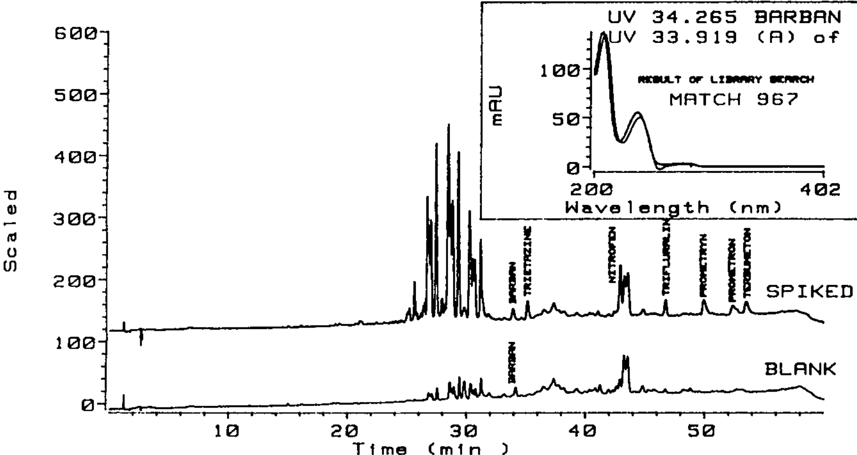

Fig. 5. LC–DAD traces of 200 ml of diluted methanol extracts of suspended particulate matter in River Rhine water (Lobith, Netherlands). A 200-ml volume of sample was spiked with a mixture of six triazines (terbumeton, prometron, prometryn, trietazine, atrazine and simazine) and two nitrophenols (nitrophen and trifluralin) at a concentration of 10 μg/l and filtered through a 0.45-μm filter after 20 h. The filter was extracted with 10 ml of methanol. Thereafter, 5 ml of the extract were dissolved in 200 ml of Milli-Q-purified water, enriched on a PLRP-S cartridge and analysed. In a blank sample treated the same way, barban was found at a concentration of about 0.3 μg/l. For further conditions, see text.

100 polar pollutants have been determined with the system; for 75% of these compounds identification limits of less than 1 μg/l can be obtained. In this context it should be noted that all of the chromatograms shown in this paper represent a compromise situation (see above). It is possible, of course, to optimise the detection conditions for a single or a limited number of analytes if their determination is of particular concern.

The system was also used for the analysis of particulate matter present in River Rhine samples. After off-line filtration (using a 0.45-μm cellulose acetate membrane filter) of 200 ml of sample, the filter was extracted with 10 ml of methanol and 5 ml of this extract were diluted with 200 ml of Milli-Q-purified water which was enriched as described previously on a PLRP-S cartridge. A sample from the same batch of River Rhine water was spiked with a mixture of six triazines and two nitrophenols at a concentration level of 10 μg/l. After 20 h at ambient temperature, 200 ml of the sample were filtered and analysed as described above. Trietazine, prometryn, prometron, terbumeton, nitrofen and trifluralin were found in the extract and are probably adsorbed on the particulate matter (Fig. 5). Atrazine and simazine, which are almost invariably present at low levels in River Rhine water [11,12], were not found in the extract, which suggests that these solutes are not adsorbed. In both blank and spiked River Rhine water samples barban was found, after extraction, at a concentration level of 0.3 μg/l. Obviously barban is also adsorbed on the particulate matter. As these are only preliminary results, this topic will be studied further in the future.

System control of fully automated system

In Fig. 1, an overview of the SAMOS components and corresponding interfaces is given. The HP-IB communication interface between the PAWS and the HP 1090 allows the on-line programming and control of the SDS and DAD. An RS 232C communication interface allows the on-line programming and control of the Prospekt and the SDU using a software package in the form of the "user macro" SAMOSMAC. The possibility of combining the system with a Local Area Network (LAN) will allow the transfer of data to other laboratories and authorities, *e.g.*, in the case of alarm situations.

After the PAWS computer has been switched on, the macro is automatedly loaded into the internal memory. A special soft-key set allows the handling

of the communication between the PAWS and the Prospekt. It is now possible directly to program, edit and save the Prospekt run (RP), SPP and START program on the screen of the computer. All of these can be sent directly to the Prospekt by pressing one of the soft-keys. When the appropriate RP and SPP programs have been loaded, sending of a START program will start the required sequence of analyses. The macro allows the on-line control of all activities of the Prospekt, such as information about the SPP and RP programs in current use with the number of cycles, positions of valves, auxiliaries, solvents used, flow-rates, actual run time and pressure of the Prospekt. In addition, error code messages of the Prospekt are checked at regular intervals and the information is stored in a logbook file. The programming, editing and storage of SPP and RP programs is user friendly and easy to handle. The main advantage is that a system consisting of two non-compatible devices, the Prospekt and the HP 1090, is now acting as a compact unit that can be operated and controlled from one computer.

Data handling

After optimisation of all the relevant data acquisition parameters, the optimum settings for the DAD were found to be a detection wavelength of 210 nm with a 10-nm bandwidth using a reference spectrum at 550 nm with a 100-nm bandwidth. The reference spectrum was taken at 550 nm because at this particular wavelength the absorbance of the analytes is negligible. All spectra were stored with a threshold of 2 milliabsorbance, a peak width of 0.2 min, a sampling interval of 1280 ms and a spectrum range of 200–400 nm with steps of 4 nm. All others conditions are given under Experimental.

Different integration event files were created and tested. With real samples, the best results were obtained using the option "integrate all valleys", where the integration baseline is constructed according to the baseline shape of the chromatograms. To exclude small peaks from the spectral evaluation procedure, the minimum peak area is set at 100, which corresponds to a 0.01–0.05 μg/l concentration level of peaks with a molar absorbance of about 10 000 [8]. A peak width of 0.2 min allows the integration of all peaks except for the 2-nitrophenol peak, which was too broad, at concentration levels below 1.0 μg/l.

Determination of the analytes is based on a calibration function (linear regression using an external standard) of the automated method. This function is used in the range of 0.01-7 μg/l. Three measurements are performed for each analyte at seven concentrations, to test simultaneously the repeatability of the method. The automated evaluation of the analysis of each sample is controlled by a so-called "macro program" run by the PAWS computer operating in the background mode during the analysis of the series of samples. This process is subdivided into three parts. First, the chromatogram is integrated and plotted. Next, the retention times of the peaks are compared with a library containing retention times of standard compounds. If there is a match of retention times within 2.5% the complete absorbance spectrum of the signal is compared with the spectra of the standards in the library. The comparison is based on the spectra recorded at the peak maximum after apex background subtraction.

Report parameters

At the end of the run the chromatogram is automatically evaluated; peaks are identified and quantified and a report is printed. The final report contains the most important results of the analysis and shows for each of the identified compounds: retention time, concentration in μg/l, spectral match factor and peak purity factor. Normally, a spectral match factor between 950 and 1000 and a retention time deviation of less than 2.5% were considered to indicate a positive identification. When analysing compounds with non-selective spectra in the spectral range 210–400 nm, a threshold value of 990 is recommended.

Additional subtract macro

A so-called additional subtract macro is used to subtract two consecutive chromatograms from each other, the result of this subtraction then being printed. This procedure should provide rapid information on changes in the general composition of the surface water between two runs. Results obtained with blank and spiked River Rhine water showed that subtraction of the chromatograms indeed can provide useful additional information (Fig. 6). If the concentration of a pollutant is increasing, the subtracted chromatogram will show an increasing ("upward") response (Fig. 6B and C). A decreasing

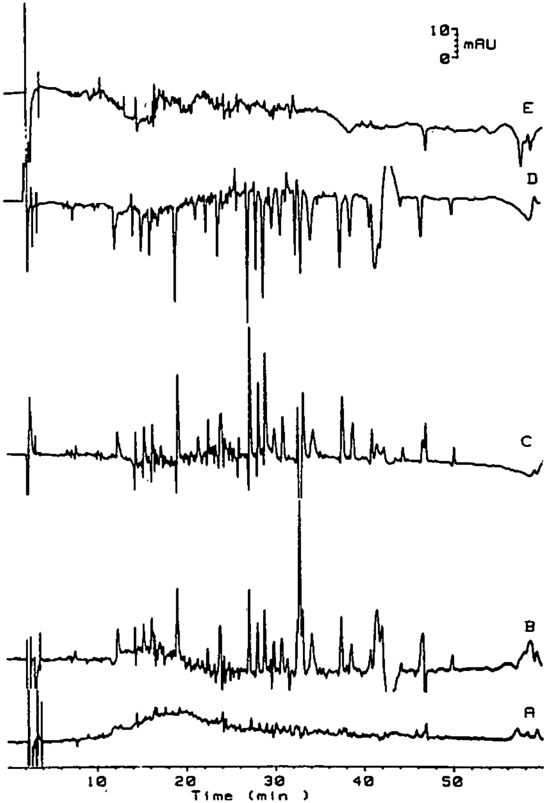

Fig. 6. Subtracted LC–DAD traces of River Rhine water blank samples and samples spiked with a mixture of 27 pesticides at different concentration levels. (A) Subtraction of blanks from two consecutive analyses; (B) subtraction of blank from 0.25 μg/l sample; (C) subtraction of 0.5 μg/l sample from 1.0 μg/l sample; (D) subtraction of 1.0 μg/l sample from 0.25 μg/l sample; (E) subtraction of blanks from two consecutive analyses. For chromatographic conditions, see text.

("downward") response (Fig. 6D) indicates a decreasing concentration level. Obviously, 0.25–0.50 μg/l differences between runs can be easily recognized in most instances.

Applications

Surface water samples were taken from six European rivers and the shape of the matrix interference was similar in all instances (Fig. 7). The samples were analysed by the standard procedure. Except for the sample from the River Mönchaltdorf, in all of them one or more of the pesticides tested in our study were found (Table IV and Fig. 8). The presence of atrazine, simazine and diuron in the River Meuse water samples was confirmed by liquid chromatography–mass spectrometry (LC–MS) [10]. Target analysis of atrazine in another River Rhine sample by means of LC–MS [10] and on-line LC–GC with thermionic detection [11] yielded closely similar concentration levels of atrazine of 0.16 and 0.17 μg/l, respectively. The SAMOS system was installed on a monitoring station on the River Meuse (Keizersveer; RIZA, Lelystad, Netherlands) in June 1992. During that month diuron was found at a concentration level of about 1.4 μg/l (Fig. 8). The results were not completely unexpected because a monitoring study of the River Meuse in 1990 showed that the concentration of diuron was gradually increasing up to about 1 μg/l in June [12]. A general conclusion may be that although LC–MS can provide more structural information, LC–DAD systems are significantly cheaper, and normally provide sufficient data for early-warning purposes.

TABLE IV

PESTICIDES FOUND IN EUROPEAN RIVERS

River	Sampling	Pesticide	Concentration (μg/l)
Garonne	November 1991	3,3′-Dichlorobenzidine	0.3
Meuse (Keizersveer)	June 1992	Atrazine	0.35
		Diuron	1.4
		Simazine	0.65
Rhine (335 km)	April 1992	Atrazine	0.2
		Barban	0.05
		Fluometuron	0.7
Rhine (Lobith)	April 1992	Atrazine	0.2
Thames	February 1992	3,4-Dichloroaniline	0.7
Uster	October 1991	Alachlor	0.25

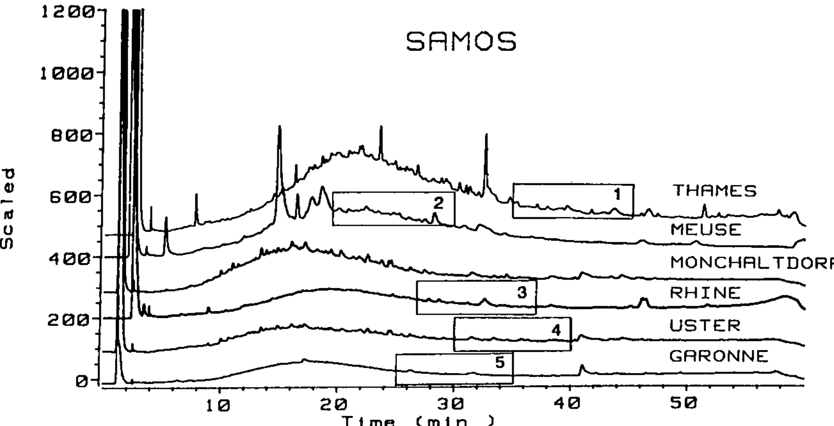

Fig. 7. LC–DAD traces of samples from various European rivers: River Thames (Westminster Bridge, London, UK), River Meuse (Keizersveer, Netherlands), River Monchaltdorf and River Uster (flowing into Lake Greiten, Switzerland), River Rhine (km 335, Germany) and River Garonne (Toulouse, France). The selected windows 1–5 are given in Fig. 8. For chromatographic conditions, see text.

Fig. 8. Blow-ups of parts of the LC–DAD traces shown in Fig. 7, and comparison of UV absorbance spectra of pollutants detected with those stored in the library. The numbers 1–5 refer to the windows in Fig. 7. The concentration levels of the various pesticides are summarized in Table IV.

CONCLUSIONS

An integrated and fully automated LC system has been developed for the monitoring of polar pesticides at trace levels, *i.e.*, low- to sub-μg/l, in surface waters. A cartridge-exchange module (Prospekt) is combined with a gradient LC system (HP 1090) with diode-array detection. The whole system is controlled by the Pascal Workstation (PAWS) of the LC system using a special laboratory-made software package. The data are automatedly evaluated, which means that compounds present at or above a certain concentration level are identified and determined and a report is printed. The SAMOS system has been validated for 27 pesticides and it has since been shown that at least 50–100 organic micropollutants can be determined at concentrations of *ca.* 1 μg/l. The robustness and reliability of the system were tested during several months in two laboratories and at a monitoring station on the River Meuse. This experience showed that the system can be used for monitoring purposes by running it unattended. The method was successfully used for the analysis of samples from six European Rivers; in nearly all instances one or more pesticides were found at concentrations over 0.1 μg/l.

Future research will be devoted to an extension of the range of applicability the system to permanently charged organic pollutants and to obtain lower identification limits by using more selective packing materials.

ACKNOWLEDGEMENTS

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Study of permanently coated columns for the high-performance liquid chromatographic determination of sulphur anions in environmental samples from metallurgical processes

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ABSTRACT

The behaviour of styrene–divinylbenzene and bonded C_{18} reversed-phases permanently coated with cetylpyridinium ion or hexadecyltrimethylammonium ion ("permanent coating" ion-interaction chromatography) were investigated for the determination of sulphur species in samples from studies on environmental control in metallurgical processes. The advantages of these types of exchanger are the ability to vary the ion-exchange capacity and the attainment of high column efficiency. Also demonstrated is the effect of an organic modifier on the control of retention and the wide linear range calibration curves ofbtained with these columns. Examples of application to the analysis of effluents and acid-tailings residues from metallurgical processing operations for the determination of sulphate, thiosulphate and thiocyanate anions are given. Detection was by indirect UV absorption.

INTRODUCTION

The Canada Centre for Mineral and Energy Technology (CANMET) is committed to the development of technology for the effective removal or disposal of potential environmental contaminants discharged from metallurgical processing operations. The analysis of mixtures of anions including sulphur anions is of interest in studies of environmental problems. Recently much effort has been applied to use of HPLC for the separation and determination of these anions.

There are two main approaches for separation methods applicable to sulphur anion analysis: (a) ion-exchange using fixed-site exchange resins of various composition and, (b) ion-interaction methods with a variety of sorbents that support dynamically exchanged or permanently bonded ionic functionalities.

It is convenient to subdivide ion-exchange methods into two groups: those in which a suppressor column is used and those in which it is not. The first group includes those methods which are predominantly based on the system originally described by Small *et al.* [1] and commercialized by Dionex and generally described by the term suppressed-ion chromatography. Single-column chromatography methods for anions make up the second group. They were introduced by Gjerde *et al.* [2]. The determination of inorganic anions by non-suppressed ion chromatography generally involves the use of low-capacity, resin-based or silica-based ion-exchange materials combined with a low-conductance eluent.

In ion-interaction chromatography, pioneered by Skelly [3], Molnar *et al.* [4], and Cassidy and Elchuk [5], an ionic hydrophobic reagent is added to the

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mobile phase of a reversed-phase chromatographic system in order to increase the retention of an oppositely charged ionic solute. Two distinct approaches for coating the column with ion-interaction reagent are dynamic coating and permanent coating. In the first approach a reagent of relatively low hydrophobicity is used initially to condition the column and is maintained in the mobile phase for all subsequent chromatography. In the permanent-coating method, a much more hydrophobic reagent is used for initial column conditioning, after which it is removed from the mobile phase.

Our studies [6] with reversed-phase columns that are permanently coated to give a charged surface that can be used for ion exchange have shown that such columns can offer distinct advantages over either dynamically coated or fixed-site ion-exchange separations. We now describe the results of a study to compare the behaviour of styrene–divinylbenzene and bonded C_{18} reversed-phase columns coated with hydrophobic reagents, cetylpyridinium bromide (CTP) and hexadecyltrimethylammonium bromide (cetrimide). Applications of these columns to the determination of sulphur anions in various types of environmental samples are shown, and the advantages of this approach are presented.

EXPERIMENTAL

Apparatus

The chromatographic pumps were a Waters M-625 pump for chromatographic determinations and a Waters M-45 pump for coating the columns (Waters, Milford, MA, USA). Sample injection was by a Rheodyne Model 9125 sample valve (Rheodyne, Berkley, CA, USA). Spectra Physics 2000 and Linear 100 variable-wavelength detectors were used for absorbency detection. Data handling was done by Spectra-Physics ChromJet integrators. (Spectra Physics, Santa Clara, CA, USA).

The columns used were 5-μm PRP-1 styrene–divinylbenzene, 150 × 4.1 mm I.D. (Hamilton, Reno, NV, USA) and 5-μm Supelcosil LC-18-DB, 150 × 4.6 mm I.D. (Supelco Canada, Oakville, Canada).

Reagents

All solutions were prepared with water which was freshly distilled and passed through a Milli-Q water system (Millipore, Bedford, MA, USA). A stock solution (5 m*M*) of the eluent acid, 1,3,5-benzenetricarboxylic, was dissolved in water by adding NaOH to a pH value of *ca.* 5. Eluents were prepared by diluting the stock solution with water and adjusting to the optium pH (refer to Experimental conditions) with tris(hydroxymethyl)aminomethane (THAM). Hexadecyltrimethylammonium bromide (cetrimide) and cetylpyridinium bromide (CTP) were used for coating the columns. These reagents were used as received from the supplier (Sigma, St. Louis, MO, USA). All solvents were HPLC-grade and were obtained from Caledon Labs. (Georgetown, Canada).

Procedure for coating columns

Solutions containing the coating reagent, CTP or cetrimide, were prepared in water–acetonitrile mixtures (0.5 m*M* of reagent with 15–30% of acetonitrile). The pH of these coating solutions was 5.5 and no further adjustment was made. The analytical column was first washed with a solution of acetonitrile without the coating reagent (the percentage of acetonitrile determined column capacity). A solution of the coating reagent containing this same percentage of acetonitrile was then pumped through the column. The breakthrough curve was recorded by monitoring the bromide ion with a UV detector at 210 nm. A 1-m*M* salicylic acid solution (5% acetonitrile and pH 6.5) was pumped through the column until breakthrough of the salicylic anion as recorded at 290 nm. This was followed by equilibrating the column with the eluent. To remove the sorbed coating reagent, a solution of 0.1 *M* potassium bromide containing 70% acetonitrile was passed through the column for at least 1 h followed by washing with acetonitrile for 10 min.

Sample preparation

Nickel process effluent. To a sample was added 0.1 *M* NaOH to give a pH of 4.8–5.2. This was followed by the addition of a solution of THAM to a pH of 6.8. The sample was then diluted to an appropriate volume with eluent.

Gold process effluent. The samples from the cyanide removal process were preserved by adding sodium hydroxide to a pH of 12. An aliquot of sample was adjusted to pH 5.5–6.0 with eluent acid and then a solution of THAM was added to give a pH of 7.0. The sample was transferred to a 50-ml volu-

metric flask and diluted to the mark wih eluent.

Sulphide concentrate and tailing samples. The procedure as described by Guillas aned Blanchette [7] for dissolving the samples and oxidation of sulphur species to sulphate ion was used. Weighed aliquots from the sample solutions were adjusted to a pH of 6.8 with a solution of THAM, and then transferred to 500 ml volumetric flasks and diluted with eluent.

Sample analysis

An aliquot (normally 50 μl) was injected into the chromatograph; chromatographic conditions recommended for the samples are those given in Figs. 3 and 4.

RESULTS AND DISCUSSION

Comparison of column systems

Both bonded silica (C_{18}) and styrene–divinylbenzene (PRP-1) reversed-phases were used in these studies. These columns were coated with the hydrophobic quaternary amines: CTP or cetrimide. The amount of reagent sorbed by a column was studied as a function of concentration of acetonitrile in the coating solution. The concentration of the coating reagent was 0.5 m*M*. The pH of the coating solution was 5.5. The anion-exchange capacity, which is calculated from the amount of sorbed quaternary amine, might not be necessarily the same as the effective capacity. This is because the amine sorbed by electrostatic interaction through a silanol group does not perform as an anion-exchange site [8]. Therefore, the anion-exchange capacities were calculated from the difference in breakthrough volumes of salicylate between the columns coated and uncoated.

The data in Fig. 1 show the amount of sorbed CTP with concentration of acetonitrile for the C_{18} and PRP-1 columns. Under the coating conditions used, the coating weights sorbed on the C_{18} column were higher than for the PRP-1 column for a given concentration of acetonitrile. In previous work [9] we showed that there were only small differences in the weights of the two quaternary amines that sorbed on the C_{18} column for a given percentage of acetonitrile. This indicated that the cetyl group in these reagents is the main contributor in the hydrophobic interactions with the octadecyl groups of the C_{18} column.

The C_{18} phase coated column was found to be more efficient than the PRP-1 column. The column efficiency as given by the height equivalent of a theoretical plate (HETP values) [10] were generally in the range of 0.1–0.15 mm at 1 ml/min for the C_{18} column when coated with either of the reagents, and the peak shapes of the anions tested, Cl^-, CNO^-, SO_4^{2-}, $S_2O_3^{2-}$, and CNS^-, were similar. Fig. 2 shows the dependence of the capacity factor k' of the anions on the concentration of the two reagents. As can be seen the effective capacity of a column coated with cetrimide is greater than for a column coated with the same weight of CTP.

Both PRP-1 and C_{18} columns were stripped and recoated several times for a period of 8 months during these studies, and only small changes in column efficiency and retention times were noted. In general, the columns did not appear to be susceptible to poisoning from extraneous ions in the samples analyzed. However, when an occasional problem was encountered, the ion exchanger was stripped and the column recoated.

Application of permanently coated column systems

Determination of µg/ml sulphate in presence of high concentrations of chloride

Sulphate ion at the μg/ml level along with transition metals are monitored in effluents from nickel

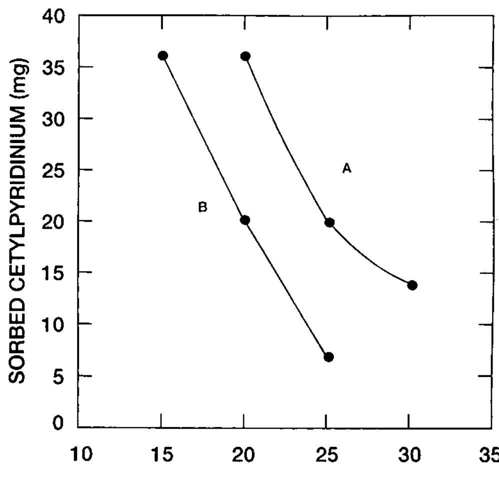

Fig. 1. Variation of amount of sorbed CTP with concentration of acetonitrile in coating solution. (A) Supelcosil LC-18-DB C_{18} column, (B) Hamilton PRP-1 styrene–divinylbenzene column.

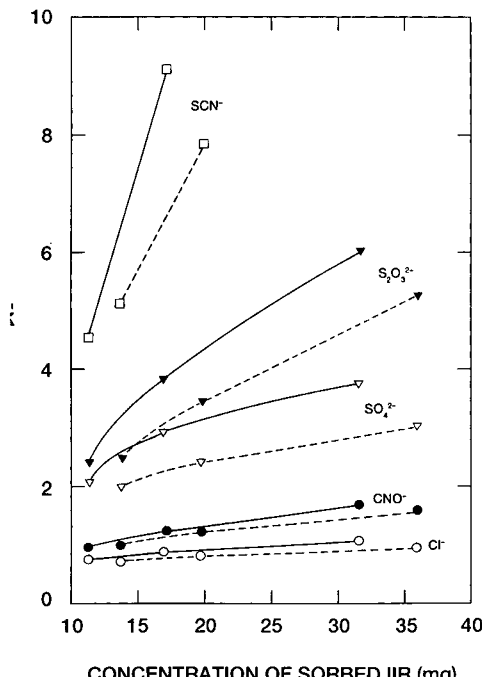

Fig. 2. Relationship between the amount of a coating reagent (IIR) sorbed on C_{18} column and k' for anions. The broken curves are for the column coated with cetylpyridinium, and the solid curves are for hexadecyltrimethylammonium coated column; eluent, $0.5 \cdot 10^{-3}$ mol/l 1,3,5-benzenetricarboxylic acid, neutralized to pH 6.8 with THAM; flow-rate, 1 ml/min; detection by indirect UV at 254 nm.

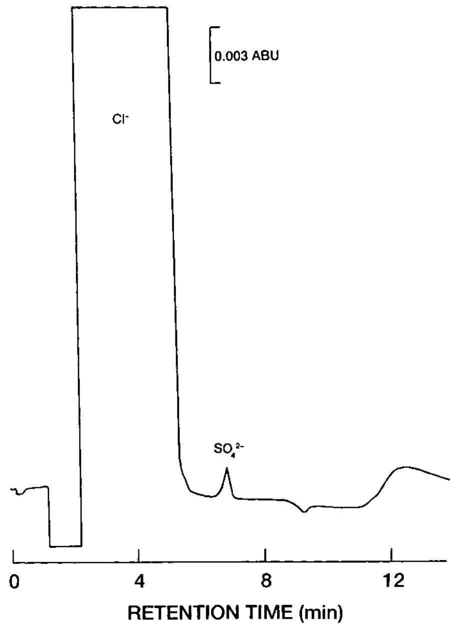

Fig. 3. Separation of sulphate in effluent containing 0.5 M HCl. Experimental conditions: Supelcosil LC-18-DB column coated with 36 mg of hexadecyltrimethylammonium; eluent, $0.5 \cdot 10^{-3}$ mol/l 1,3,5-benzenetricarboxylic acid, neutralized to pH 6.8 with THAM; flow-rate, 1 ml/min; detection by indirect UV at 254 nm (ABU = absorbance units).

processing operations. The addition of hydrochloric acid to the samples at the collection site, to prevent hydrolysis of metals, complicated the determination of sulphate.

With both suppressed-ion chromatography and single-column ion chromatography, high concentrations of chloride generally are eluted with sulphate and obscures the sulphate peak in the chromatogram. Pretreating the sample with silver compounds to separate large amounts of chloride has been used [11], but this is time consuming and introduction of contaminants into the sample may take place. Also, peak broadening occurs with this technique.

A high-capacity (32 mg of cetrimide), permanently coated column system gave efficient separation of the sulphate ion from the chloride matrix ion, as shown in Fig. 3. In this example the concentration of the sulphate was determined to be 1 ppm in a sample containing 0.5 M of chloride. There is little tailing of the large chloride peak, and this reflects the rapid mass transfer characteristics of permanently coated ion exchangers. A small "reaction peak" from an overloading effect of the high chloride appears after the sulphate. With a lower-capacity column this peak increased in size and obscured the sulphate peak. The ability to adjust the ion-exchange capacity was an important factor in being able to optimize separation of low levels of sulphate from high concentrations of chloride ion.

Determination of thiosulphate, thiocyanate, and sulphate in gold process effluents

There is an urgent need to develop technology for the effective removal of cyanide from gold process effluents. In projects carried out in this area at CANMET, thiosulphate, thiocyanate, and sulphate, as well as chloride and cyanate, needed to be closely monitored.

TABLE I

EFFECT OF ORGANIC MODIFIER ON RETENTION TIMES

Eluent acid was $0.5 \cdot 10^{-3}$ mol/l 1,3,5-benzenetricarboxylic acid adjusted to pH 7.0 with THAM, and contained 5% (v/v) of the solvent; flow-rate was 1 ml/min; detection by indirect UV at 254 nm.

Anion	Retention times (min)		
	Acetonitrile	Methanol	*n*-Butanol
Cl^-	2.56	2.46	2.33
SO_4^{2-}	5.05	4.51	4.30
$S_2O_3^{2-}$	6.42	5.84	5.35
CNS^-	12.54	9.28	6.08

One of the major problems in the determination of thiosulphate and thiocyanate by available chromatography techniques is that the conditions required to separate chloride and cyanate results in rather long retention times for thiosulphate and thiocyanate. Also the sensitivity of reported methods was found to be insufficient [12,13].

In our studies to devise chromatographic conditions for the determination of these anions in one chromatographic run, it was found that the type of organic solvent added to the eluent caused differences in the relative retention of the anions. Table I summarizes some of the retention patterns observed. A change in the solvent concentration from 2 to 6% had much smaller effects than observed when the nature of the solvent was changed. The data shows that a change in solvent from acetonitrile to *n*-butanol decreased the retention of thiosulphate and thiocyanate ion significantly relative to sulphate. This effect was likely due to specific solvation effect on the more polar thiosulphate and thiocyanate anions. Retention time for a pure aqueous system were similar to that observed with methanol. The different solvents had little effect on the retention times of cyanate and chloride.

Fig. 4 shows the separation of chloride, cyanate, sulphate, thiosulphate, and thiocyanate in a typical gold process effluent. The pH of the eluent affected peak shape for cyanate and the size of the system peak, which could interfere with thiocyanate quantitation. The eluent pH was tested over the pH range of 5.5 to 8.0 and interference effects were minimized when the eluent was adjusted to a pH of 7.0–7.2. The initial system peak in this chromatogram was caused by the high concentration of NaOH present in the sample. Both the early-eluted anions and the more highly retained sulphur species were determined in less than 8 min with good resolution. This system illustrates that exploitation of eluent composition is an attractive feature of these permanently coated reversed-phases, as it is another parameter that can be used to optimize separations.

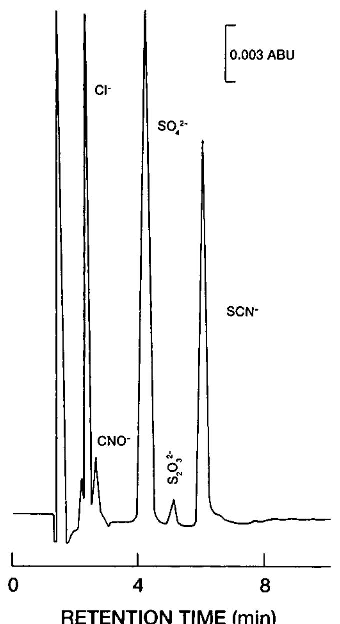

Fig. 4. Separation of chloride, cyanate, sulphate, thiosulphate and thiocyanate in a gold process effluent. Experimental conditions: Supelcosil LC-18-DB column coated with 24 mg of cetylpyridinium; eluent, 0.5×10^{-3} mol/l 1,3,5-benzenetricarboxylic acid, neutralized to pH 7.0 with THAM; modifier, 2.5% (v/v) *n*-butanol; flow-rate, 1 ml/min; detection by indirect UV at 254 nm; Sample, 50 μl of diluted sample (10:1) (ABU = absorbance units).

Analysis of sulphide concentrates and tailings for total sulphur

The emission of sulphur dioxide in the pyrometallurgy of sulphide concentrates can be monitored by measuring the sulphur content in feed and in materials remaining after processing, *i.e.* tailings. Total sulphur in these materials is determined routinely by combustion techniques. The accuracy of these determinations is dependent on the availabil-

ity of matrix matched and certified standards to be used for instrument calibration.

Methods used to collect analytical data for certification of sulphur standards have been restricted to the classical gravimetric procedure and inductively coupled plasma–atomic emission spectroscopy (ICP–AES) [14], since these are the only techniques for the analysis of total sulphur which are not matrix-dependent and are independent of compositional reference materials for calibration purposes.

The present authors experience with HPLC dynamic ion-exchange techniques has shown that a peak-height repeatability (1σ) of $<1\%$ was obtained for the determination of rare earths in samples from uranium ore refining processes [15]. Further, our application of permanently coated anion-exchange systems has indicated that wide linear range calibration curves are obtained, and large weights of an anion, up to 10 μg, can be used for sample injection [9]. Errors associated with the sample loop are not a concern, as repeatabilities are expected to be $\pm$ 0.1% [16]. Consequently, the permanently coated column chromatographic approach was investigated for the determination of total sulphur in tailings and concentrates as an alternative technique to the gravimetric and ICP–AES methods.

Samples used in this study were Canadian Certified Reference Materials from CANMET. Three samples of acid tailings and a copper concentrate were analyzed. The wet chemical procedure described by Guillas and Blanchette [7] for dissolving the samples and oxidation of sulphur species to sulphate ion was used. Dilutions were made of accurately weighed aliquots from the sample solution. The chromatographic conditions for the samples are those given in Fig. 3. The weight of sulphate ion in the injected samples was 5–7 ng.

The results obtained for the samples are shown in Table II along with the corresponding values for these samples from the CANMET certification program for comparison. The HPLC data were obtained from the average of five samples, each analyzed in duplicate. The average relative standard deviation (R.S.D.) was 0.48%. The data show that excellent agreement was obtained between the values recommended by the Canadian Certified Reference Materials Project and the HPLC results. The peak area calibration curves prepared from standards that did not contain ions to simulate the sample matrix, and illustrates non-biased recovery of the sulphur by this HPLC system. This study has shown that permanent coated column techniques can be used to determine total sulphur in tailings and concentrates with a precision (1σ) of $<1\%$.

TABLE II

HPLC ANALYSIS OF CANMET CANADIAN CERTIFIED REFERENCE MATERIALS FOR TOTAL SULPHUR

Reference material	Type of material	CCRMP[a] value	HPLC[d] value	HPLC S.D.
RTS-2	Tailings	19.5[b]	19.5	0.076
RTS-3	Tailings	10.3[b]	10.2	0.042
RTS-4	Tailings	35.6[b]	34.6	0.17
CCU-a1a	Copper concentrate	33.8[c]	33.8	0.18

[a] Canadian Certified Reference Materials Project (CCRMP) of CANMET.
[b] CCRMP recommended value.
[c] Certified value, $\pm$ 95%; confidence interval = 0.3%.
[d] Average of results of 5 samples, each analyzed in duplicate.

CONCLUSIONS

Anion exchangers formed by the sorption of hydrophobic cations on reversed-phase columns can offer significant advantages over bonded fixed-site exchangers for the separation of sulphur anions. These exchangers give greater chromatographic efficiency and greater flexibility with regard to choice of column capacity and eluent composition for the development of separations that may be difficult on other types of ion exchangers. This kind of chromatography has given reliable analysis of samples from the environmental area of metallurgical processing for anions and is applied in our Laboratory to the resolution of difficult sample matrices.

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CHROMSYMP. 2731

Retention characteristics of some volatile compounds on Tenax GR

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ABSTRACT

Breakthrough volumes (maximum sample volumes, $V_{max.}$) of low-molecular-mass compounds on graphite-filled Tenax have been measured. Using Tenax GR the sampling volumes are greater than with Tenax GC or Tenax TA. Tenax GR can be used in environmental analysis for trapping and enriching traces of low-molecular-mass organic compounds.

INTRODUCTION

Tenax TA (GC) is frequently used in environmental analysis as an adsorbent for enriching traces in the ppb (v/v) range [1–4]. Tenax GR is a new adsorbent for trapping low-molecular-mass organic compounds. It consists of Tenax matrix (poly-*p*-2,6-diphenylphenylene oxide) filled with 23% graphite.

To extend the usefulness of sorbents of the Tenax type, the effects of air flow-rate, pollutant concentration and other parameters have been investigated. The most important of these parameters is the estimated column capacity, *i.e.*, the volume of sampled air at which the compound being collected begins to be eluted from the sampling tube [5].

The column capacity depends on the retention time of the compound being sorbed at ambient temperature (using the Tenax stationary phases).

References to the properties and typical applications of Tenax GC and Tenax TA are given in refs. 4 and 6–8. The sources in the literature do not offer any information regarding the properties of Tenax GR.

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EXPERIMENTAL

Apparatus

A Chrom 5 gas chromatograph with a flame ionization detector (Laboratorní přístroje, Prague, Czech Republic) was used.

All chemicals (*n*-alkanes, aromatics, alcohols, ketones, esters, amines and halogenated hydrocarbons; see Table I) were of analytical-grade purity and were obtained from Fluka, Merck and Janssen; helium was obtained from Messer Griesheim (Austria), nitrogen from Linde Technoplyn (Prague, Czech Republic), Tenax GR (60–80 mesh) was purchased from Chrompack (Middelburg, Netherlands). The U-shaped glass tested column, 3 mm I.D. with a bed length of sorbent 37 cm (mass 1 g), was homemade and was connected to the injection and detection ports of a gas chromatograph.

Procedure

The glass U column was filled with 1 g of Tenax GR and conditioned using helium as a carrier gas; after 0.5 h at a laboratory temperature the column temperature was programmed at a rate of 2°C min^{-1} from 22 to 250°C, then for 4 h at 250°C. Retention volumes and theoretical plates were determined by injection of 20–40 μl of the vapours of tested compounds saturated at 20°C, *i.e.*, in the

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TABLE I

EXTRAPOLATED SPECIFIC RETENTION VOLUMES AND MAXIMUM SAMPLE VOLUMES FOR ORGANIC VAPOURS SAMPLED ON A 1.0-g TENAX GR ADSORBENT TUBE

A, B = empirical parameters of eqn. 3; N = number of theoretical plates; r = correlation coefficient; V_g^{20} = extrapolated specific retention volume at 20°C; $V_{max.}^{20}$ = maximum sample (breakthrough) volume at 20°C.

Organic compound	Boiling point (°C)	A	B	r	V_g^{20} (l)	N	$V_{max.}^{20}$ (l)
Hexane	68.4	3231.01	−6.55	0.9998	29.4	185	25.1
Heptane	98.3	3607.01	−7.32	0.9995	95.7	250	83.6
Benzene	80.5	3054.62	−5.83	0.9999	39.3	290	34.7
Toluene	110.8	3290.36	−6.01	0.9994	252.3	288	222.6
Xylene(s)	138–144	3827.24	−6.89	0.9974	1464.2	285	1290.7
Dichlormethane	40.7	2342.32	−4.70	0.9994	2.1	390	1.9
Trichlormethane	61.5	2679.50	−5.33	0.9974	7.9	325	7.0
Tetrachlormethane	76.7	2935.44	−5.74	0.9999	18.5	159	15.6
1,2-Dichlorethane	84.1	2917.04	−5.20	0.9989	49.5	405	44.6
Trichloroethylene	86.9	3042.63	−5.50	0.9962	49.0	484	44.5
Tetrachloroethylene	121.0	3262.83	−5.66	0.9992	292.0	561	267.3
Chlorobenzene	131.7	3547.92	−6.50	0.9999	402.6	362	360.3
Ethanol	78.5	2205.99	−4.50	0.9974	1.1	530	1.0
1-Propanol	97.2	2611.49	−5.10	0.9978	6.5	482	5.9
1-Butanol	117.5	3020.85	−5.72	0.9980	38.0	431	34.3
Acetone	56.3	2695.87	−5.58	0.9969	4.4	466	4.0
Methyl-isobutyl ketone	118.0	3714.12	−7.22	0.9993	279.6	131	230.8
Ethylacetate	77.1	3042.94	−6.00	0.9987	23.9	410	21.5
Propylacetate	101.6	3477.10	−6.69	0.9978	149.5	351	133.5
Butylacetate	126.5	3879.96	−7.42	0.9990	646.1	304	572.0
Propylamine	47.8	2471.95	−4.93	0.9947	3.2	442	2.9
Butylamine	77.8	2892.02	−5.54	0.9966	21.0	308	18.6
Pentylamine	104.0	3182.70	−5.90	0.9961	91.3	318	81.1
Diethylamine	56.3	2617.37	−5.10	0.9923	6.8	349	6.1
Dipropylamine	110.0	3491.30	−6.58	0.9974	194.1	264	170.2
Dibutylamine	160.0	4126.93	−7.53	0.9978	3521.0	191	3011.5
Epichlorhydrine	116.5	3062.74	−5.69	0.9997	57.9	949	54.1

range 10^{-6} to 10^{-7} g per injection, at a carrier gas (nitrogen) flow-rate of 20 ml min^{-1}.

Solute retention times were measured at a different temperatures ranging from 160 to 220°C.

RESULTS AND DISCUSSION

Determination of the maximum sample volume

The trapping tubes filled with Tenax GR may be regarded as chromatographic columns. At a given temperature, the vapour of each compound being eluted through the trapping tube is quantitatively adsorbed inside the trap until the gas volume flowing through the tube is equal to the retention volume of that substance less the volume corresponding to the elution of half of the solute band obtained in elution analysis. The maximum sample volume ($V_{max.}$) is given by the following equation [9–11]:

$$V_{max.} = V_R\left(1 - \sqrt{\frac{4}{n}}\right) \tag{1}$$

To apply this equation, it is necessary to know the retention volume (V_R) and the number of theoretical plates (n) for each substance at trapping temperature.

Combining eqn. 1 with the equation for specific retention volume, the relationship for $V_{max.}$ reads:

$$V_{max.} = \frac{V_g \cdot w_L \cdot T_E \cdot 101.32}{p_B \cdot 273.15} \cdot \left(1 - \sqrt{\frac{4}{n}}\right) \tag{2}$$

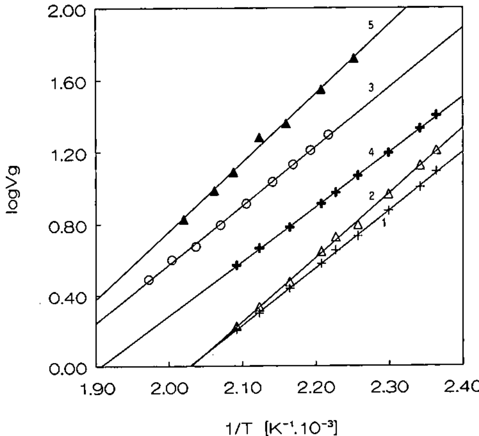

Fig. 1. Plot of log V_g *versus* reciprocal absolute temperature for hexane (1), heptane (2), toluene (3), benzene (4) and xylene(s) (5).

in which $V_{max.}$ is the volume of sampled gas (= breakthrough volume, often non-accurately called safe sampling volume), V_g is the specific retention volume, T_E is the absolute temperature of exposition (K), p_B is the atmospheric pressure (kPa) and w_L is the mass of sorbent in column tube (g).

It is necessary to apply another model [12] for very low plate numbers.

The maximum sample volumes were then found indirectly from measured solute retention times and the theoretical plates of the adsorbent tube. The amount of vaporized sample injected into the gas chromatograph system was selected in such a way

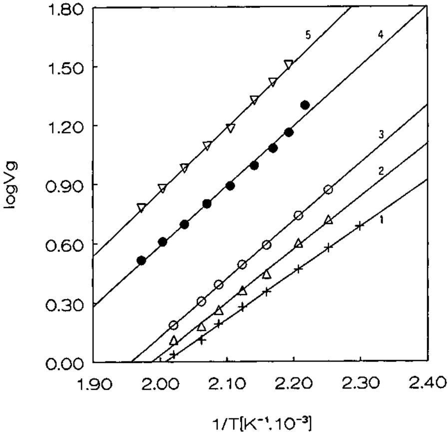

Fig. 2. Plot of log V_g *versus* reciprocal absolute temperature for dichloromethane (1), trichloromethane (2), tetrachloromethane (3), trichloroethylene (4) and tetrachloroethylene (5).

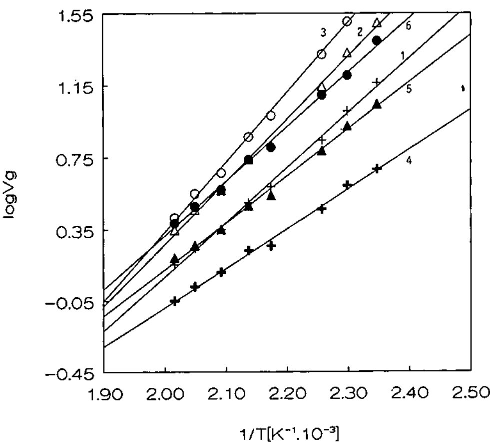

Fig. 3. Plot of log V_g *versus* reciprocal absolute temperature for ethyl acetate (1), propyl acetate (2), butyl acetate (3), ethanol (4), 1-propanol (5) and 1-butanol (6).

that the relationship between V_R was independent of sample volume (concentration) in the range studied (10^{-6} to 10^{-7} g).

Specific retention volumes at 20°C were estimated by extrapolation from temperature dependence using measured values for temperatures of 160–220°C:

$$\log V_g = \frac{A}{T} + B \quad (3)$$

where A and B are empirical parameters (see Table I).

Measured dependences for different homologous series of compounds are given in Figs. 1–5. Good

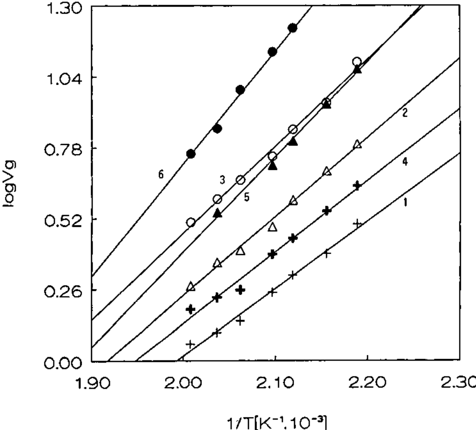

Fig. 4. Plot of log V_g *versus* reciprocal absolute temperature for propylamine (1), butylamine (2), pentylamine (3), diethylamine (4), dipropylamine (5) and dibutylamine (6).

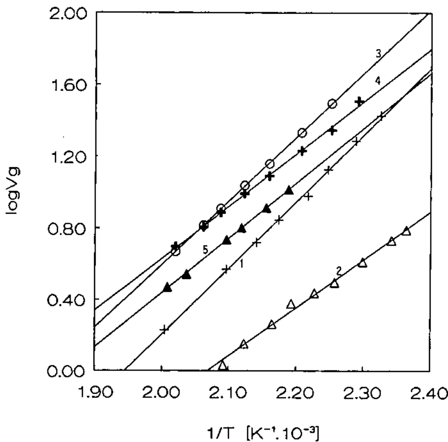

Fig. 5. Plot of log V_g *versus* reciprocal absolute temperature for methyl isobutyl ketone (1), acetone (2), chlorobenzene (3), 1,2-dichloroethane (4) and epichlorhydrine (5).

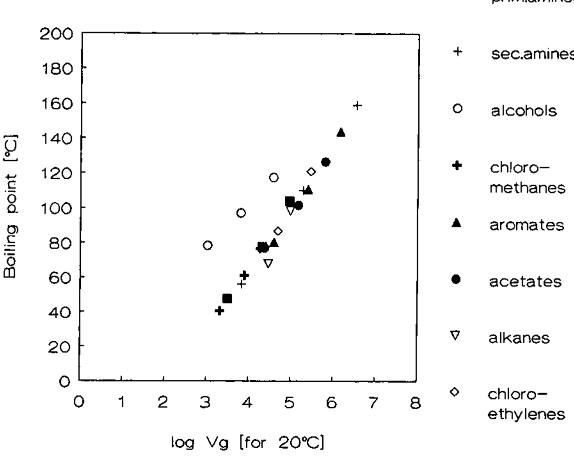

Fig. 6. Log V_g for Tenax GR as a function of boiling point.

linearity was obtained for all investigated compounds, as shown in Table I. Correlation coefficients are better than 0.997 for most of the compounds.

There is a good correlation between the logarithm of specific retention volumes and boiling points [8] of investigated compounds. The correlation for most compounds fits the straight line (Fig. 6), with the exception of aliphatic alcohols.

The flow-rate in the range 10–100 ml min^{-1} did not affect the retention volumes significantly. A flow-rate of 20 ml min^{-1} is sufficient (the minimum of the van Deemter equation is less than 5 ml min^{-1}).

CONCLUSIONS

The maximum sample volumes ($V_{max.}$) for organic vapours of low-molecular-mass compounds on Tenax GR sorption tubes have been measured. Quantitative sample enrichment is limited by the minimal detectable amount of the compounds which must be at least trapped, and thus by the breakthrough volume of the least-retained compound of the sample mixture.

This paper shows the possible use of graphitized Tenax as a trapping material. It has been observed that relatively large sample volumes can be used at ambient temperature for compounds of similar vapour pressure and at very low concentrations. This is usually valid for a great number of atmospheric pollutants. When the sampling volumes of most of the compounds used in the present work are compared with Tenax GC(TA) [4,13], values nearly twice as high are obtained. All the advantages of "Tenax", for example good thermal desorption, are retained.

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CHROMSYMP. 2681

Sensitive flame ionization detector for the determination of traces of atmospheric hydrocarbons by capillary column gas chromatography

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ABSTRACT

A sensitive flame ionization detector was developed for capillary column gas chromatography. The detector used no make-up gas and lower flow-rates of hydrogen and air to suppress detector noise and to achieve maximum response. The detector was responsive down to the 10^{-13} g level for C_2–C_{11} hydrocarbons. Atmospheric hydrocarbons could be determined at parts per billion or parts per trillion levels by using gas chromatography with the detector via cryogenic concentration of a small volume of air sample.

INTRODUCTION

Flame ionization detection (FID) has been usually employed for gas chromatographic (GC) determination of atmospheric hydrocarbons in studies of air pollution [1–14]. In such cases, a large volume of air sample has been often sampled by using cryogenic or adsorptive concentration techniques to compensate the detector response, thus lowering the GC performance because of contamination of analytical columns and the detector. On the other hand, water vapour in the sample may be condensed to clog the sampling tubes in the cryogenic process. Use of a large amount of dehydration agent could cause an unsuccessful determination of polar or unstable components owing to their adsorption or reactivity.

In this study, a sensitive flame ionization detection (FID) system was developed for capillary column GC analysis and successfully applied to the determination of trace levels of atmospheric C_2–C_{11} hydrocarbons via a cryogenic concentration of a small volume of air sample.

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EXPERIMENTAL

Reagents and materials

All the reagents used for calibration were of special grade from Wako (Osaka, Japan). Tedlar bags (10 l) were used to prepare a standard sample and to sample an air sample.

The 50 ppb (v/v) toluene standard sample was prepared as follows. A 23.7-μl aliquot of toluene was diluted with 100 ml of hexane and 10 μl of the solution were placed into a 1-ml glass ampoule. The ampoule was sealed by fusing its end, then placed into a 1000-ml glass flask. The flask was evacuated, the ampoule broken by shaking the flask, and the flask heated at 80°C for 10 min in a oven. Nitrogen was introduced into the flask to atmospheric pressure, 10 l of nitrogen were passed through the flask at room temperature and the gas sample was collected into a 10-l Tedlar bag.

Apparatus

A Varian (Walnut Creek, CA, USA) 1400 gas chromatograph equipped with a Hewlett-Packard (Avondale, PA, USA) 3380A integrator was employed. The analytical columns used were a 42 m × 0.25 mm I.D. stainless-steel squalane capillary col-

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TABLE I

RESPONSES OF S-FID FOR HYDROCARBONS AND RELATIVE RESPONSES OF HYDROCARBONS TO TOLUENE

No.	Hydrocarbon	Detection limit[a] (pg)	Sensitivity[b] (pg/s)	Relative response[c]
1	Ethylene	0.5	0.036	0.953
2	Acetylene	0.5	0.034	1.00
3	Propylene	0.5	0.036	0.953
4	Propane	0.5	0.037	0.916
5	Isobutane	0.3	0.021	1.02
6	Isobutene + 1-butene	0.3	0.022	0.953
7	*n*-Butane	0.3	0.021	1.02
8	*trans*-2-Butene	0.3	0.022	0.953
9	*cis*-2-Butene	0.3	0.022	0.953
10	3-Methyl-1-butene	0.2	0.011	0.953
11	Isopentane	0.2	0.011	0.972
12	1-Pentene	0.2	0.011	0.953
13	2-Methyl-1-butene	0.2	0.011	0.953
14	*n*-Pentane	0.2	0.011	0.972
15	*trans*-2-Pentene	0.2	0.011	0.953
16	*cis*-2-Pentene	0.2	0.011	0.953
17	2-Methyl-2-butene	0.2	0.011	0.953
18	Cyclopentane	0.2	0.011	0.972
19	2,3-Dimethylbutane	0.1	0.008	0.963
20	2-Methylpentane	0.1	0.008	0.963
21	3-Methylpentane	0.1	0.008	0.963
22	*n*-Hexane	0.1	0.008	0.963
23	Methylcyclopentane	0.1	0.008	0.963
24	2,4-Dimethylpentane	0.1	0.008	0.963
25	Benzene	0.1	0.008	1.05
26	Cyclohexane	0.1	0.008	0.963
27	2-Methylhexane	0.1	0.008	0.963
28	2,3-Dimethylpentane	0.1	0.008	0.963
29	3-Methylhexane	0.1	0.008	0.963
30	*n*-Heptane	0.1	0.008	0.963
31	Methylcyclohexane	0.1	0.008	0.963
32	Toluene	0.1	0.008	1.00
33	2-Methylheptane	0.1	0.009	0.907
34	4-Methylheptane	0.1	0.009	0.907
35	3-Methylheptane	0.1	0.009	0.907
36	*n*-Octane	0.1	0.009	0.907
37	Ethylbenzene	0.1	0.008	0.953
38	*p*-Xylene	0.1	0.009	0.935
39	*m*-Xylene	0.1	0.008	0.972
40	*o*-Xylene	0.1	0.008	0.953
41	*n*-Nonane	0.1	0.009	0.916
42	Isopropylbenzene	0.1	0.009	0.907
43	*m*-Ethyltoluene	0.1	0.008	0.944
44	*p*-Ethyltoluene	0.1	0.009	0.935
45	*o*-Ethyltoluene	0.1	0.008	0.953
46	1,3,5-Trimethylbenzene	0.1	0.009	0.916
47	1,2,4-Trimethylbenzene	0.1	0.009	0.907
48	*n*-Decane	0.1	0.009	0.915
49	1,2,3-Trimethylbenzene	0.1	0.009	0.916
50	*n*-Undecane	0.1	0.009	0.915

[a] Absolute detection limit at three times the noise level on the squalane capillary column (which showed an excellent performance for simultaneous separation of the aliphatic and aromatic hydrocarbons).

[b] Calculated by using a chromatogram of the hydrocarbons simultaneously separated on the squalane column.

[c] Relative response of hydrocarbon to toluene (w/w).

umn (Hitachi, Tokyo, Japan) and a J&W Scientific (Folsom, CA, USA) 30 m × 0.2 mm I.D. DB-1 fused-silica bonded-phase capillary column system. A two-stage concentration technique was employed for air sampling. The first cryogenic trap was a 30 cm × 2.1 mm I.D. stainless-steel tube packed with Flusin GU 60–80 mesh (GL Science, Tokyo, Japan). For making the second trap, a 300 cm × 0.25 mm I.D. stainless-steel squalane capillary column (Hitachi) was deactivated at 300°C for 120 min under a nitrogen stream, spiralled and covered with glass-fibre ribbons 1 mm thick. The GC analytical conditions were as follows: carrier, nitrogen 0.6 ml/min; column temperature, (1) 0°C for 10 min, raised rapidly to 30°C and programmed from 30 to 90°C at 1°C/min for the squalane column and (2) 0°C for 10 min and programmed from 0 to 150°C at 4°C/min for the DB-1 column; sensitive FID, hydrogen 25 ml/min, air 200 ml/min (no make-up gas was used).

Sampling and analytical procedure

Air was sampled at 300 ml/min for 30 min into a Tedlar bag by using a lung-type pneumatic sampler. A 50–1000-ml volume of the sample was passed at 200 ml/min through the first sampling trap at liquid oxygen temperature. The condensed components were then moved into the second trap at liquid oxygen temperature by heating the first trap at 100°C and by passing the carrier for 15 min in the opposite direction to that used in sampling. The retrapped substances were released by exposing the second trap at room temperature. When the second trap was exposed at room temperature, GC analysis was started. Hydrocarbons with C_2–C_{11} were identified by their retention times and quantified by the peak areas. The concentration of the hydrocarbons was determined by using 50 ml of 50 ppb toluene and the relative responses to toluene shown in Table I.

RESULTS AND DISCUSSION

Sensitive FID

A Varian 1400 FID assembly was modified so as to insert a capillary column outlet to the hydrogen path in the manner to keep the hydrogen flow streamlined (see Fig. 1). No make-up gas was used in the capillary GC analysis to avoid turbulent flow owing to mixing with the hydrogen and the carrier gases. The flow-rates of hydrogen and air were reduced to 25 and 200 ml/min, respectively, to minimize the noise. The position of the column outlet was adjusted by using a screw nut to minimize detector noise. The best position of the outlet was just at the inner wall of the gas confluent cell of the detector (see Fig. 1). A too deep or too shallow position resulted in a highly noisy output. These conditions made the flame stable and minimized the noise output. The more turbulent flow in the flame seemed to produce more noise in the detector output. Fig. 2 shows plots of detector output under both flame-on and flame-off conditions. The noise observed was approximately $1 \cdot 10^{-14}$ A/mV, being at the same level as inherent in one of the electronic circuits. Little noise derived from the flame was detected. Once the detector conditions were set, the noise level and the analytical response were very constant whenever the detector was used throughout a year.

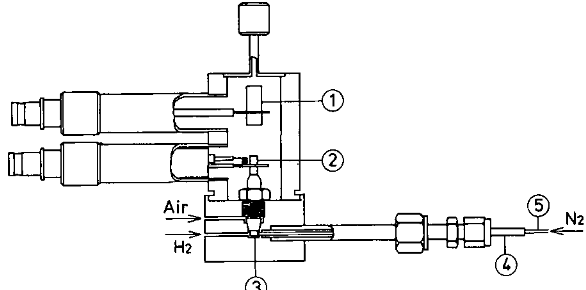

Fig. 1. Sensitive FID system. 1 = Electrode, ion collector; 2 = jet flame tip (orifice diameter, 0.51 mm); 3 = gas confluent cell; 4 = stainless-steel tube (1.5 mm I.D.); 5 = analytical column.

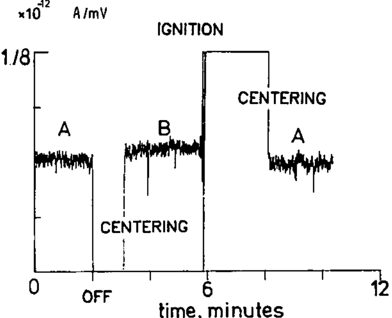

Fig. 2. Noise level of the detector output. (A) Flame-on; (B) flame-off.

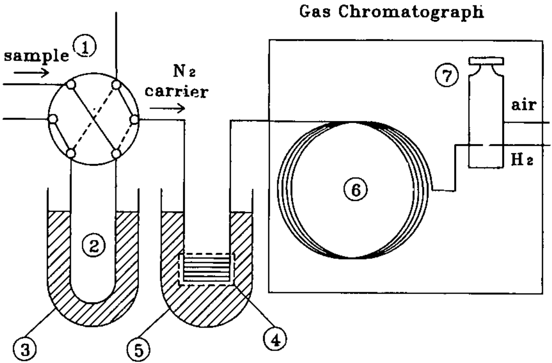

Fig. 3. Analytical system of air sample. 1 = Six-way switching valve; 2 = first cryogenic trap; 3 = liquid oxygen bottle (−183°C) or hot water bottle (100°C); 4 = second trap (squalane-coated 300 cm × 0.25 mm I.D. stainless-steel capillary tube spiralled and covered with 1-mm-thick glass-fibre ribbon); 5 = liquid oxygen bottle (−183°C); 6 = analytical column; 7 = sensitive FID system

Detection of hydrocarbons

The above modification was for minimizing the FID noise under the flame-on condition and not for maximizing an absolute response output to hydrocarbons. Table I indicates the detection limits and the sensitivities of typical C_2–C_{11} hydrocarbons as observed in the atmosphere. As a result of suppressing the detector noise, the sensitivities were 20–100 times better than those of conventional FID assemblies [15–18]. The linear dynamic range was 10^{-12} to 10^{-4} g for the hydrocarbons. Bleeding-free capillary columns and the sensitive FID system may be useful to determine ultra-traces of such hydrocarbons at the low noise level.

Determination of atmospheric hydrocarbons

A 50–1000 ml volume of air sample, subjected to the cryogenic concentration, was enough for determination of the atmospheric hydrocarbons. In this case, no clogging due to water vapour occurred in the packed column and the capillary tubes during the concentration. The water vapour and other substances coexisting in the sample had little effect on the separation efficiency of the analytical columns and the detector performance. In this case, two-stage concentration was used to determine simultaneously a number of C_2–C_{11} hydrocarbons.

The second trap was exposed at room temperature after retrapping the organics at a liquid oxygen temperature (see Fig. 3). When the C_2–C_4 hydrocarbons were eluted from the analytical column, the temperature-programmed GC analysis was started. In this way, the C_2–C_{11} hydrocarbons were simultaneously separated and determined at ultratrace levels.

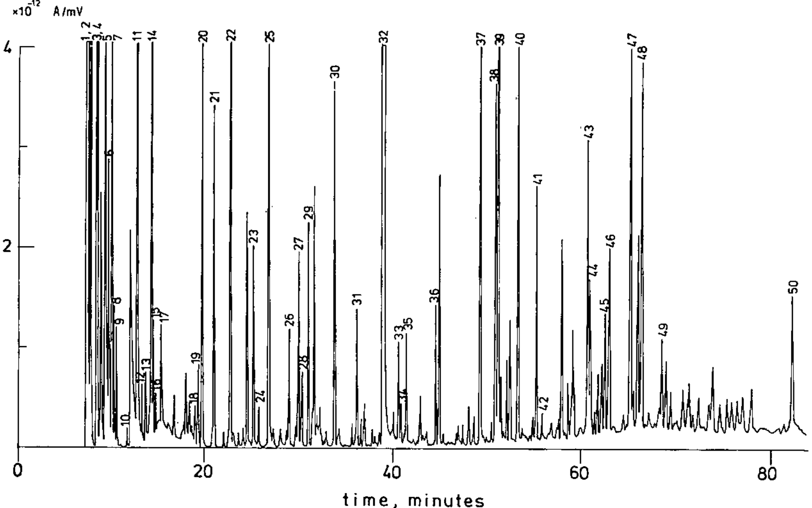

Fig. 4. Typical chromatogram of the determination of trace levels of atmospheric hydrocarbons: the sample volume was 1000 ml; the analytical column used was the squalane capillary column; see Table II for the hydrocarbons.

TABLE II

TYPICAL ANALYSIS OF ATMOSPHERIC HYDROCARBONS AT THE ENVIRONMENTAL POLLUTION CONTROL CENTER, OSAKA CITY

Sample volume, 200 ml; analytical column used, squalane capillary column. Concentration (v/v) in parts per American billion (10^9).

No.	Hydrocarbon	Concentration (ppb)	No.	Hydrocarbon	Concentration (ppb)
1	Ethylene	23.3	26	Cyclohexane	0.8
2	Ethane + acetylene	24.3[a]	27	2-Methylhexane	1.5
3	Propylene	6.1	28	2,3-Dimethylpentane	0.6
4	Propane	8.9	29	3-Methylhexane	1.7
5	Isobutane	5.1	30	*n*-Heptane	2.0
6	Isobutene + 1-butene	3.6	31	Methylcyclohexane	0.7
7	*n*-Butane	11.0	32	Toluene	31.1
8	*trans*-2-Butene	1.1	33	2-Methylheptane	0.6
9	*cis*-2-Butene	0.9	34	4-Methylheptane	0.3
10	3-Methyl-1-butene	0.2	35	3-Methylheptane	0.7
11	Isopentane	10.6	36	*n*-Octane	0.6
12	1-Pentene	0.4	37	Ethylbenzene	3.8
13	2-Methyl-1-butene	0.7	38	*p*-Xylene	2.3
14	*n*-Pentane	7.7	39	*m*-Xylene	5.4
15	*trans*-2-Pentene	0.7	40	*o*-Xylene	2.8
16	*cis*-2-Pentene	0.3	41	*n*-Nonane	0.7
17	2-Methyl-2-butene	0.5	42	Isopropylbenzene	0.1
18	Cyclopentane	0.4	43	*m*-Ethyltoluene	2.0
19	2,3-Dimethylbutane	0.8	44	*p*-Ethyltoluene	1.1
20	2-Methylpentane	3.9	45	*o*-Ethyltoluene	0.7
21	3-Methylpentane	3.1	46	1,3,5-Trimethylbenzene	1.2
22	*n*-Hexane	5.5	47	1,2,4-Trimethylbenzene	2.9
23	Methylcyclopentane	1.7	48	*n*-Decane	0.9
24	2,4-Dimethylpentane	0.3	49	1,2,3-Trimethylbenzene	0.6
25	Benzene	5.1	50	*n*-Undecane	0.4

[a] The value was obtained by using the relative response of acetylene in Table I because *ca.* 90% of the peak was occupied by acetylene.

Table II indicates an example of determination of hydrocarbons in the atmospheric environment by using the squalane column and sensitive FID, and Fig. 4 shows a typical chromatogram of the atmospheric hydrocarbons. The hydrocarbons could be determined at ultra-trace levels without any problems, such as column-clogging, column/detector contamination and disappearance of polar or reactive components, as often seen in conventional analyses of atmospheric samples.

CONCLUSIONS

The proposed sensitive FID was useful to determine sensitively hydrocarbons in the atmospheric environment without effects of water vapour and other coexistent substances. A more sensitive detector signal could be available if a modern electronic data-processing system is applied. In any case, sensitive FID may be useful for trace analysis of many other types of samples on bleeding-free capillary columns.

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CHROMSYMP. 2716

Analysis of semivolatile organic compounds by headspace gas chromatography

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ABSTRACT

Through an unconventional application of static headspace gas chromatography (GC), organic solutes of low volatility were determined at sub mg/l or lower levels in organic matrices of higher volatility as well as in aqueous matrices. Organic compounds such as phenol, biphenyl, diphenyl ether, 1,3,5-triethylbenzene, diphenylethanes, and monochlorobiphenyl with boiling points as high as 291°C were determined in complex matrices by headspace GC without preconcentration or other sample handling.

INTRODUCTION

Static and dynamic headspace analysis have been widely used with gas chromatography (GC) for analysis of trace amounts of volatile organic components. Dynamic headspace analysis is used for concentration of volatiles in matrices such as biological fluids and environmental samples. The main reasons for using headspace analysis are to avoid injecting compounds with high boiling points that may not elute from the column and to increase the amount of solutes of interest reaching the detector. Dirty or complex samples drastically affect quantitative results, shorten the lifetime of capillary columns, and increase the down time of the instrument. Headspace analysis, when applicable, alleviates the problems associated with dirty and complex samples. Headspace analysis of volatile components in a wide range of matrices is covered in the literature [1–8].

Determination of residual solvents in high-boiling pharmaceutical formulations is an excellent example of using static headspace analysis. Due to the difference in volatility between residual solvents and drugs, the solvents can be selectively injected into the GC system without injecting the drug. Residual monomers in the associated polymers are often analyzed by headspace GC.

Determinations of semivolatile and non-volatile components are usually made by direct injection of the sample into the gas or liquid chromatograph or by selective extraction into another solvent followed by gas or liquid chromatographic analysis. Adsorption onto solid adsorbents followed by solvent extraction is another popular approach for analysis of non-volatile components. Non-volatile compounds such as lactic acid and succinic acid [9] have been methylated in a headspace vial, and then the volatile methyl esters were determined by headspace analysis.

The sensitivity of static headspace analysis depends on several factors such as the volume ratio of the gas and liquid phases and the partition coefficient of the analyte between the liquid and gas phases at the equilibrium temperature used [10]. The sensitivity of the headspace technique can be improved by increasing the concentration of the analyte in the headspace in equilibrium with the liquid phase. Depending on the matrix, this can be done by adjusting the pH, adding a solvent, adding salt, or increasing the equilibration temperature. For example, the sensitivity of the headspace analysis of residual monomers in polymers has been increased by spiking the polymer solution with water [11]. This sensitivity enhancement has been attributed to decreased solubility of the monomers in the solution

which increases the equilibrium concentration of the monomers in the headspace. For a detailed discussion of the methods for increasing sensitivity of static headspace analysis and applications of these techniques, see refs. 10 and 12.

Many improvements have been made in headspace technology. The most important is automation, which reduces deviations in the sample size injected. Another improvement is the ability to work at higher temperatures. Some headspace analyzers allow the sample to be heated to 200°C and the valve, sample loop, and transfer line to 250°C. The higher temperature of the sample increases the sensitivity of the analysis, while the higher temperature of the transfer line reduces carry-over effect. Thus, the higher temperature capabilities extend the range of compounds that can be determined by the headspace technique. However, the risk of degrading the sample or bursting the container or losing the analyte due to chemical interaction with the vial septum must be considered when using the high temperatures.

In this work, headspace analysis with matrix modifiers and/or high equilibrium temperatures was used to determine semivolatile components such as phenol, biphenyl, diphenyl ether, 1,3,5-triethylbenzene, 1,1-diphenylethane, and 1,2-diphenylethane in an aqueous matrix and monochlorobiphenyls in an organic matrix of moderate volatility.

EXPERIMENTAL

Chemicals

n-Propylbenzene, 1,3,5-triethylbenzene, 1,2-diphenylethane, and biphenyl were obtained from Aldrich (Milwaukee, WI, USA). Dimethyl sulfoxide and phenol were obtained from Fisher Scientific (Pittsburgh, PA, USA). 2-Phenylphenol was obtained from Mobay Chemical (now known as Miles). 1,1-Diphenylethane was synthesized in the laboratory, and diphenyl ether was obtained from the Dow Chemical (Midland, MI, USA).

Chromatographic system

Chromatographic studies were carried out with a Varian 3400 gas chromatograph equipped with both flame ionization and electron-capture detectors and a Varian Genesis headspace analyzer. The headspace analyzer was connected to the gas chromatograph via a transfer line through the split injector. A 30 m × 0.25 mm I.D. column with 0.25 μm DB-FFAP was obtained from J & W Scientific (Folsom, CA, USA) and a 60 m × 0.32 mm I.D. column with 1.0 μm Rt_x-20 was obtained from Restek (Bellefonte, PA, USA). Other GC conditions are described in the figure captions.

Non-aqueous samples

Several solutions of *o*-, *m*- and *p*-chlorobiphenyls were prepared in the range from 2 mg/l to 47 mg/l in *n*-propylbenzene. The solvent, *n*-propylbenzene, was chosen as representative of organic matrices with moderate volatility. Aliquots (5 ml) were transferred to 20-ml headspace vials.

The vials were equilibrated at 150°C for 15 min. A 1-ml aliquot of the headspace above the solution was injected into the gas chromatograph via a transfer line that was held at 190–230°C. Electron-capture detection (ECD) was used with these analyses.

Aqueous samples

A stock solution of *n*-propylbenzene, 1,3,5-triethylbenzene, biphenyl, diphenyl ether 1,1-diphenylethane, 1,2-diphenylethane, and 2-phenylphenol was prepared in dimethyl sulfoxide. Dilute solutions were prepared by successive dilutions of the stock solution in water. The vials were equilibrated at 95°C for 20 min. For comparison, dilute solutions were also prepared in *n*-propylbenzene. These were equilibrated at two temperatures, 95°C and 135°C, for 20 min. The transfer line was held at 230°C. Flame ionization detection (FID) was used with these analyses. Other conditions are the same as for non-aqueous analysis. Higher equilibration temperatures of aqueous samples was not attempted because of the associated complexity of the analysis at those conditions.

A water solution containing 5 μg/l phenol was prepared. Analysis of a 5-ml aliquot of the phenol solution was performed. Analysis of phenol solutions that were spiked with either 1-ml or 2-ml aliquots of *n*-propylbenzene to make a total sample volume of 5 ml was performed and compared to the unspiked sample analysis.

RESULTS AND DISCUSSION

Non-aqueous samples

Determination of monochlorobiphenyls in complex matrices has been routinely done by GC, however, the procedure is tedious. A typical sample preparation consists of concentrating the analytes on a solid adsorbent and then washing them off with a solvent. The resulting solution is analyzed by GC. Headspace analysis is an appealing alternative to the adsorption and concentration of the monochlorobiphenyl analytes. However, due to their low volatility this would be an unconventional application of the headspace technique. Nonetheless, headspace GC analysis with high equilibration and vapor transfer temperatures was investigated for the determination of low levels of monochlorobiphenyls. The boiling points and vapor pressures of the compounds of interest at various temperatures are listed in Table I. The data in Table I are only indicative of what happens in the vials during equilibration. The partitioning in the phases is a very important factor and affects the sensitivity of the analysis. A 15-min equilibration at 150°C produced adequate analyte concentrations in the vapor phase for the purpose of this study. No optimization of the equilibration time was done. Steady-state conditions might not have been achieved for all solutes in a 15-min period. Manual injection of the headspace sample via a syringe is problematical because vapors can condense in the syringe. Instead, a valve injector was used. By maintaining the valve and transfer line at 190–230°C vapor condensation was avoided.

TABLE I

PHYSICAL PROPERTY DATA

Data obtained from the Dow physical property data bank.

Compound	Boiling point (°C)	Vapor pressure data	
		Temperature (°C)	Pressure (Pa)
o-Chlorobiphenyl	275.7	95	198
		135	1418
		150	2640
m-Chlorobiphenyl	291.5	95	109
		135	867
		150	1661
p-Chlorobiphenyl	291.5	95	116
		135	863
		150	1636
n-Propylbenzene	159.2	95	13 823
		135	52 191
		150	79 549
1,3,5-Triethylbenzene	218.7	95	1675
Biphenyl	253.1	95	570
Diphenyl ether	258.1	95	359
1,1-Diphenylethane	272.6	95	214
1,2-Diphenylethane	280.8	95	161
o-Phenylphenol	286.2	95	116
Phenol	181.9	95	4343

The partitioning of the analytes between the liquid phase and the vapor phase is an important factor in determining the sensitivity of the analysis. Several factors contribute to the low concentration of such high boiling compounds in the vapor phase: the high boiling point, the low vapor pressure and favorable partitioning in the liquid phase. However, the use of sensitive, selective detection, such as ECD, can overcome the low concentrations of those semivolatiles in the vapor phase. This is especially useful in complex samples where many compounds may be present and may complicate the analysis if a universal detection system, such as FID, were used.

Fig. 1 shows a chromatogram for *o*-, *m*- and *p*-chlorobiphenyls in *n*-propylbenzene. The combination of high equilibration temperature and a selective detector allows the analysis of such semivolatiles by the static headspace technique.

Linearity plots for concentrations that range from 2 to 47 mg/l gave a correlation coefficient, *r*, of 0.99 for each isomer. The relative precision ranged between 24 and 27% at the 95% confidence level for 6, 2, and 6 mg/l concentration level of *o*-, *m*- and *p*-chlorobiphenyls, respectively.

Aqueous samples

Semivolatile solutes in an aqueous matrix represent a difficult challenge for headspace GC because the sample equilibration temperature must be below the boiling point of the matrix. In order to avoid the hazards associated with high pressure in the headspace vial, the equilibration temperature of aqueous samples should be limited to a maximum of 100°C.

To demonstrate the analysis of semivolatile compounds in an aqueous matrix, a standard solution that contained *n*-propylbenzene, 1,3,5-triethylben-

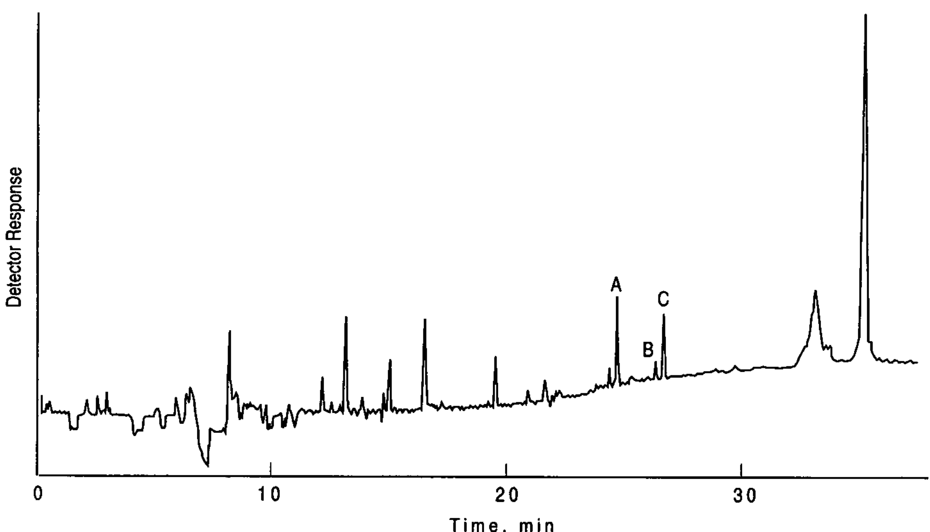

Fig. 1. Gas chromatogram of a headspace sample of a solution that contained monochlorobiphenyls. Oven temperature: 80°C for 1 min then to 280°C at 8°C/min with a 10-min final hold; column: 60 m × 0.32 mm I.D. coated with 1.0-μm Rt_x-20; detector: electron capture at 350°C; injector: split (68 ml/min), 300°C. Peak identification: A = *o*-chlorobiphenyl (6.4 mg/l), B = *m*-chlorobiphenyl (2.2 mg/l), C = *p*-chlorobiphenyl (6.6 mg/l).

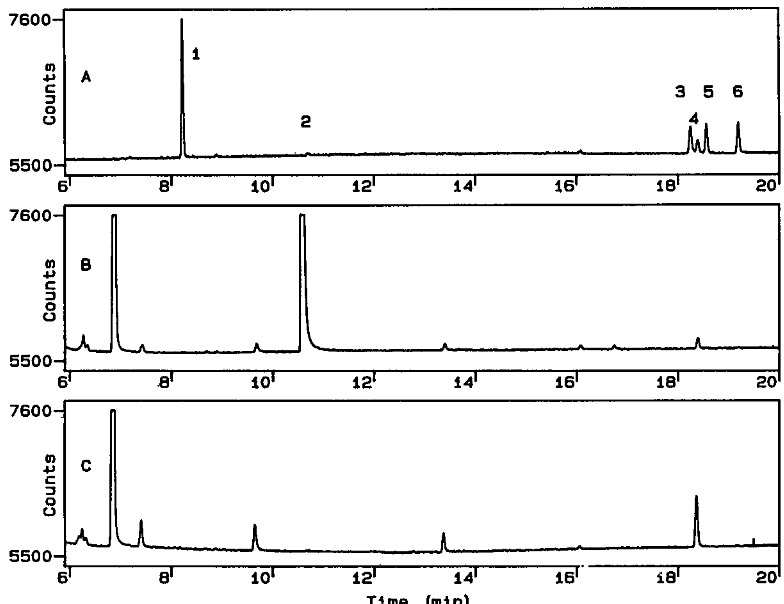

Fig. 2. (A) Gas chromatogram for semivolatiles in water. Oven temperature: 80°C for 1 min then to 230°C at 6°C/min; column: 30 m × 0.25 mm I.D. coated with 0.25-μm BD-FFAP; detector: flame ionization at 300°C; injector: split (68 ml/min), 250°C. Peak identification: 1 = 1,3,5-triethylbenzene (0.40 mg/l), 2 = dimethyl sulfoxide, 3 = biphenyl (0.23 mg/l), 4 = diphenyl ether (0.28 mg/l), 5 = 1,1-diphenylethane (0.19 mg/l), 6 = 1,2-diphenylethane (0.50 mg/l). Sample size: 5 ml. (B) Same as in (A) except solution was prepared in *n*-propylbenzene. (C) Same as (B) except that 2 ml *n*-propylbenzene was replaced with 2 ml water.

zene, biphenyl, diphenyl ether, 1,1-diphenylethane, 1,2-diphenylethane, and 2-phenylphenol was prepared in dimethyl sulfoxide and diluted with water to concentrations ranging from 13 to 693 μg/l. To contrast the behavior of these analytes in water to their behavior in an organic matrix, a solution that contained all the solutes except for *n*-propylbenzene was prepared in *n*-propylbenzene. Solute concentrations in this solution ranged from 170 to 500 μg/l. The sample prepared in *n*-propylbenzene was equilibrated at two different temperatures, 95°C and 135°C, before analysis by headspace.

Only diphenyl ether and dimethyl sulfoxide were detected at both equilibration temperatures. However, analysis of the water solution showed very good response for all the components except dimethyl sulfoxide and 2-phenylphenol. The relative precision for the determination of each of these components (*i.e.*, all except dimethyl sulfoxide and 2-phenylphenol) at a concentration of 200 μg/l ranged from 8.6 to 12.9% at the 95% confidence level. In a separate experiment the detection limit for 2-phenylphenol was found to be 3.4 mg/l with a signal-to-noise ratio (S/N) of 3. Thus, the conditions reported here were not suitable for the determination of sub mg/l concentrations of 2-phenylphenol.

The difference in responses of dimethyl sulfoxide and diphenyl ether is related to the difference in partition coefficients of those solutes between *n*-propylbenzene and water. Due to the different interaction and partition of the solutes in the different solvents, the response of certain compounds will be more favorable in one solvent *versus* another. Addition of water to the *n*-propylbenzene solution enhanced the response of diphenyl ether but decreased the response of dimethyl sulfoxide which is more soluble in water than is diphenyl ether. Representative chromatograms that demonstrate the difference in responses are shown in Fig. 2.

The importance of the matrix effect described above is clearly illustrated in the determination of phenol. Determination of μg/l levels of phenol by headspace analysis under the conditions described above cannot be achieved in an aqueous matrix because of the favorable partitioning into the water phase. However, addition of an immiscible solvent, such as *n*-propylbenzene, in which phenol has considerable partitioning, enhances the sensitivity tremendously. Fig. 3 shows a comparison of chromatograms obtained for 5 μg/l phenol in water. Fig. 3A is for a water blank. Fig. 3B, C, and D show the enhancement of the response of phenol due to the addition of 0-, 1- and 2-ml aliquots of *n*-propylbenzene. Several experiments were performed to determine how changes in the relative volumes of water, *n*-propylbenzene, and gas phase would affect the analysis. In all cases, the addition of *n*-propylbenzene improved the sensitivity of the analysis. This sensitivity enhancement cannot be explained by favorable partitioning of the solute since phenol was found to partition almost equally between the two solvents. The addition of a non-electrolyte, *n*-propylbenzene, increases the activity coefficient of phenol. This increases the vapor pressure of phenol which enhances the sensitivity of the analysis.

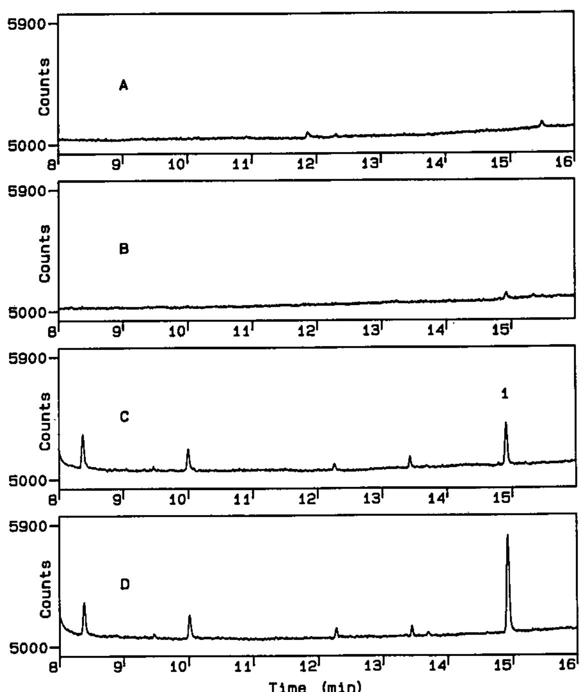

Fig. 3. Gas chromatograms of phenol in water. (A) Water blank; (B) sample size: 5 ml water solution containing 5 μg/l phenol; (C) sample size: 4 ml water solution + 1 ml *n*-propylbenzene; (D) same as (C) but sample size 3 ml water + 2 ml *n*-propylbenzene. Peak identification: 1 = phenol. Oven temperature: 70°C for 3 min then to 210°C at 12°C/min. Other conditions are same as in Fig. 3.

Carry-over effects were studied for non-aqueous and aqueous analysis. A solvent blank run was

analyzed after each sample. No interference or carry-over was noticed when this procedure was practiced.

CONCLUSIONS

The range of compounds that can be determined by static headspace GC has been extended to include semivolatile materials with boiling points as high as 291°C. High equilibration temperatures and matrix modifiers were used to increase the vapor concentration of semivolatile solutes in aqueous and organic matrices of moderate volatility. A selective detector can be used to enhance the response of the analytes relative to other matrix components. Sub mg/l detection levels were demonstrated for some of the semivolatile solutes.

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CHROMSYMP. 2659

Gas chromatographic–mass spectrometric determination of lower aliphatic tertiary amines in environmental samples

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ABSTRACT

A method is described for determining lower aliphatic tertiary amines in environmental samples such as river water and bottom sediments. The method consists of distillation of aliphatic tertiary amines and determination by headspace gas chromatography–mass spectrometry. The detection limits for final 40-ml samples were 50 ng for trimethylamine, 10 ng for triethylamine, 5 ng for triallylamine, 10 ng for tripropylamine and 5 ng for tributylamine. The recoveries were over 70% and the relative standard deviations of the recoveries were below 12% (n = 5). The method is easy to operate, shows excellent sensitivity and selectivity and is applicable to environmental samples.

INTRODUCTION

Lower aliphatic tertiary amines are important as industrial raw materials for agricultural medicines, medicines, surfactants, dyes, etc., and are used in vast quantities. These compounds have such an unpleasant smell and high toxicity that working environmental standards are imposed on many of them. Tripropylamine in particular is included among high-toxicity chemical substances in Japan. For such reasons, it is necessary to develop a microanalytical method for aliphatic tertiary amines in order to monitor their concentration levels in environmental samples.

Analysis for aliphatic tertiary amines have frequently been reported [1–12], mainly involving analytical methods for trimethylamine in the atmosphere and for trimethylamine in tissues to determine the freshness of fish and shellfish. A few reports have appeared on triethylamine, triallylamine, tripropylamine and tributylamine [13–19]. However, little has been reported on these amines in aqueous or bottom sediment samples; only Prokopeva *et al.* [15], Yamamoto [20] and Selyutina and Vinnikov [18] have presented some methods. Prokopeva *et al.*'s method, for the analysis of amines in industrial waste waters at μg/ml concentrations directly by GC with flame ionization detection (FID), is too low in sensitivity to apply directly to environmental samples. In Yamamoto's method the sample water is acidified and concentrated to make

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it strongly alkaline, amines are vaporized by purging with nitrogen and trapped in water, then the aqueous solution is subjected to GC with flame thermionic detection (FTD). The drawbacks of this method are that a large amount of sodium hydroxide is used and the detector has low sensitivity. In Selyutine and Vinnikov's method, potassium hydroxide solution is added directly to the sample water to make it alkaline, then it is purged with nitrogen and amines are trapped in sulphuric acid. The solution obtained is concentrated and made alkaline, then the headspace gas is subjected to GC–FID. As with Yamamoto's method, this method also uses large amounts of alkali and is low in sensitivity.

With the above in mind, we report here the development of a method with a detection limit for amines below the μg/l level using a headspace analysis method that minimizes solvent and reagent use and analysis by capillary column GC–MS that has increased selectivity.

EXPERIMENTAL

Reagents

Sodium hydroxide and sodium chloride were of analytical-reagent grade. Trimethylamine (TMA), triethylamine (TEA), triallylamine (TAA), tripropylamine (TPA) and tributylamine (TBA) were purchased at the highest purity available. $[^2H_{15}]$Triethylamine (TEA-d_{15}) was obtained from MSD Isotopes.

Sodium chloride was heated for 3 h at 600°C before use. Water was distilled and passed through a cation-exchange resin for purification.

Apparatus

The gas chromatograph was a Hewlett-Packard Model 5890 and the mass spectrometer was a JEOL Model JMA-AX505W.

GC–MS measurement conditions

The conditions for GC–MS with selected ion monitoring (SIM) were as follows: column, TC-1, 5-μm film thickness 30 m × 0.53 mm I.D. (GL Science); column temperature, initially 40°C for 2 min, increased to 180°C at 20°C/min and held at 180°C 5 min; injection port temperature, 180°C; separator and ion source temperatures, 250°C; ionization voltage, 70 V; ionization current, 300 μA; carrier gas (helium) linear velocity, 75 cm/s; monitored ions (*m/z*), 59 (TMA), 86 and 101 (TEA), 110 and 137 (TAA), 114 and 143 (TPA), 142 and 185 (TBA) and 98 (TEA-d_{15}).

Standard procedure

Aqueous samples. A water sample of 500 ml is placed in a 1 distillation flask, 5 ml of 2.5 *M* sodium hydroxide solution and 1.0 μg of TEA-$_{15}$ are added and 40 ml of the sample solution are distilled out into 5 ml of 0.025 *M* sulphuric acid as absorption solution at a distillation rate of about 1 ml/min. The 40-ml distillate is transferred into a 75-ml vial containing 8 g of sodium chloride, 10 ml of 15 *M* sodium hydroxide solution are added and immediately the vial is stoppered. The vial is shaken well and kept in a water-bath at 40°C for 1 h, then 1 ml of the headspace gas is injected into the GC–MS system for analysis.

Bottom sediment samples. A 50-g wet sediment sample is placed in a 1-l distillation flask containing 300 ml of purified water, 1.0 μg of TEA-d_{15} and 5 ml of 2.5 *M* sodium hydroxide solution are added and 40 ml of the sample solution are distilled out into 5 ml of 0.025 *M* sulphuric acid as absorption solution at a rate of about 1 ml/min. The 40-ml distillate is treated in the same way as for aqueous samples.

RESULTS AND DISCUSSION

Examination of distillation conditions

Lower aliphatic amines can be distilled out relatively easily under alkaline conditions, so in the present method distillation is used for both clean-up and concentration.

For the examination of recovery, each of the standard substances was placed in 500 ml of purified water 5 ml of 2.5 *M* sodium hydroxide solution were added and distillation was conducted to give 10-ml fractions. As the results in Table I show, the tertiary amines may be almost 100% recovered when 20 ml have been distilled; a 40-ml distillation is sufficient even for TBA, which is lowest to distil. For bottom sediment samples, 300 ml of purified water were added to 50 g of bottom sediment and the recovery was measured in the same way as for aqueous samples. The results were similar to those

TABLE I

RELATIONSHIP BETWEEN RECOVERY AND PORTIONS OF DISTILLATE FROM AQUEOUS SAMPLE

Portion of distillate (ml)	Recovery (%)					
	TMA	TEA-d_{15}	TEA	TAA	TPA	TBA
0–10	95.8	98.0	97.8	92.6	96.7	83.6
10–20	3.5	1.5	1.6	5.4	2.5	11.9
20–30	0.7	0.5	0.6	1.3	0.6	3.2
30–40	0	0	0	0.7	0.2	1.3
40–50	0	0	0	0	0	0

for the aqueous samples. For this reason, the present method adopts the addition of 5 ml of 2.5 *M* sodium hydroxide solution and the 40-ml distillate into 5 ml of 0.025 *M* sulphuric acid as adsorption solution. In addition, a distillation rate as slow as 1 ml/min has been found to be suitable.

Examination of headspace conditions

As the present method involves a headspace procedure, which is simple to be operate and not susceptible to interfering substances, the optimum vaporizatin conditions were examined.

Effect of sodium chloride concentration on vaporization efficiency. Into five 75-ml vials, 0, 2.5, 5.0, 7.5 and 10 g of sodium chloride were placed, followed by 40 ml of purified water, 5 μg of each standard substance and 10 ml of 15 *M* sodium hydroxide solution. The vials were immediately stoppered and kept in a water-bath at 40°C for 1 h, then the headspace gases were analysed by GC–MS.

The relationship between the sodium chloride concentration and the vaporization efficiency was thus examined. The results (Fig. 1) show that the vaporization efficiency at saturation increased *ca.* 5 times for TMA, TEA and TEA-d_{15}, *ca.* 3 times for TAA and TPA and *ca.* 1.5 times for TBA relative to no addition of sodium chloride. The salting-out effect increased as the molecular size decreased. For this reason, saturation with sodium chloride was adopted.

Effect of temperature on vaporization efficiency. For the headspace method, it was expected that higher temperatures would increase the vaporization efficiency. The vaporization efficiency was ex-

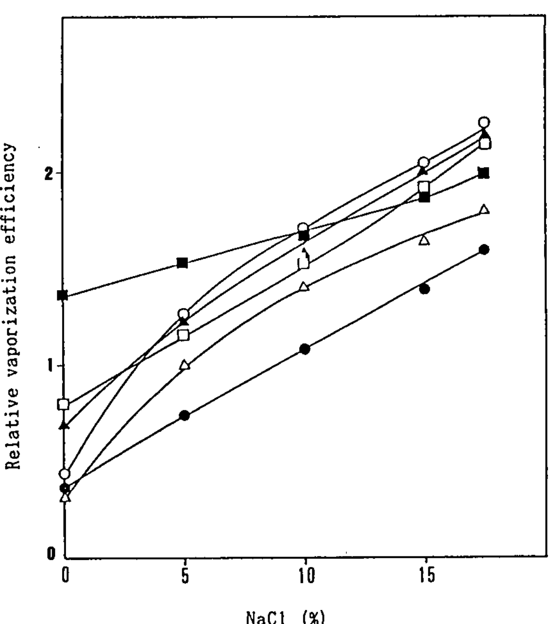

Fig. 1. Relationship between vaporizatin efficiency and sodium chloride concentration. ● = TMA; ○ = TEA; ▲ = TAA; □ = TPA; ■ = TBA; △ = TEA-d_{15}.

amined by adding sodium chloride to saturation under the above-mentioned conditions and varying the water-bath temperature from 20 to 50°C at intervals of 10°C. For TMA, the vaporization efficiency increased *ca.* 4 times at 50°C relative to that at 20°C, and for the other substances 1.5–2 times (Fig. 2). In addition, it was found that higher temperatures tended to increase the variation in the measured values, so that 40°C was adopted.

Effect of sodium hydroxide concentration on vaporization efficiency. Conventionally, potassium hydroxide, sodium carbonate or sodium hydroxide has been used for the vaporization of aliphatic amines. On the precondition that the present method should use the cheapest salt, vaporization at different sodium hydroxide concentrations in the range 0–6 *M*, was examined, with saturated sodium chloride and the water-bath temperature kept at 40°C.

As the results in Fig. 3 show, the relationship between the alkali concentration and the vaporization efficiency was linear for TMA without giving any maximum vaporization efficiency, but TEA, TAA, TPA and TBA yielded maximum vaporization effi-

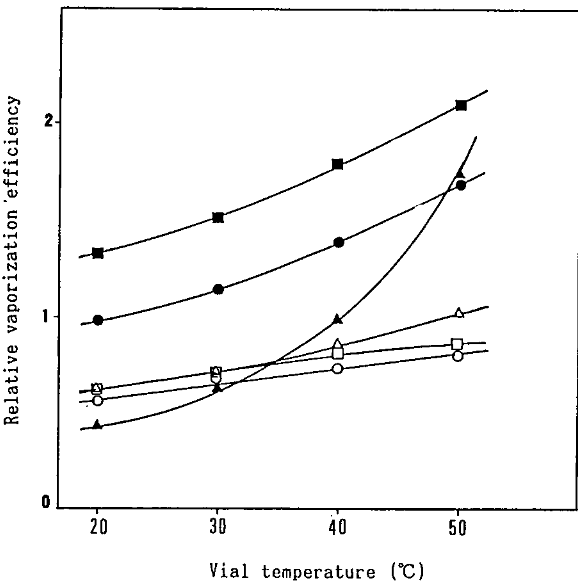

Fig. 2. Relationship between vaporization efficiency and vial temperature. ▲ = TMA; ● = TEA; ○ = TAA; □ = TPA; ■ = TBA; △ = TEA-d_{15}.

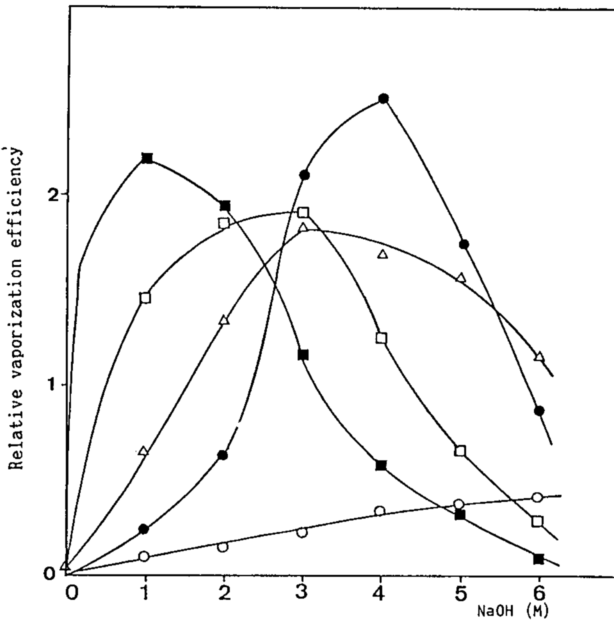

Fig. 3. Relationship between vaporization efficiency and sodium hydroxide concentration. ○ = TMA; ● = TEA; △ = TAA; □ = TPA; ■ = TBA.

ciencies 4, 3, 2.5–3 and 1 *M*, respectively. This indicates that the alkali concentrations for the maximum vaporization efficiency tend to decrease with increasing length of the alkyl group. Based on this result, an intermediate concentration of 3 *M* for the sodium hydroxide solution was adopted.

Examination of GC–MS measurement conditions

The columns used for analyses for aliphatic amines are typically packed columns in which Tenax GC or Chromosorb 103 is coated with potassium hydroxide. These columns are low in efficiency and induce adsorption at low concentrations and therefore, have been considered inapplicable to the microanalysis of environmental samples. In this work, satisfactory separations were obtained with a wide-bore capillary column using a thick layer of a non-polar liquid phase such as OV-1 rather than a high-polarity phase such as PEG or FFAP.

To select the monitoring ions for SIM measurement, MS measurements were made in the electron impact mode. It was found that, except for TMA, monitoring can be effected with base ions. For TMA, no selective fragments were found except *m/z* = 59, which was therefore adopted for monitoring. The SIM chromatograms of the standard substances are shown in Fig. 4.

Calibration and detection limits

To construct the calibration graphs, 8-g amounts of sodium chloride were placed in 75-ml vials, then 40 ml of purified water, 1.0 μg of TEA-d_{15} and variing amounts of a standard substance were added, finally 10 ml of 15 *M* sodium hydroxide solution were added. The vials were immediately stoppered, shaken well and kept in a water-bath at 40°C for 1 h, then 1 ml of the headspace gas was injected into the GC–MS system. The peak-area ratios with respect to the internal standard were used to construct calibration graphs. The detection limits for the final 40-ml sample solution were found to be 50, 10, 4, 10 and 5 ng for TMA, TEA, TAA, TPA and TBA, respectively.

Recovery experiments and application to real samples

The recovery obtainable by the method was examined by adding 0.5 μg of a standard substance

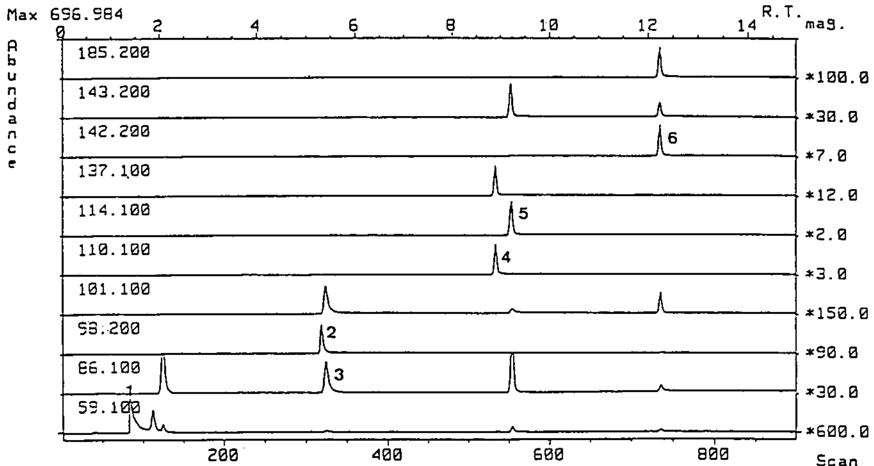

Fig. 4. SIM chromatogram of aliphatic amines. Peaks: 1 = TMA; 2 = TEA-d_{15}; 3 = TEA; 4 = TAA; 5 = [illegible] Retention time in min.

TABLE II

RECOVERY OF ALIPHATIC TERTIARY AMINES FROM RIVER WATER AND BOTTOM SEDIMENT

Compound	River water			Bottom sediment		
	Added (μg)	Recovery (%)	R.S.D.[a] (%)	Added (μg)	Recovery (%)	R.S.D.[a] (%)
TMA	0.5	69.4	11.7	50	85.3	4.9
TEA	0.5	89.0	9.3	0.5	87.6	10.2
TAA	0.5	80.4	8.3	0.5	73.7	12.2
TPA	0.5	81.0	8.7	0.5	84.3	7.3
TBA	0.5	73.4	12.0	0.5	82.3	5.1

[a] $n = 5$.

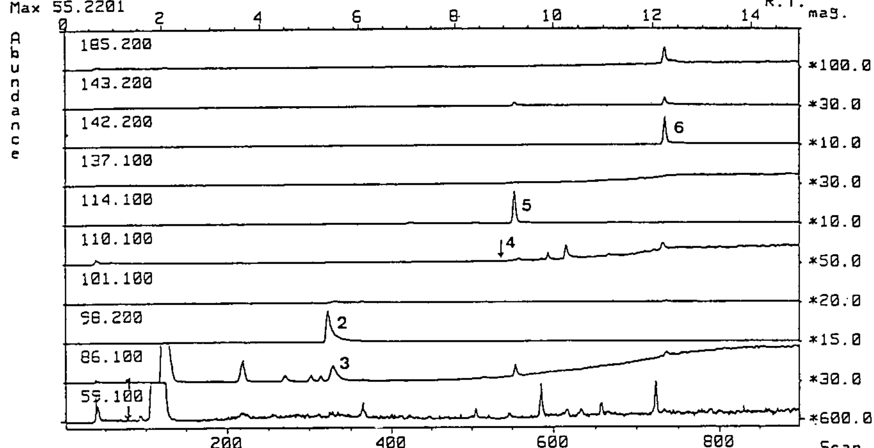

Fig. 5. SIM chromatogram of pond water from Suwa Lake. Peaks as in Fig. 4.

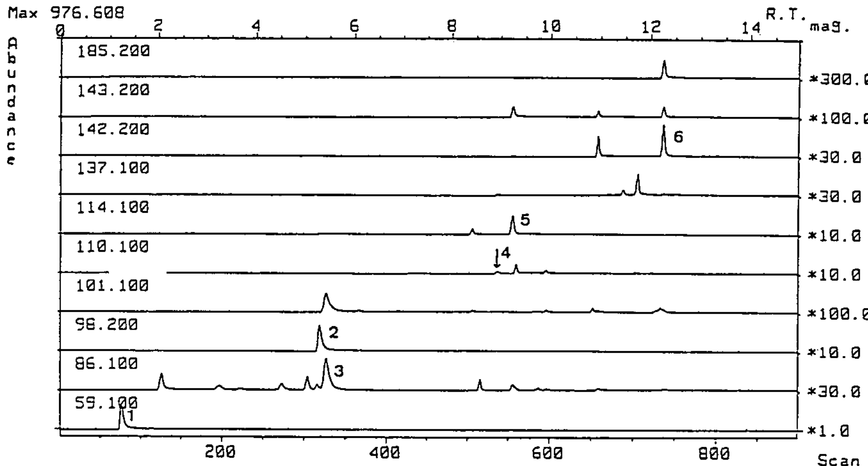

Fig. 6. SIM chromatogram of bottom sediment from Tokyo Bay. Peaks as in Fig. 4.

(50 μg of TMA for bottom sediment samples having high blank TMA values) to either 500 ml of water from the Susobana River in Nagano City or 50 g of bottom sediment from Tokyo Bay. As the results in Table II shows, the recovery was over 70% with a relative standard deviation below 12%.

As application examples, the method was applied to waters and bottom sediments from rivers, lakes and marshes in Nagano Prefecture and bottom sediment from Tokyo Bay (n = 12). Fig. 5 shows the results for water from Suwa Lake and Fig. 6 those for bottom sediment from Tokyo Bay. The water from Suwa Lake contained 0.25 μg/l of TEA and 0.23 μg/l of TBA and the bottom sediment from Tokyo Bay contained 0.53 μg/g of TMA, 4.0 μg/kg of TEA, 0.26 μg/kg of TPA, 1.0 μg/kg of TBA and no TAA.

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Development of automated gas chromatographic–mass spectrometric analysis for natural volatile organic compounds in the atmosphere

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ABSTRACT

An automated system for the trace determination of natural volatile organic compounds (dimethyl sulphide, bromoform, isoprene and its reaction products) was developed. A volume of 200 ml of air was collected on a small Tenax TA trap cooled electrically to 15°C. After desorption it was subjected to capillary GC–MS (selected ion monitoring) analysis. After each five air analyses, a standard gas generated with permeation tubes was analysed for calibration. With a sampling volume of 200 ml, the detection limits were dimethyl sulphide 2.4, isoprene 1.9 and bromoform 1.3 ppt (v/v) at a signal-to-noise ratio of 3. This method was applied successfully to the monitoring of natural organics at Tsukuba, Japan, where 1800 data sets were obtained during the period July–December 1991.

INTRODUCTION

Naturally derived organics in the atmosphere are now of major interest, as some are intimately involved in global atmospheric chemistry. Dimethyl sulphide (DMS), produced mainly by marine algae, is photochemically oxidized to non-sea salt sulphate in the atmosphere [1], potentially contributing to cloud formation and the Earth's radiation budget [2]. Bromoform and some other bromomethanes, also emitted from the ocean, may be important in the springtime surface-ozone destruction in the Arctic [3]. Isoprene, a biogenic hydrocarbon from terrestrial plants, and its reaction products, methacrolein (MAC) and methyl vinyl ketone (MVK), can contribute to the photochemical production of ozone and organic acids [4]. Globally, they might be a significant source of carbon monoxide and also be important in determining tropospheric OH concentration [5].

As these naturally derived organics are often present at ppt–ppb (v/v) levels in the atmosphere, their measurement is difficult, usually being performed by collection and preconcentration either on adsorbents or in canisters or cryogenic traps, with final analysis by GC. Because the mixing ratios of these compounds are likely to change drastically owing to the fluctuation of source- and sink-strength, serial measurements on-site are necessary to understand better their impact on atmospheric chemistry. To accomplish this, we developed a fully automatic analysis for on-site ambient monitoring of volatile organics, based on capillary GC–MS, especially focused on DMS, bromomethanes and isoprene and its reaction products.

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EXPERIMENTAL

The method is based fundamentally on normal cryogenic concentration followed by thermal desorption and GC–MS analysis. Special attention was paid to the reduction of the dead volume, adsorptive losses of organics on sampling lines, undesirable water vapour effects and good chromatographic separation, all necessary for the precise measurement of organics in the ppt range.

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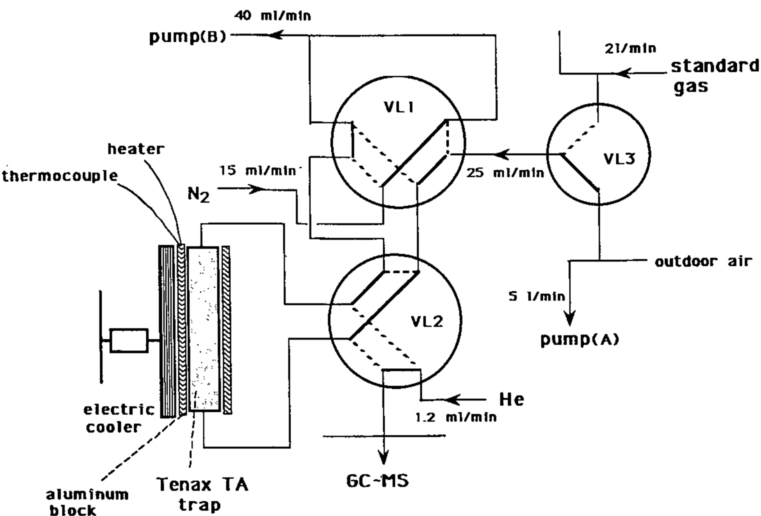

Fig. 1. Schematic diagram of preconcentration system.

Preconcentration unit

The concentration unit for automatic GC analyses of hydrocarbons reported elsewhere [6] was used after some modifications (Fig. 1). Automatic selection of sampling air and standard gas was accomplished by adding another switching valve (VL3). An electric cooler (based on the Peltier effect) was used to lower the trap temperature instead of flushing with liquid CO_2 as in previous work [6]. Electric cooling, which requires no cryogen, is more suitable for field studies, although liquid CO_2 can easily give a much lower temperature. The trap contained 0.08 g of Tenax TA in a stainless-steel tube (5 cm × 4 mm O.D.), set inside a small aluminium block with two ceramic heaters and thermocouples inserted. The electric cooler was mounted on a cylinder and only for cooling was it pressed on the block to lower the trap temperature. Air was drawn in at a high flow-rate (5 l/min) using pump A, and some was supplied to the concentration system, to reduce adsorptive losses of sampled organics on the sampling tube (PTFE). A diaphragm pump (B) drew both sample air and purging nitrogen gas (15 ml/min) at a flow-rate of 40 ml/min, resulting in 25 ml/min of air passing through the trap. All flow-rates were controlled with thermal mass flow controllers.

To avoid adsorption of components in the sampled air on the valves and connecting tubes, their temperature was maintained at 150°C with a heating cable.

Gas chromatograph–mass spectrometer

GC–MS was performed using a Hewlett-Packard Model 5971 mass-selective detector directly interfaced with an HP 5890 gas chromatograph. A Poraplot Q column (10 m × 0.32 mm I.D.) was used for GC separation. This had been used successfully for the separation of bromomethanes without problems of subambient cooling of the capillary column [7]. The column oven temperature was initially maintained at 55°C for 7 min, then programmed to 210°C at 12°C/min. The flow-rate of helium carrier gas was controlled at 1.2 ml/min by a thermal mass flow controller.

The mass-selective detector was used in the selected ion monitoring (SIM) mode. Table I lists the target compounds with their retention times and monitored ions. The time programme of the monitored ions was m/z 68, 62 and 58 for 10–14 min, m/z 70, 82, 130 and 174 for 14–16.5 min and m/z 94 and 173 for 16.5–23 min. In order to confirm that there was no interference with the target compounds, the qualifier ions in Table I were also monitored in several air analyses.

TABLE I

RETENTION TIMES AND MONITORED IONS FOR THE TARGET COMPOUNDS

Compound	Retention time (min)	Monitored ion (m/z)	Qualifier ion (m/z)
Dimethyl sulphide (DMS)	12.4	62	(47)
Acetone	12.7	58	(43)
Isoprene	13.1	68	(67)
Methacrolein (MAC)	14.3	70	(41)
Methyle vinyl ketone (MVK)	14.8	70	(55)
3-Methylfuran	14.9	82	(53)
Dibromomethane	15.9	174	(172)
Trichloroethylene	16.0	130	(132)
Dimethyl disulphide (DMDS)	17.2	94	(79)
Bromoform	19.6	173	(171)

Standard gas

A standard gas mixture of DMS, dibromomethane and bromoform at the ppb level was generated using thermostated permeation tubes. Certification of the mixing ratios of the generated standard gas was done from the mass loss of the permeation tubes, measured several times during 5 months. This standard was analysed in the same manner as the atmospheric samples, but with a smaller sampling volume, and was used for calibration during the analytical runs.

To determine components other than DMS, dibromomethane and bromoform, the relative sensitivity was calculated from liquid standard analyses at the beginning and end of the serial measurements. Liquid standard containing *ca.* 100 pg of each standard (DMS, dibromomethane, bromoform, isoprene, trichloroethylene, MAC, MVK) in 0.5 μl of methanol was injected into a 5-ml glass vial on-line via helium to the preconcentration trap. The vial was heated to 100°C and the standards were collected on the trap and then desorbed in the usual way. Both methods provided a good correlation between permeation tubes and the liquid standards for the compounds. Liquid standard was also used for the studies of linearity of the response and detection limits.

Procedure

The time programme of the sequential analyses was as follows. The procedure starts with cooling the trap to 15°C. For quantitative trapping of the target compounds in a 200-ml air sample, 15–20°C was cool enough (see below), and no water filter was required because of the low trapping efficiency of water at this temperature. The next steps are trapping of the air sample (200 ml total) for 8 min, thermal desorption and sweeping the trapped compounds into the capillary column for 7 min and start of the GC–MS analysis. During the analysis, the trap was baked for 5 min under a nitrogen flow. These processes were repeated automatically every 45 min, using a time controller.

After every five air analyses, standard gas was analysed using the same procedure, but with a sampling time of 2 min.

Field study

The method was applied successfully for the automatic measurement of DMS, bromoform, isoprene and its reaction products at Tsukuba, located at 36°05′N, 140°10′E and 50 km inland of the Pacific Ocean. Field research was conducted at the monitoring station of the National Institute for Environmental Studies, adjacent to pine forests and some farms. Some 2–3-week serial measurements were repeated during the period July–December 1991.

RESULTS AND DISCUSSION

Analytical method

Figure 2 shows chromatograms of an air sample. The chromatographic separation is excellent with-

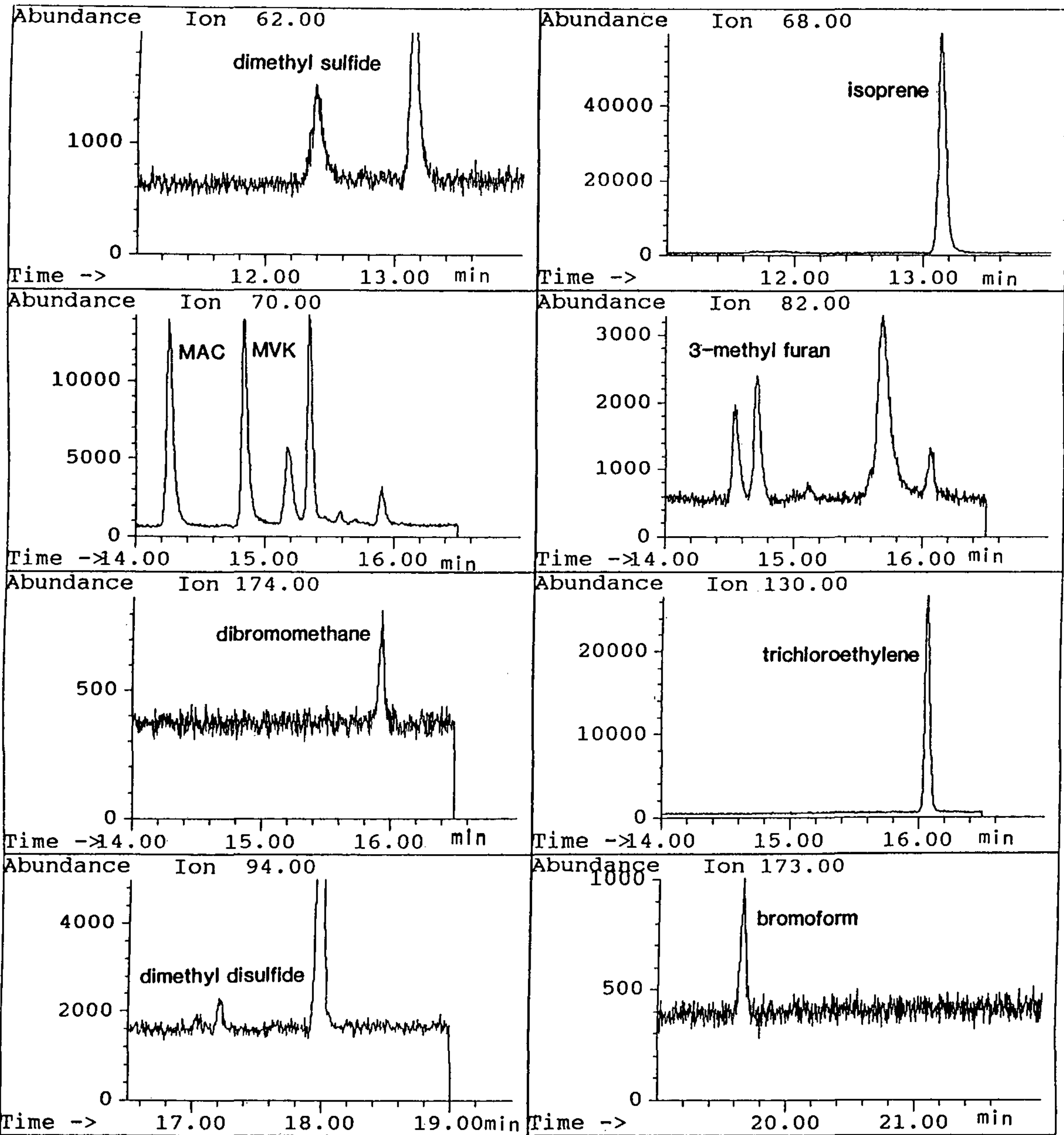

Fig. 2. Ion chromatograms of an air sample (200 ml) collected at Tsukuba at 13:18 h on August 31st, 1991.

out cryogenic cooling of the capillary column. The peak width of DMS is larger than those of the other compounds, but can be improved by lowering the initial temperature to 40–45°C and focusing more precisely on the top of the colum.

The method was validated by determining breakthrough volume, recovery, linearity of detector response and detection limit.

Breakthrough volume. The breakthrough volume was determined by extrapolating the retention vol-

umes of each compound on Tenax TA used as a GC column. DMS has the smallest breakthrough volume for Tenax TA of the five compounds examined. That of DMS was 24, 12 and 6.8 l per gram of Tenax TA at 0, 10 and 20°C, respectively, corresponding to 1.9 l 960 ml and 540 ml breakthrough volumes for the trap containing 0.08 g of Tenax TA. Therefore, under these sampling conditions, where 200 ml of air was collected at 15°C, 100% of these compounds in the sample can be trapped. Quantitative sampling (sampling volume smaller than breakthrough volume) was also confirmed directly by trapping standards on the trap, passing a known volume of helium through it and analysing as for atmospheric samples.

Recovery. The recovery efficiencies for the compounds from the trap were determined by changing the thermal desorption time for the collected standards on the Tenax TA trap to the capillary GC column. It was found that 7 min was sufficient to desorb all the compounds with helium carrier gas (1.2 ml/min) at 210°C. Quantitative recovery was also confirmed by comparison with direct injection of the liquid standard onto the capillary GC column.

The relative standard deviation of five analyses of standard gas was excellent: 2.4 % for DMS, 1.5% for dibromomethane and 0.49% for bromoform.

Linearity and detection limit. Linearity in the range 10 pg–2 ng was excellent for all the compounds. Detection limits at a signal-to-noise ratio of 3 were determined by extrapolation from the analysis of 10 pg of standards and were 1.3 pg for DMS, 1.6 pg for dibromomethane, 2.9 pg for bromoform and 1.2 pg for isoprene. Based on a 200-ml air sample, the relative detection limits by volume are 2.4 ppt for DMS, 1.0 ppt for dibromomethane, 1.3 ppt for bromoform and 1.9 ppt for isoprene.

The analytical procedure described above was developed for naturally derived organics. It is also applicable to trichloroethylene, tetrachloroethylene, benzene and some other anthropogenic organics. Given the trapping efficiency of Tenax TA and the adsorptive property of the Poraplot Q column, this method is potentially useful for non-polar organic compounds with boiling points approximately in the range 30–160°C.

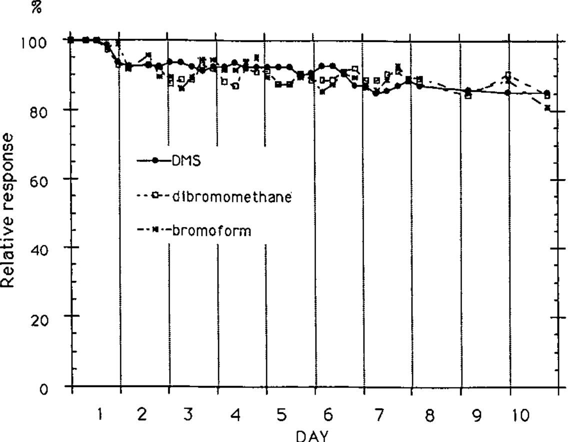

Fig. 3. Change of response of detector for standard gas after tuning. Analysis of a standard was performed after every five air analyses, except for on the 8th–10th days, where it was performed after every 21 air analyses. Monitored ions are m/z 62 for DMS (●), m/z 174 for dibromomethane (□) and m/z 173 for bromoform (×).

Serial measurements of naturally derived organics in the atmosphere

The response for standard gas was generally stable, although it showed a gradual decrease. The raw data for the responses for the standards during a serial measurement with no adjustment for of the mass-selective detector are shown in Fig. 3. Therefore, tuning of the mass-selective detector was not often required (usually once every 1–2 weeks), demonstrating its practical usefulness for field monitoring. The change in response for DMS (m/z 62) differs from those for dibromomethane (m/z 174) and bromoform (m/z 173) (Fig. 3). This may have resulted from a change in the relative response of high mass to low mass.

During the July–December 1991 field study 1800 data sets were obtained. These appear the first to be on-site automatic serial measurements for bromoform, DMS and the reaction products of isoprene using GC–MS. Our objective here was not a precise study of the variation of these compounds; those results will be reported elsewhere. However, as an example, the variations of isoprene, DMS and bromoform over 1 week are shown in Figs. 4–6.

The diurnal variations of isprene, DMS and bromoform differed. The mixing ratio of isoprene was much higher in daytime than at night (Fig. 4),

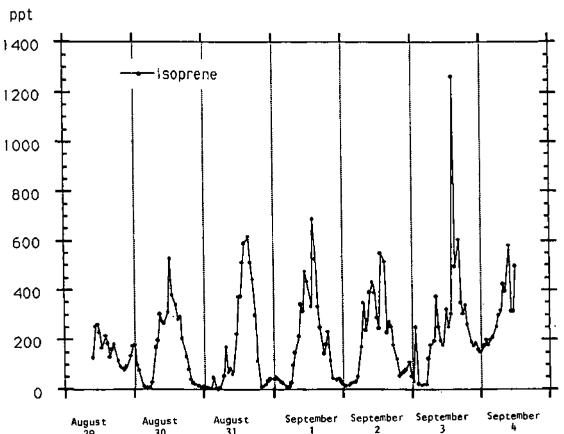

Fig. 4. Variation of the mixing ratio of isoprene in the atmosphere (Tsukuba, August 29th–September 4th, 1991).

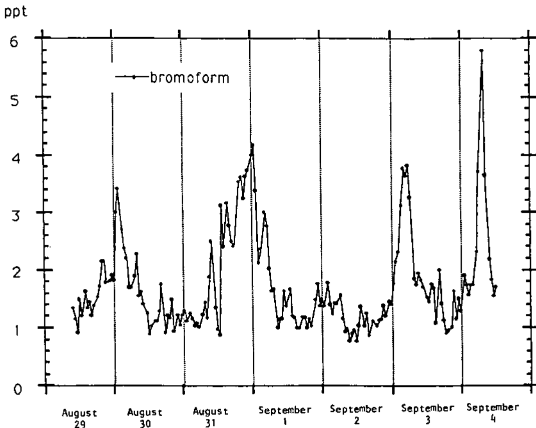

Fig. 5. Variation of the mixing ratio of DMS in the atmosphere (Tsukuba, Augst 29th–September 4th, 1991).

whereas DMS and bromoform were most abundant at night or in the early morning (Figs. 5 and 6).

A higher mixing ratio of isoprene in clear daylight hours was observed previously in forest air [8]. This was explained by isoprene emission being greatly increased in the daytime, although chemical reaction and dilution are also active. It is reasonable to assume, therefore, that the mixing ratio of isoprene was relatively low, even in the daytime on cloudy days (August 29th). Two reaction products of isoprene, methacrolein and methyl vinyl ketone, showed afternoon maxima, just after the maximum of isoprene.

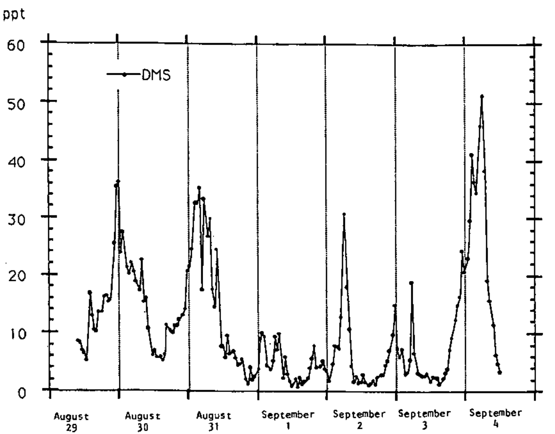

Fig. 6. Variation of the mixing ratio of bromoform in the atmosphere (Tsukuba, August 29th–September 4th, 1991).

A much higher mixing ratio was observed at night than during the day for DMS (Fig. 5). In clean marine air, Andrea *et al.* [9] reported tha the mixing ratio of DMS in the afternoon was about one third lower than that during the night maxima. This agreed with model simulations involving OH oxidations of DMS in the atmosphere. In this study, however, a much more significant diurnal variation was often observed. Possible explanations for the greater differences of the DMS mixing ratio between day and night at Tsukuba are (1) the atmosphere over land is much more stable at night, thereby suppressing dilution and leading to a high mixing ratio, and (2) the loss of DMS through atmospheric reaction with OH and ozone occurring during transportation from the ocean to Tsukuba was lower at night.

Another marine organic compound, bromoform, is decomposed through photolysis in the atmosphere. This is effective only in daytime. Therefore, its mixing ratio is also expected to be higher at night owing to suppressed dilution and photolysis at that time, which is similar to the condition for DMS. However, the diurnal variations of bromoform and DMS are not always coincident (for example, August 30th–31st). This results mainly from the change in the relative strength of their source: DMS is emitted from the soil and the ocean, whereas the ocean is considered the only source for bromoform. Even in the ocean, the relative ratio of DMS and bromoform in sea-water might change daily.

CONCLUSIONS

The automated preconcentration–GC–MS system described here is a very powerful method for trace determinations of such natural volatile organic compounds as bromoform, isoprene and DMS. The detection limits for these compounds in a 200-ml of sample air were *ca.* 2 ppt at a signal-to-noise ratio of 3. The response of the mass-selective detector was stable enough to be compensated for by occasional standard gas analyses.

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Application of gas chromatographic retention properties to the identification of environmental contaminants

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ABSTRACT

Gas chromatographic retention indices (*I*) were measured for congeners of commonly encountered classes of environmental contaminants, which are often reported as tentatively identified compounds. Several commonly encountered polycyclic aromatic hydrocarbons were employed as *I* reference standards. These indices were related to boiling points with reasonable accuracy, allowing the estimation of *I* for additional members of those classes.

INTRODUCTION

Gas chromatography (GC) has long been applied to environmental analysis for both separation and identification of target analytes. High-resolution capillary columns have greatly increased the separation power, while selective detectors have improved the identification power of GC. In addition to selective detectors such as electron-capture, nitrogen–phosphorus and Hall's electrolytic conductivity detectors, both mass spectrometers and Fourier-transform infrared spectrometers have been widely employed for identification of compounds separated by GC.

The US Environmental Protection Agency (EPA) has monitored pesticides in the environment for many years using GC with selective detectors [1]. Confirmation may be performed by a second analysis using a GC column having different polarity from that of the first. The relative retention times of analytes on the two columns are then compared against a data base for identification. As another example of the multiple-column confirmation technique, Albro *et al.* [2] analyzed polychlorinated biphenyl commercial mixtures on 13 GC column liquid phases and calculated retention index (*I*) values to assist in compound identification.

It is often easier to apply the power of gas chromatographic relative retention times for the identification of large numbers of compounds when multiple reference compounds are used; *I* values are calculated against those reference standards. Temperature programming is generally the technique of choice due to the wide variety of structures and retention properties of target environmental contaminants.

Van den Dool and Kratz [3] described a temperature-programmed *I* scheme using the normal hydrocarbons. This approach permitted successful *I* modelling of the 1700 brominated, chlorinated, and bromochlorinated dioxins [4], and of the chlorinated dibenzofurans [5]. In these studies, the mass spectra of different isomers with the same level of chlorina-

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tion/bromination were indistinguishable. Hence, mass spectrometry povided insufficient data for isomer-specific identifications. The gas chromatographic I data were essential to obtain isomer specificity.

Vassilaros *et al.* [6] have reported a similar scheme using several polynuclear aromatic hydrocarbons (PAHs). Their I scheme possesses advantages for many types of environmental analyses, because the reference compounds may be indigenous to the samples. Archived data may be studied, and tentative compound identification can be made. Furthermore, the chromatographic behavior against temperature and temperature programming rates of the PAH reference compounds may more closely parallel the behavior of other compounds of interest. The Lee and Kováts indices have been employed for certain members of other compound classes having environmental significance, including phenols, alcohols, ketones, nitriles and esters [7].

GC–MS has emerged as an extremely important routine technology for environmental analysis and confirmation. The EPA conducts thousands of analyses each year by GC–MS for compounds of environmental interest, generally using a combination of user-generated mass spectral libraries and a commercial data base of mass spectra. Many of these analyses are performed under the Contract Laboratory Program (CLP), under rigorous, prescribed conditions [8]. Components of environmental extracts that are not identified by the computerized mass spectral library search are labeled "tentatively identified compounds" (TICs). When subjected to manual mass spectral interpretation, many of these TICs turn out to be aliphatic hydrocarbons or other compounds of minor toxicological or ecological interest. Some retention time windows, however, are believed to include the retention times of compounds having more serious implications for the environment.

It was decided to tabulate the I of several classes of compounds having environmental significance, against the PAH reference compounds indigenous to many environmental samples. These tabulated I values could then be used along with the mass spectral libraries for identification of TICs found while analyzing samples, and while reviewing data packages from previous analyses.

EXPERIMENTAL

Retention indices were determined on a Hewlett-Packard 5988 gas chromatograph–mass spectrometer, with 200°C injector temperature, and autosampler-controlled 2-μl injections. The mass spectrometer was operated in full-scan, electron ionization mode (70 eV). A J&W Scientific DB-5 30 m × 0.32 mm fused-silica capillary column with 0.25-μm film thickness was temperature programmed as follows: 3 min at 40°C, 8°C/min to 285°C, hold for 29.5 min. Helium carrier gas was used at 2.5 ml/min. Data presented in Table VIII were obtained with a 30 m × 0.25 mm DB-5 column with 0.25-μm film thickness, under the above conditions. Retention indices were calculated according to the method reported by Rostad and Pereira [7]. Solutions of commercially available standards were prepared in methylene chloride at 100 ng/μl. Retention index values were determined using high-purity analytical standards purchased from commercial suppliers. The following classes of target analytes were studied: carboxylic acids, 1-*n*-alkenes, esters, alkyl phenols, aldehydes, *n*-alkylbenzenes and cyclic alkenes. Duplicate determinations were made on three separate days.

RESULTS AND DISCUSSION

From the experimental retention time measurements, the average I were calculated for each analyte (see Tables I–VIII). In most cases, the R.S.D. for individual measurements of I did not exceed 0.1%. Indices were determined relative to naphthalene, phenanthrene, chrysene, and benzo[*ghi*]perylene.

The calculations of I were performed according to the procedure described by Rostad and Pereira [7] which was based on the equation suggested by Van den Dool and Kratz [3]. For index (I_X) of a compound X:

$$I_X = 100\left[\frac{T_X - T_n}{T_{n+1} - T_n}\right] + 100n$$

where T_X is the retention time of compound X; T_n and T_{n+1} are the retention times of the PAHs used as reference compounds; n is the largest ring number of the reference compounds eluting before compound X; and $n+1$ is the smallest number of fused

TABLE I

ALKYL-SUBSTITUTED PHENOLS

Compound	B.p. [9]	Calc. b.p.	Calc. *I*	Exptl. *I*	%Diff.
2-Methylphenol[a]	191		164.42	170.89	3.79
4-Methylphenol[a]	201.9		177.79	175.32	−1.41
3-Methylphenol[a]	202.2		178.16	175.41	0.56
2,6-Dimethylphenol[a]	212		190.18	182.78	−4.05
2-Ethylphenol[a]	207		184.04	188.28	2.25
2,4-Dimethylphenol[a]	210		187.72	190.86	1.65
2,5-Dimethylphenol[a]	211.5		189.56	190.94	0.72
4-Ethylphenol[a]	219		198.76	194.51	−2.19
3-Ethylphenol[a]	214		192.63	194.63	1.03
2,3-Dimethylphenol[a]	218		197.53	196.83	−0.46
3,4-Dimethylphenol[a]	225		206.12	199.63	−3.25
2,4,6-Trimethylphenol[a]	221		201.21	202.51	0.64
2-*n*-Propylphenol[a]	220		199.99	204.88	2.39
3-Propylphenol	228		209.80		
2,3,5-Trimethylphenol[a]	230		212.25	214.69	1.14
2,4,5-Trimethylphenol	232		214.70		
4-Propylphenol	232.6		215.44		
2-*n*-Butylphenol	236		219.61		
2,3,5,6-Tetramethylphenol[a]	247		233.10	231.28	−0.79
3-*n*-Butylphenol	248		234.32		
4-*n*-Butylphenol	248		234.32		

[a] Data used for derivation of the *I* relationship yields the following equation: b.p. $= 0.815I + 56.93$; $R^2 = 0.935$.

TABLE II

n-ALKYLCARBOXYLIC ACIDS

Compound	B.p.[b]	Calc. b.p.	Calc. *I*	Exptl. *I*	%Diff.
Acetic acid	117		57.68		
Propionic acid	141		84.70		
Butanoic acid	165.5		112.28		
Pentanoic acid[a]	186		135.37	137.02	1.20
Hexanoic acid[a]	205		156.76	158.12	0.86
Heptanoic acid[a]	223		177.03	177.85	0.46
Octanoic acid[a]	239.3		195.38	196.58	0.61
Nonanoic acid[a]	268 [10]		227.69	214.27	−6.26
Decanoic acid[a]	270		229.94	231.58	0.71
Undecanoic acid[a]	280		241.20	247.95	2.72
Dodecanoic acid	131^{1}	299.58		263.25	
Tridecanoic acid	236^{100}	312.30		277.57	
Tetradecanoic acid	250.5^{100}	324.89		291.75	
Pentadecanoic acid	257^{100}	337.82		306.30	
Hexadecanoic acid	350, 227^{100}	351.89	320.02	322.15	0.66
Heptadecanoic acid	227^{100}	365.03		336.97	
Octadecanoic acid	360d	377.45		350.93	
Eicosanoic acid	328d	401.36		377.85	

[a] Data used for derivation of the *I* relationship yields the following equation: b.p. $= 0.888I + 65.78$; $R^2 = 0.976$.

[b] Ref. 9, except as noted in brackets. Superscripted numbers indicate reduced pressure boiling points, mmHg (1 mmHg = 133.322 Pa). Decomposition upon boiling is noted with d.

TABLE III

n-ALKYL ESTERS

Compound	B.p.[b]	Calc. b.p.	Calc. I	Exptl. I	%Diff.
Ethyl acetate	77.06		78.71		
Methyl butyrate	102.3		101.58		
Ethyl butyrate	124		121.23		
Propyl butyrate[a]	143		138.44	135.60	−2.09
Methyl hexanoate[a]	151 [10]		145.69	141.95	−2.64
Butyl butyrate	166.6		159.82		
Ethyl hexanoate[a]	168 [10]		161.09	159.04	−1.29
Methyl heptanoate[a]	172		164.71	164.64	−0.04
Butyl acetate	186		177.39		
Ethyl heptanoate	188 [10]		179.20		
Methyl octanoate[a]	194 [10]		184.64	185.69	0.57
Ethyl octanoate[a]	207 [10]		196.41	200.00	1.80
Methyl nonanoate[a]	213 [10]		201.85	205.26	1.66
Hexyl acetate	223		210.91		
Butyl heptanoate	226.2		213.80		
Octyl butyrate	244.1		230.02		
Ethyl decanoate[a]	245 [10]		230.83	235.83	2.12
Methyl dodecanoate[a]	262^{766} [10]	273.8	246.23	256.91	4.16
Decyl acetate	270		253.48		
Ethyl dodecanoate		285.6		267.59	
Octyl heptanoate	290		271.59		
Methyl tetradecanoate[a]	323 [10]		301.49	286.83	−5.11

[a] Data used for derivation of the I relationship yields the following equation: b.p. $= 1.104I - 9.838$; $R^2 = 0.982$.
[b] Ref. 9, except as noted in brackets. Superscripted numbers indicate reduced pressure boiling points, mmHg.

TABLE IV

n-ALKYL-SUBSTITUTED BENZENES

Compound	B.p.[b]	Calc. b.p.	Calc. I	Exptl. I	%Diff.
Benzene	80.1		73.25		
Toluene	110.6		102.53		
Ethylbenzene	136		126.92		
Propylbenzene[a]	159.2	159.3	149.20	149.31	0.07
Butylbenzene	183		172.06		
Pentylbenzene	205.4		193.57		
Hexylbenzene	226 [11]	225.7	213.43	213.07	−0.17
Heptylbenzene[a]	245.5	245.0	232.07	231.69	−0.16
Octylbenzene	262 [11]	263.3	248.01	249.13	0.45
Nonylbenzene[a]	280.1	280.3	265.30	265.56	0.10
Decylbenzene	298 [11]	296.4	282.59	280.92	−0.60

[a] Data used for derivation of the I relationship yields the following equation: b.p. $= 1.041I + 3.821$; $R^2 = 1.00$.
[b] Ref. 9, except as noted in brackets.

TABLE V

CYCLIC ALKENES

Compound	B.p.[b]	Calc. b.p.	Calc. *I*	Exptl. *I*	%Diff.
Cyclohexene	83		77.06		
4-Methylcyclohexene[a]	102.7		95.91	95.57	−0.36
3-Methylcyclohexene	104		97.16		
1-Methylcyclohexene	110		102.90		
1,3,5-Cycloheptatriene[a]	116 [10]		108.64	108.93	0.27
4-Vinylcyclohexene[a]	128.9		120.98	120.10	−0.73
1,5-Cyclooctadiene[a]	150.8^{757}	153.37	141.94	144.40	1.70
d-Limonene[a]	178		167.97	166.62	−0.81

[a] Data used for derivation of the *I* relationship yields the following equation: b.p. $= 1.045I + 2.472$; $R^2 = 0.957$.
[b] Ref. 9, except as noted in brackets. Superscripted numbers indicate reduced pressure boiling points, mmHg.

TABLE VI

PRIMARY ALKENES

Compound	B.p. [9]	Calc. b.p.	Calc. *I*	Exptl. *I*	%Diff.
Hexene	63.3		53.98		
Heptene	93.6		83.28		
Octene	121.3		110.08		
Nonene	146		133.97		
Decene[a]	170.5		157.66	157.55	−0.07
Dodecene[a]	213.4		199.16	199.34	0.09
Pentadecene	268		251.87		
Hexadecene[a]	284.4		267.83	267.76	−0.03

[a] Data used for derivation of the *I* relationship yields the following equation: b.p. $= 0.951I + 17.18$; $R^2 = 0.998$.

TABLE VII

ALDEHYDES

Compound	B.p.[b]	Calc. b.p.	Calc. *I*	Exptl. *I*	%Diff.
Butanal	76 [12]		35.78		
Pentanal	103 [12]		72.21		
Hexanal[a]	128	133.0	105.97	112.67	5.95
Heptanal[a]	152.8	151.0	139.44	137.01	−1.77
Octanal[a]	171	168.6	164.01	160.75	−2.03
Nonanal[a]	191	184.8	191.00	182.61	−4.59
Decanal[a]	208	213.5	213.95	221.34	3.34
Undecanal	117^{18}	226.6		239.08	
Tridecanal	156^{13}	254.7		277.01	
Tetradecanal	166^{24}	255.8		278.43	

[a] Data used for derivation of the *I* relationship yields the following equation: b.p. $= 0.741I + 49.49$; $R^2 = 0.974$.
[b] Ref. 9, except as noted in brackets. Superscripted numbers indicate reduced pressure boiling points, mmHg.

TABLE VIII

RETENTION INDICES FOR SOME ENVIRONMENTAL MONITORING TARGET ANALYTES

Compound	Average I	Compound	Average I
[$^2H_4$]1,4-Dichlorobenzene	161.50	2,6-Dinitrotoluene	250.02
2-Fluorophenol	128.01	3-Nitroaniline	254.17
[$^2H_6$]Phenol	154.35	Acenaphthene	254.81
Phenol	154.73	2,4-Dinitrophenol	257.01
Bis(2-chloroethyl)ether	156.16	4-Nitrophenol	260.36
2-Chlorophenol	156.75	Dibenzofuran	259.69
1,3-Dichlorobenzene	160.41	2,4-Dinitrotoluene	261.83
1,4-Dichlorobenzene	162.02	Diethylphthalate	269.94
Benzyl alcohol	167.16	4-Chlorophenyl phenyl ether	270.89
1,2-Dichlorobenzene	167.36	Fluorene	270.45
2-Methylphenol	171.29	4-Nitroaniline	273.55
Bis(2-chloroisopropyl)ether	171.64	[$^2H_{10}$]Phenanthrene	299.25
4-Methylphenol	175.76	4,6-Dinitro-2-methylphenol	274.09
N-Nitroso-di-*n*-propylamine	176.04	N-Nitrosodiphenylamine	274.93
Hexachloroethane	176.00	2,4,6-Tribromophenol	278.46
[$^2H_8$]Naphthalene	199.33	4-Bromophenyl phenyl ether	285.82
[$^2H_5$]Nitrobenzene	178.88	Hexachlorobenzene	289.85
Nitrobenzene	179.46	Pentachlorophenol	295.77
Isophorone	186.60	Phenanthrene	300.00
2-Nitrophenol	188.87	Anthracene	301.64
2,4-Dimethylphenol	191.20	Di-*n*-butylphthalate	324.02
Benzoic acid	196.52	Fluoranthene	344.75
Bis(2-chloroethoxy)methane	193.99	[$^2H_{12}$]Chrysene	398.90
2,4-Dichlorophenol	196.26	Pyrene	351.40
1,2,4-Trichlorobenzene	198.23	[$^2H_{14}$]Terphenyl	358.16
Naphthalene	200.00	Butylbenzylphthalate	378.40
4-Chloroaniline	202.75	3,3′-Dichlorobenzidine	395.28
Hexachlorobutadiene	205.81	Benz[*a*]anthracene	398.07
4-Chloro-3-methylphenol	218.50	Chrysene	400.00
2-Methylnaphthalene	221.13	Bis(2-ethylhexyl)phthalate	402.21
[$^2H_{10}$]Acenaphthene	253.79	[$^2H_{12}$]Perylene	442.31
Hexachlorocyclopentadiene	228.14	Di-*n*-octylphthalate	420.38
2,4,6-Trichlorophenol	231.04	Benzo[*b*]fluoranthene	430.03
2,4,5-Trichlorophenol	231.91	Benzo[*k*]fluoranthene	430.83
2-Chloronaphthalene	236.02	Benzo[*a*]pyrene	440.32
2-Fluorobiphenyl	233.54	Indeno[1,2,3-*cd*]pyrene	488.94
2-Nitroaniline	241.07	Dibenz[*a,h*]anthracene	488.15
Dimethylphthalate	247.99	Benzo[*ghi*]perylene	500.00
Acenaphthylene	248.87		

rings in the reference compounds eluting after compound X.

The tabulated I values often make it possible to identify environmental contaminants that cannot be readily determined by the mass spectra alone. Since the reference PAH are present in many environmental samples, it is possible to calculate I values for the TICs reported in those analytical data packages. Tentative identifications of those TICs can then be made based upon both the mass spectra and the I. It may also be possible to use I windows to identify which TICs may be compounds of particular significance because they elute in specific I windows.

This study demonstrated a practical application for calculation of I from boiling points, when non-polar or very slightly polar GC columns are used for the analysis. Using the general equation $y = mx + b$, boiling points (y) could be related to I

values (x) multiplied by a factor (m), with a constant value (b) added. This simple relationship provided satisfactory approximations (see Tables I–VIII) for the I of suspected TICs that did not have readily available standards for verification of the structural assignments. Most cases of the calculated and experimental values differed by less than 3%, demonstrating the utility of this technique for calculating I of these classes of compounds. The percent differences (%Diff.) were calculated as follows: %Diff. = [(experimental I − calculated I)/experimental I] × 100.

Two potential limitations of the technique are (1) the need for a number of compounds having similar structures in order to establish values for m and b in the equation $y = mx + b$, and (2) the equation may be too simple to adequately describe some compound classes, or those with multiple functional groups.

Once the relationship between I and boiling point has been determined, it is possible to predict the I for other members of the same compound class. It is then possible to identify additional I windows that may or may not be likely to contain TICs from these classes of compounds. Investigations and data reporting could then focus on I windows occupied by compound classes of interest, or on different I windows that do not contain commonly encountered classes of little interest.

Experimental I values were determined for 71 environmental target compounds (see Table VIII). Reproducibility was good for these target compounds, with R.S.D.s less than 1.8% for the analytes (except benzoic acid, 2.4%) and for the six deuterated internal standards.

CONCLUSIONS

To assist in the identification of tentatively identified compounds found during environmental monitoring analyses, retention time characteristics were expressed as retention indices relative to commonly encountered polynuclear aromatic hydrocarbons. For a relatively non-polar GC column, these indices could be related to boiling points with reasonable accuracy for various members of the chemical classes (or homologous series) tested. This relationship allows for predicting the I of other members of those classes, which may not be readily available as analytical standards.

NOTICE

Although the research described in this article has been funded wholly or in part by the US Environmental Protection Agency through contract 68-CO-0049 to Lockheed Engineering & Sciences Company, Inc., it has not been subjected to Agency review. Therefore it does not necessarily reflect the views of the Agency. Mention of trade names or commercial poducts does not constitute an endorsement or recommendation for use.

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CHROMSYMP. 2755

Use of an automated thermal desorption system for gas chromatographic analysis of the herbicides trifluralin and triallate in air samples

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ABSTRACT

An automated solid particulate adsorption-based air sampling and thermal desorption gas chromatographic system has been evaluated for monitoring trifluralin and triallate vapours in air. Utilizing mini-tubes packed with Tenax-TA resin, capillary gas chromatographic separations of these herbicides with electron-capture detection were equivalent to those obtained with autosampler on-column injection. Linear detector responses and precision exhibiting standard deviations of $<1\%$ with respect to retention time and $<2\%$ with respect to peak area were obtained following thermal desorption of mini-tubes fortified with 0.1 to 50 ng of both compounds. Relative humidity (0–100%) of the air being sampled had little effect on mini-tube breakthrough, with $<1\%$ breakthrough of either herbicide following 28-h sampling periods with a flow-rate of 100 ml min^{-1}. Recoveries of trifluralin and triallate from fortified tubes maintained at room temperature for up to 168 h was quantitative with insignificant cross-contamination between mini-tubes stored in the sampling carousel over this time frame.

INTRODUCTION

Pesticide deposits may dissipate from soil or plant surfaces by volatilization [1] and it is now well established that significant off-target transport of pesticides occurs in the atmosphere as vapour drift [2]. Various air sampling procedures have been used to quantitate off-target vapour drift. Initial monitoring studies utilized liquid sampling trains to trap atmospheric residues [3–5]. Later, solid particulate sorbents, such as silica gel or XAD resins, were employed [6,7]. More recently, organic polymer foams, such as polyurethane foam, which have the advantage of sampling air at much higher flow-rates, have been utilized [8–10].

Each of the above sampling media offers advantages/disadvantages. Although liquid sorbents permit direct gas chromatographic (GC) analysis of sorbed pesticides, a preconcentration step is often required to enhance sensitivity. Polymer foams are the most convenient to use and permit air sampling at high flow-rates, however, as with solid particulate adsorbents, extensive solvent cleanup, usually by Soxhlet extraction, is required prior to sampling. Removal of the adsorbed pesticides from particulate or foam sorbents has typically been accomplished by solvent desorption which, in the case of foams, has generally involved Soxhlet extraction.

Thermal desorption has also been used to strip adsorbed analytes from particulate sorbents directly onto a GC column [11–14]. Since particulate sorbents can also be thermally conditioned prior to sampling and are effectively conditioned for reuse during subsequent analyses, solvent use is essentially eliminated. In addition, sensitivity is greatly enhanced because total analyte residues in the air sample are desorbed directly onto the GC column. Another advantage of thermal desorption is that the process can be automated. Recently, a system utilizing thermal desorption has been described that automates both the sampling and the analysis pro-

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cedures [15]. Integration of the field sampling and laboratory analysis was achieved through a sampling component common to both an automated air sampler and an automated thermal desorption unit mounted on a gas chromatograph. This component was a sample carousel capable of holding 50 mini-tubes packed with a particulate sorbent.

The objective of this study was to evaluate this system for its effectiveness in determining residues of the herbicides trifluralin and triallate in air with a view to utilizing the system in the aerodynamic gradient method of determining post-application herbicide vapour losses from treated fields [16,17].

EXPERIMENTAL

Mini-tube air sampling system (MASS)

The MASS (Canadian Centre for Advanced Instrumentation, Saskatoon, Canada) consisted of a sequence-programmable air sampling unit (Model SAM) and an automated thermal desorption unit (ATDU) together with a common 50-position carousel. The carousel was fitted with borosilicate glass mini-tubes (38 mm × 2 mm I.D.) packed with approximately 14 mg of Tenax-TA resin. The resin was centered within each tube and held in place by a pressed stainless-steel screen on either side. For the field study, the air pump in the SAM was operated at its maximum capacity which produced an air flow-rate of 0.1 l min^{-1} through the mini-tubes. The SAM was programmed for sampling time (15, 30, 60 and 120 min) and with respect to mini-tube position such that some positions were bypassed, these being filled with empty mini-tubes for the later insertion of mini-tubes fortified with standards. For mini-tube analysis, the ATDU was mounted onto a Hewlett-Packard Model 5890A gas chromatograph equipped with a ^{63}Ni electron-capture detector (ECD) and controlled with a Model 5895A data section. The ATDU, directly interfaced to the GC system via an HP-1 fused-silica column (Hewlett-Packard; 25 m × 0.53 mm I.D., 0.88 μm film thickness), was operated isothermally (desorption oven temperature, 210°C) with a desorption cycle time of 5 min. The column oven temperature program of the GC system was as follows: 70°C for 1 min, then 5°C min^{-1} to 270°C, and hold for 5 min. The carrier gas (helium UHP) flow-rate was 6.5 ml min^{-1} while the detector make-up gas (nitrogen UHP) flow-rate was maintained at 70 ml min^{-1}. ECD temperature was set at 350°C. Under the above operating conditions, trifluralin and triallate had retention times of 24.5 and 28.1 min, respectively.

Thermal conditioning of the mini-tubes

A manifold, constructed from stainless-steel tubing and Swagelok components to accomodate eight mini-tubes, was used for the preliminary conditioning of the mini-tubes. The manifold was housed in a GC oven maintained at 325°C (maximum recommended temperature for Tenax-TA) and helium passed through the mini-tubes at 25 ml min^{-1} for 2 h. Then, with the GC column removed, the mini-tubes were cycled three times using the ATDU–GC with an ATDU oven temperature of 210°C and a 10-min desorption cycle. With the GC column installed, this process was repeated with a 5-min desorption cycle until desired background interferences were obtained.

Desorption temperature optimization

Mini-tubes were fortified with trifluralin and triallate by adding to the Tenax-TA resin 1 ng each of both herbicides contained in 10 μl of hexane using a 10-μl syringe. The mini-tubes were then placed in the carousel such that the fortified end of the resin was towards the GC column when the carousel was positioned in the ATDU. The optimum ATDU oven temperature using a 5-min desorption cycle was determined by thermally desorbing the fortified mini-tubes using temperatures from 150 to 220°C at 10°C intervals.

ATDU–GC–ECD linearity/reproducibility

Six-point (0.1, 0.5, 1.5, 10 and 50 ng) calibration curves for the thermal desorption of both herbicides from fortified mini-tubes were determined using the ATDU–GC–ECD. Peak area and retention time reproducibilities were established following replication of the above experiment nine more times.

Carousel storage/cross-contamination study

A carousel was fitted with 8 thermally conditioned mini-tubes, wrapped in aluminum foil, and then placed in a polypropylene container (with screw-cap lid) at room temperature. At weekly intervals, the carousel was inserted into the ATDU–GC–ECD and a single mini-tube analysed to deter-

mine background interferences. To determine whether the carousel contributed to the mini-tube backgrounds, this study was repeated except that the mini-tubes were placed in a 20-ml glass scintillation vial and a mini-tube removed at weekly intervals for analysis using the ATDU–GC–ECD.

Trifluralin and triallate cross-contamination between mini-tubes was studied by interspacing in a carousel mini-tubes fortified with either 10 or 100 ng of each herbicide with unfortified thermally conditioned mini-tubes. The carousel was wrapped in aluminum foil and placed in a polypropylene container (with screw-cap lid) at room temperature for 2 weeks. A mini-tube fortified at each level along with an adjacent unfortified mini-tube were analyzed using the ATDU–GC–ECD at the following times: 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168 and 336 h.

Mini-tube retention/breakthrough study

The PTFE U-tube (Fig. 1) was fortified with 1000 ng each of trifluralin and triallate in 100 μl of hexane and then emersed in the water bath (50°C). Air at approximately 0, 65 or 100% relative humidity (monitored with a Model 911 Dew-All digital humidity analyzer; EG & G Environmental Equipment, Waltham, MA, USA) was continuously drawn through the U-tube at 0.1 l min^{-1} and subsequently through the two mini-tubes arranged in series. The down-stream mini-tube was analyzed using the ATDU–GC–ECD after the following times of continuous air flow: 2, 4, 8, 12, 14, 16, 18, 20, 24, 32 and 48 h. After 48 h, the U-tube was rinsed with 100 ml of hexane and the rinsing concentrated and analyzed by GC–ECD to determine the amount of each herbicide remaining in the U-tube. This experiment was repeated two more times using 5000 and 10 000 ng of each herbicide, except that, rather than fortifying the U-tube, the upstream mini-tube was fortified directly.

Field evaluation

The SAM was mounted at a 1.5-m height on a mast positioned approximately in the centre of a 7.1-ha circular plot. Immediately after surface application of a tank mix of trifluralin and triallate, each at 2.0 kg ha^{-1}, air sampling was commenced. Sampling times of 15 min were used immediately after application when large vapour losses were expected and then, as time after application increased, the sampling program included sampling times of increasing duration. After 120 h, the carousel was removed from the SAM and, after insertion of mini-tubes fortified with trifluralin and triallate standards, loaded into the ATDU–GC–ECD and the air samples analyzed.

RESULTS AND DISCUSSION

The SAM and the ATDU combine to make an integrated system because of the common sample carousel. Once air sampling has been completed,

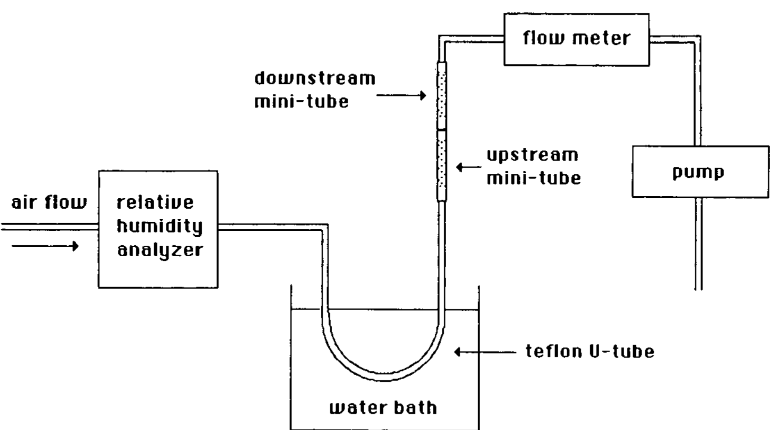

Fig. 1. Apparatus used to determine the breakthrough characteristics of the mini-tubes.

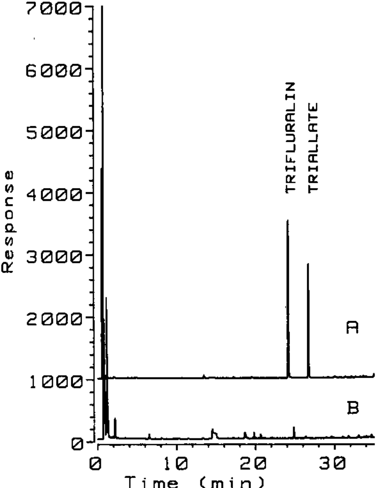

Fig. 2. Mini-tube backgrounds. (A) Background following the extended desorption procedure and three 10-min ATDU–GC desorption cycles at 210°C; (B) 5.0 ng of trifluralin and triallate, respectively.

the carousel is removed from the SAM and, after mini-tubes fortified with appropriate standards have been inserted, positioned in the ATDU on the gas chromatograph. The mini-tubes are then automatically sequentially desorbed onto the GC column.

Prior to the thermal desorption of a mini-tube, carrier gas is routed through the ATDU via a switching valve into the GC column. Once a mini-tube has been inserted into the desorption oven, the valve position is changed and the carrier gas is routed sequentially through the mini-tube and then into the GC column. The mini-tube is inserted into the carrier gas stream such that the end of the mini-tube through which the air was sampled is placed towards the GC column. The desorption oven can be operated isothermally, or temperature programmed once the mini-tube is in place. Pesticide residues sorbed on the particulate sorbent are thermally desorbed and then focussed at the head of the GC-column, essentially effecting a cool on-column injection.

Extensive thermal conditioning of the Tenax-TA resin in the mini-tubes was necessary prior to evaluation of the effectiveness of the mini-tubes for air sampling and analysis of trifluralin and triallate. After the extended desorption procedure and three 10-min ATDU–GC desorption cycles at 210°C, backgrounds acceptable for the quantitation of 1 ng amounts of both herbicides were obtained (Fig. 2). However, several (4–5) additional 5-min cycles were required to obtain backgrounds acceptable for the detection of 100 pg of both herbicides (Fig. 3). Thus, longer desorption [14] at the maximum recommended temperature for Tenax-TA may have more effectively conditioned the resin. A major background peak occurred for all mini-tubes at 35.8 min (Figs. 3 and 5) and, thus far, the compound responsible for this peak has not been identified.

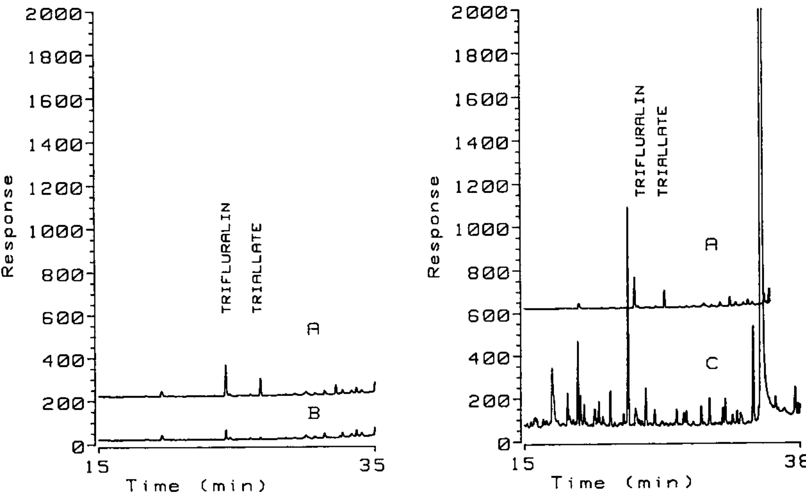

Fig. 3. Mini-tube backgrounds. (A) 0.1 ng trifluralin and triallate, respectively; (B) background following the additional four to five 5-min desorption cycles; (C) background after 8 weeks storage at room temperature in carousel wrapped in aluminum foil.

Following storage at room temperature in a carousel wrapped in aluminum foil, background peaks in the chromatograms of thermally conditioned mini-tubes increased somewhat after 8 weeks (Fig. 3). Interfering background peaks at the retention times for trifluralin and triallate remained small, being less than the equivalent of 100 pg. Similar results were obtained when the mini-tubes were stored in a glass scintillation vial over the same storage period except that, at 8 weeks, interfering peaks were smaller and none were detected at the retention times for trifluralin and triallate. Thus, it seems that during storage of the mini-tubes in the carousel, some of the increased background interferences may have originated from the carousel. This would indicate that when analytes in amounts of 100 pg or less are to be quantitated, the time intervals between thermal conditioning and air sampling, and air sampling and analysis should kept as short as possible.

Using a 5-min desorption cycle, the lowest oven temperature at which quantitative recovery of both trifluralin and triallate was obtained from fortified mini-tubes was 210°C. Because these herbicides have similar vapour pressures (trifluralin, 14.80 mPa [18]; triallate, 25.73 mPa [19], programming the ATDU oven temperature was not necessary and an isothermal desorption cycle was used. ATDU–GC–ECD analysis of fortified mini-tubes under these operating conditions produced trifluralin and triallate peaks which were equivalent to those obtained with on-column injection (Fig. 4). This would indicate that, under these conditions; both herbicides were efficiently focussed at the head of the GC column by the desorption process. These desorption parameters were then used for all other analyses.

A linear ECD response was observed for both trifluralin and triallate following thermal desorption of mini-tubes fortified with amounts of the two herbicides ranging from 0.1 to 50 ng ($r^2 = 0.998$ for both herbicides). The reproducibility of the retention times and area counts for both herbicides over this fortification range was very good. Standard deviations ($n = 10$ at each fortification level) of retention times were generally <0.2%, whereas those for area counts were <2% (Table I).

Cross-contamination between mini-tubes stored at room temperature for 2 weeks in a carousel wrapped in aluminum foil was minimal. At the 100-ng fortification level, cross-contamination between fortified and unfortified adjacent mini-tubes after 2 weeks was <0.1% for trifluralin and <0.2% for triallate. Somewhat higher percentages cross-contamination were observed at the 10-ng level, and this may reflect the presence of small interfering

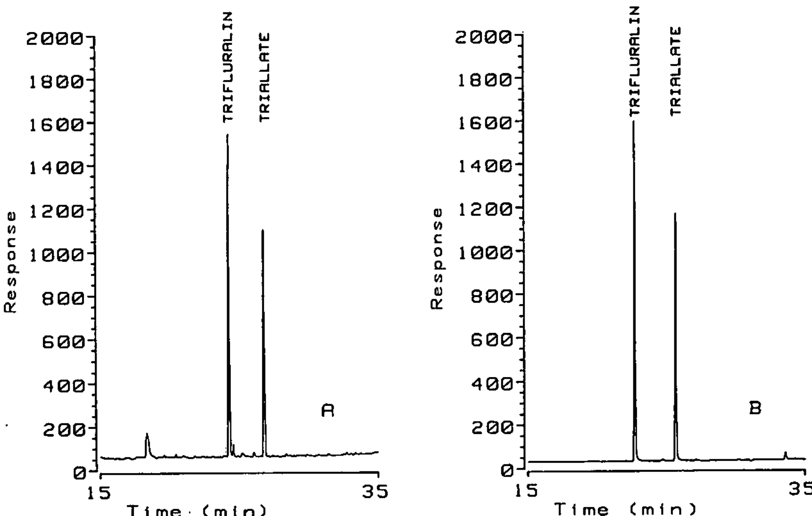

Fig. 4. Comparison of mini-tube thermal desorption and on-column injection for analysis of 1.0 ng each of trifluralin and triallate. (A) Mini-tube thermal desorption; (B) on-column injection.

TABLE I

REPRODUCIBILITY OF AREA COUNTS AND RETENTION TIMES FOR THE ATDU–GC–ECD ANALYSIS OF TRIFLURALIN AND TRIALLATE OVER A MINI-TUBE FORTIFICATION RANGE OF 0.1 TO 50 ng

Fortification level (ng)	Number of replicates (n)	Area counts · 10^{-3} (mean ± S.D.)		Retention times (min) (mean ± S.D.)	
		Trifluralin	Triallate	Trifluralin	Triallate
0.1	10	8.48 ± 0.11	6.22 ± 0.07	24.38 ± 0.01	26.94 ± 0.02
0.5	10	29.60 ± 0.46	22.42 ± 0.18	24.37 ± 0.02	26.92 ± 0.02
1	10	50.24 ± 0.69	38.11 ± 0.40	24.36 ± 0.03	26.91 ± 0.02
5	10	203.5 ± 1.7	153.4 ± 1.3	24.51 ± 0.02	27.07 ± 0.04
10	10	411.8 ± 2.8	309.9 ± 2.9	24.46 ± 0.16	27.06 ± 0.04
50	10	1200 ± 7.7	1157 ± 5.0	24.57 ± 0.03	27.13 ± 0.03

background peaks from the thermally conditioned mini-tubes at the retention times for trifluralin and triallate.

Breakthrough of trifluralin and triallate through the mini-tubes was studied using the maximum air sampling flow-rate (0.1 l min^{-1}) possible with the SAM and relative humidities ranging from 0 to 100%. As observed previously for several workplace air pollutants [14], relative humidity had little effect on the breakthrough of either herbicide through the Tenax-TA resin. Less than 1% breakthrough of either herbicide was detected for each relative humidity level after 168 l of air (air flow-rate of 0.1 l min^{-1} for 28 h) was passed through each mini-tube. This magnitude of breakthrough was observed regardless of whether a 1000-, 5000- or 10 000-loading of each herbicide per mini-tube was used.

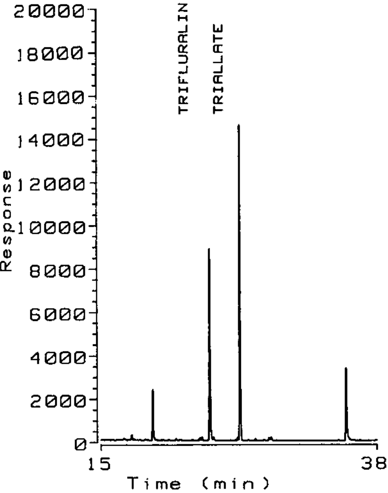

Fig. 5. Chromatogram resulting from the thermal desorption of a 120-min air sample collected 97.5–99.5 h after spraying the field with a surface application of a tank mixture of trifluralin and triallate at (2.0 + 2.0) kg ha^{-1}.

This study also confirmed the feasibility of using the mini-tube system for field sampling of herbicide vapours. In all air samples collected above the treated field over the 120-h sampling period following application, trifluralin and triallate peaks were large relative to atmospheric background peaks (Fig. 5). Thus, a sampling period longer than 120 h would have been required to determine the lowest level at which these herbicides could be realistically detected with a 2-h sample collection time. However, air sample analysis using the mini-tube system did show that herbicide vapour concentrations in the air were maximum immediately after application, and that increased vapour concentrations were present in the air during periods of dew deposition or after a rainfall, as would be expected [17].

As mentioned previously, one advantage of thermal desorption is that all of the analyte(s) in an air sample are desorbed onto the GC column and this can result in enhanced sensitivity. For example, a polyurethane foam sampler aspirated at 25 l min^{-1} will sample 3.0 m^3 over a 2-h sampling period. In contrast, a mini-tube sampling at 0.1 l min^{-1} over the same time period would sample only 0.012 m^3. In previous studies [16,17], the solvent extracts from the polyurethane foam samplers were concentrated to a volume of 10 ml. Thus, the GC analysis of 2.0 μl injection would represent only 0.0006 m^3 of sampled air. In this comparison, a 20-fold sensitivity

enhancement would be realized using the mini-tube technology. Presumably, sensitivity could be increased further with greater air sampling flow-rates through the mini-tubes.

In addition to enhanced sensitivity, the mini-tube based system: (i) offers automated/integrated sampling and analysis as well as convenient sample handling and transportation; (ii) essentially eliminates the use of organic solvents; and (iii) should provide field application for the simplified measurement of herbicide vapour concentrations in air. Studies are currently underway to compare the trapping efficiences of the mini-tubes with those of the polyurethane foam samplers currently used in herbicide vapour flux determinations using the aerodynamic gradient method of measurement.

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CHROMSYMP. 2829

Chromatographic techniques in accurate analysis of chlorobiphenyls

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ABSTRACT

Accurate congener-specific determination of chlorobiphenyl congeners (all 209 congeners) is finally possible with the use of multidimensional gas chromatography–electron-capture detection techniques. The effectiveness of this technique for environmental analyses is enhanced by ultraclean laboratory practices, non-destructive extraction and clean-up steps and the use of low-volume, high-efficiency HPLC separation for various classes of organic contaminants. In the light of these new developments conventional procedures for chlorobiphenyl analysis are evaluated.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are anthropogenic compounds. Their presence in the environment has been studied in great detail for several reasons.

(1) Many representatives of this class of compounds (chlorobiphenyls; CBs) are persistent. Their ubiquitous presence in the environment has been demonstrated by the last four decades of research [1].

(2) Laboratory studies using *in vivo* and *in vitro* bioassays indicate that several CBs are inducers of drug-metabolizing enzymes, being able to affect various physiological processes such as reproduction, embryonic development, carcinogenesis and hormone- and vitamin-related control systems of these physiological processes [2].

(3) Several of these toxic symptoms noticed in laboratory experiments in animals have also been observed in victims of PCB poisoning and in occupationally exposed workers [3].

(4) Marine health programmes utilize biological indicators such as mussels to judge coastal contamination by CBs. It is proposed that "mussel watch" could be an early warning system for ecological catastrophes [4].

(5) CBs are even used as chemical tracers for observing certain biological phenomena such as population structure in marine mammals and migration pattern of birds and other animals [5].

(6) PCB mixtures are composed of many individual constituents (of all 209 CB congeners). These cover a large range of chemical and physicochemical properties in a systematic way [6]. Their distribution in the environment has been interpreted in theoretical models, involving molecular properties [7]. These models can be used to forecast the environmental behaviour of other less well studied compounds.

All the above-mentioned approaches, in one way or another, depend on reliable, accurate and unambiguous measurement of CBs in the material of choice.

PCBs differ in their physicochemical characteristics as well as in their toxic potencies. For example, non-*ortho* chlorine-substituted congeners such as 3,3′,4,4′-tetrachlorobiphenyl (IUPAC No. 77), 3,3′,4,4′,5-pentacholrobiphenyl (126) and 3,3′,4,4′,5,5′-hexachlorobiphenyl (HCB, 169) are potent inducers of enzymes, being far more toxic for certain biological end-points than other congeners [1]. On the

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other hand, lower chlorinated biphenyls (di- and trichlorobiphenyls, for example) and *ortho* chlorine-substituted congeners are more potent inhibitors of dopamine, an important neurotransmitter, than other congeners in pre-human primates [8]. The composition of PCBs in biological tissues and other environmental matrices differs greatly from the original source, *i.e.*, commercial PCBs, for various reasons such as metabolism, physical weathering etc. [9]. Taking into consideration such factors, the need for congener-specific analysis of CBs was stressed in the beginning of the last decade [10].

Although the use of high-resolution single-column gas chromatography (HRSCGC) is essential to reach this goal, no single column available can separate all 209 CB congeners. The use of mass spectrometry (MS) detection techniques to solve co-elution problems [11] may be not foolproof as fragmentation of co-eluting congeners with higher mass can generate interfering signals [12,13].

Mixtures of chlorobiphenyl can be analysed unambiguously in terms of the individual CBs by multidimensional gas chromatographic (MDGC) techniques [in combination with electron-capture detection (ECD)] [14–16]. Applying several thousand of "heart-cuts", the composition of a range of commercial PCB mixtures was determined [16,17]. Additional problems arise when "PCB" fractions of environmental samples are studied. Appropriate extraction and clean-up procedures are essential to minimize the presence of interfering compounds. We have developed an HPLC technique that is effective for biological tissues and other environmental matrices [15].

MDGC–ECD has been successfully used in the direct analysis of toxic non-*ortho* CBs, as they are usually present in very low concentrations; moreover they co-elute with other CBs that are present at considerably higher levels. Other methods have been suggested and applied for the analysis of this class of CBs, *e.g.*, various charcoal enrichment techniques [18]. The application of MDGC–ECD has shown some inherent weakness in the latter pre-GC separation techniques [19].

In view of these developments, it is highly desirable to sum up the procedures that are used for the accurate measurement of CBs. The current paper deals with important topics in CB analysis, such as clean laboratory practices (including solvent purity and blank problems), sampling, extraction, clean-up and pre-GC separation of environmental contaminants in column chromatography and quantitation of CBs.

MATERIALS AND METHODS

Since our laboratory procedures are directed towards meeting the severe requirements of MDGC–ECD, a brief description of this technique is given first.

MDGC–ECD

MDGC allows a selected small fraction of the eluate of a high-resolution capillary column to enter a second capillary column with different characteristics for further separation. Thus, co-eluting compounds can be separated on the second column. The columns are in separate ovens and connected to separate detectors. The one connected to the first column is referred to as the "monitoring detector"; the other is the "main detector". Transfer of the selected fraction is regulated by a pneumatically controlled, valveless "live T-piece" unit. This is achieved without loss and dead volume problems. The system is optimized by regulating two gas flows in such a way that maximum and zero signals are obtained on the monitor and main detectors, respectively, when the flow is directed through the monitor detector and maximum and zero signals are obtained on the main and monitor detector, respectively, when the "heart-cut" is made.

A Siemens SiChromat-2 MDGC-ECD system is used. The first column is a 0.25-μm fused-silica SE-54 (25 m × 0.32 mm I.D.) column. The second column is a 0.25-μm fused-silica OV-210 (25 m × 0.32 mm I.D.) column. The gas pressure (hydrogen) was maintained at 0.8 bar on the first column and at 0.4 bar on the second column. Temperature programming conditions were: first column 140–250°C at 4°C min^{-1}, 12 min at 250°C; second column at 170°C until 20 min after injection, then increase to 240°C at 4°C min^{-1}.

MDGC–ECD is very sensitive and selective for CB components. Usually, the main detector is at least 50 times more sensitive than the monitor detector because the column load is small and the sample is pre-cleaned. The normal detection limit for CBs in this detector is 0.01 pg. MDGC–ECD

determination has certain advantages over MS detection. For example, owing to high-resolution separation combined with high-sensitivity ECD, even congeners with low relative contribution to a peak are determined with a better accuracy than is attainable by electron impact (EI) MS (*e.g.*, CB-77 and -110). For similar reasons, congeners with the same number of chlorine atoms are separated and measured accurately in MDGC–ECD, which is not possible in single-column EI-MS.

The high sensitivity and selectivity of the MDGC–ECD technique as well as the extremely low levels of CBs in some of the samples (*e.g.*, open ocean waters [20,21]) have forced us to apply severe measures to guarantee the integrity of samples with respect to the analytes. These are related to the quality of solvents, the effect of the atmosphere and pre-GC separations to eliminate interfering compounds.

It is a regular practice in our laboratory to screen every sample extract by GC–flame ionization detection (FID) and single-column GC–ECD, prior to MDGC–ECD analysis.

Reagents

The use of an MDGC–ECD system demands extremely pure solvents. Commercial products do not normally meet this requirement. Solvents are distilled under a nitrogen blanket without contact with laboratory air; in most cases two cycles are required. Distilled solvents are stored in glass ampoules (250 ml) sealed under a nitrogen atmosphere.

Laboratory chemicals such as chromatographic alumina and sodium sulphate are purchased commercially, cleaned overnight in organic solvents in a Soxhlet extractor and activated/dried. The clean materials are stored in clean and sealed glass ampoules; alumina in 2-g batches. Prior to their use with samples, their purity and activity is routinely checked in blank procedures.

Extraction and clean-up of environmental samples

A 2–3 g aliquot of biological tissue is homogenized with sodium sulphate and extracted in a special Soxhlet type of extractor [22] with 150 ml of 10% water in acetonitrile for 6 h. CBs and other contaminants are re-extracted from this solvent three times using 100 ml of double-distilled hexane. An aliquot is used for lipid determination.

Alumina column chromatography

The hexane extract is reduced in volume using a rotary evaporator in a nitrogen atmosphere. A 2-g aliquot of deactivated (10% with distilled water) alumina is packed on a glass column (8 mm I.D., 130 mm high, with solvent reservoir at the top) using *n*-hexane, and topped with sodium sulphate. The sample extract (*ca.* 200 μl) in hexane is transferred to the column and eluted with 12 ml of *n*-hexane under nitrogen pressure. This allows the separation of lipids from other organics.

HPLC clean-up

Sample extracts after alumina clean-up are reduced further in volume (*ca.* 100 μl) in a rotary evaporator in a nitrogen atmosphere. HPLC is carried out with a Constametric III pump with a Rheodyne injector on a stainless-steel column (200 × 4 mm I.D.) packed with Nucleosil 100-S, at a flow-rate of 0.5 ml/min. Details of the fractionation are given elsewhere [15]. The volume of the second HPLC fraction containing CBs and *p,p'*-DDE is reduced under nitrogen to *ca.* 50 μl and sealed in specially prepared glass microvials until analysis by MDGC–ECD.

RESULTS AND DISCUSSION

Most aspects of recent methodology for analysis of chlorobiphenyls are based on methods originally developed for packed-column GC–ECD analysis. They have been modified and refined in many respects to meet the requirements of capillary column GC–ECD. The use of MDGC–ECD has only further fortified this requirement. We have found some inherent problems associated with some of the original extraction, clean-up and chromatographic procedures. Great emphasis has also been placed in recent years on the measurement of certain toxic CB congeners at extremely low levels previously never attempted [12–14,23–25]. It is important to check whether conventional procedures are good enough for today's demands. Some experiments conducted in our laboratory to answer this question revealed that existing procedures require modifications. The results and comments are given in the following sections.

CB contamination through laboratory solvents

In the process of extraction, clean-up and GC–ECD analysis of environmental samples, solvents such as dichloromethane (DCM) are used in 200–500-ml volumes; these are concentrated to 2–100 μl before final determination in GC–ECD or GC–MS. Contamination originally present in the solvent is concentrated to possibly unacceptable levels. This can also occur when solvents are exposed to the atmosphere for longer periods.

Fig. 1 is a GC–ECD chromatogram of 100 ml of dichloromethane after HPLC clean-up. This solvent had been transported in a car in a capped bottle on a long drive during a pine needle-sampling exercise. The level of CB contamination that occurred in the solvent during the drive is unacceptable, especially when pine needles are used as indicators of atmospheric contamination [26]. Similar problems were noticed when DCM was transported on ships during research cruises. The significance of this factor in the analysis of water has been described before [20,21]. This problem can be solved by storing solvents under a nitrogen blanket in sealed ampoules, after distillation under nitrogen.

CB contamination from laboratory chemicals (solids)

Alumina or silica gel is often contaminated with compounds that interfere on the analysis of CBs. When activated in a drying cabinet, it becomes a very efficient sorbent for all vapours in the oven atmosphere. Thus, Soxhlet extraction and subsequent reactivation in a not perfectly clean oven may result in a product that is even more contaminated than before the intended purification. Drying, reactivation under vacuum and storage in sealed glass ampoules in small units (2–3 g of alumina) are recommended.

Alterations in CB concentration/composition during acid or alkali treatments

It is a practice in many laboratories to use strong acid or alkali to remove chemicals that interfere in CB analyses. We have checked whether such treatments maintain the integrity of CB residues in biological matrices. Four different tissues from a harbour seal (*Phoca vitulina*) with high (blubber), medium (liver) and low levels of CBs (brain and blood) were considered for this experiment.

These tissues were processed under completely

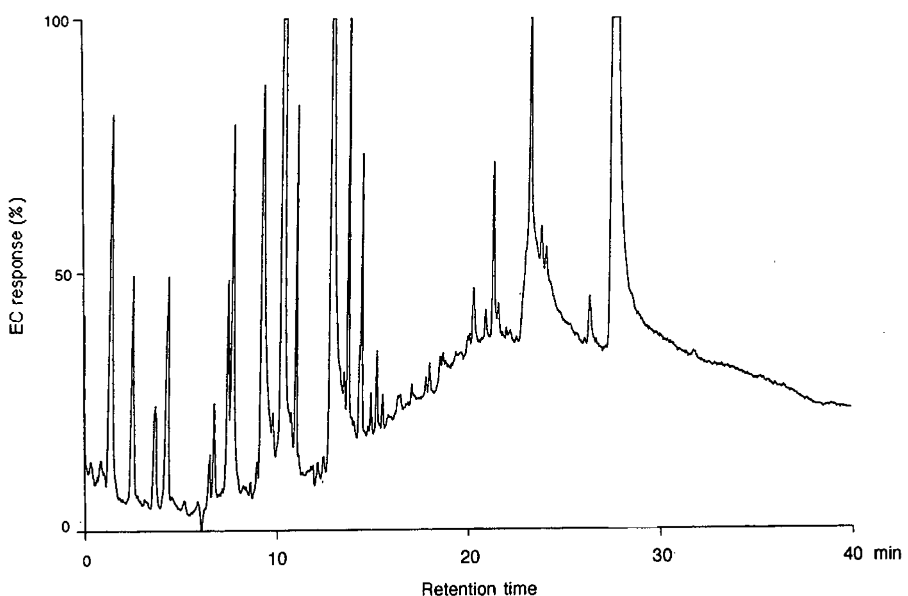

Fig. 1. A blank chromatogram of solvent dichloromethane. This solvent was carried in a closed glass bottle in a car for over 1000 km during pine needle sampling. A 100-ml portion of this solvent was tested after flash vacuum evaporation (under nitrogen) and HPLC clean-up. The HRGC–EC chromatogram shows air-borne contaminants which could interfere in the quantitation of CBs.

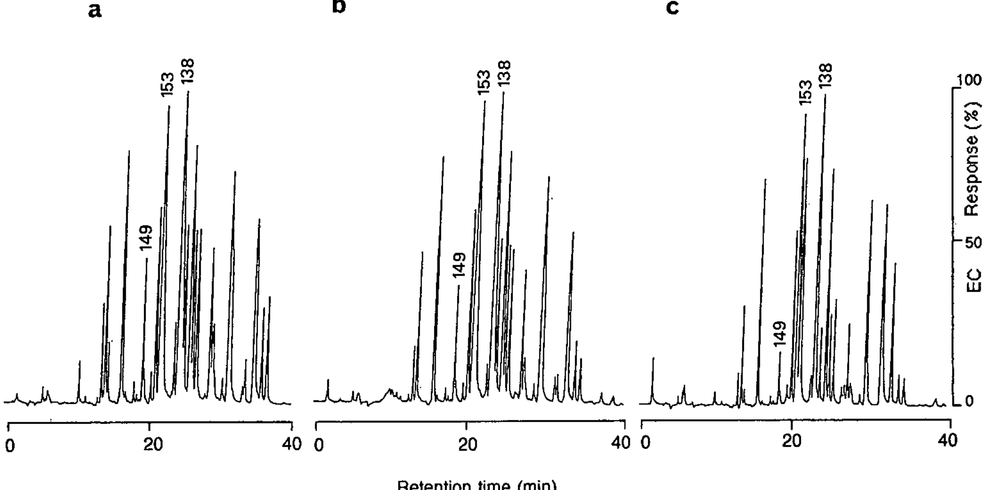

Fig. 2. HRGC–ECD: chromatogram of blubber (harbour seal) extract. (a) Processed according to our non-destructive methodology (see Materials and methods section for details). (b) Sample initially digested in 0.5 *M* ethanolic potassium hydroxide for 1 h, extracted and processed as in (a). (c) The extracted sample was treated with chromic acid, re-extracted and processed as in (a). Three CBs are identified by their IUPAC numbers as "markers".

non-destructive procedures as practised in our laboratory. Another batch was digested in 0.5 *M* ethanolic potassium hydroxide at 80°C for 1 h. These samples were then extracted and processed as the first. A third batch was extracted like the first one; the concentrated extract was treated with chromic acid. After re-extraction of CBs in hexane the sample was processed like the first batch. The CB compositions in all these samples were determined by HRGC–ECD.

The chromatograms for blubber were very similar (Fig. 2). Three persistent congeners are labelled as markers. The ΣCB concentrations in samples are in the μg/g level. Apparently no dramatic changes are visible at these concentrations.

Fig. 3 shows chromatograms of treated and

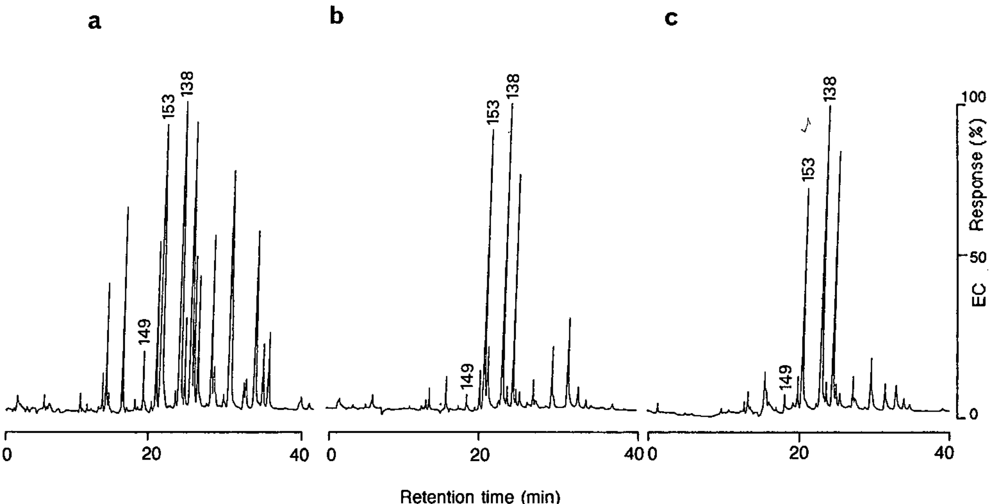

Fig. 3. HRGC–ECD: chromatogram of the liver of a harbour seal. For explanation, see legend to Fig. 2.

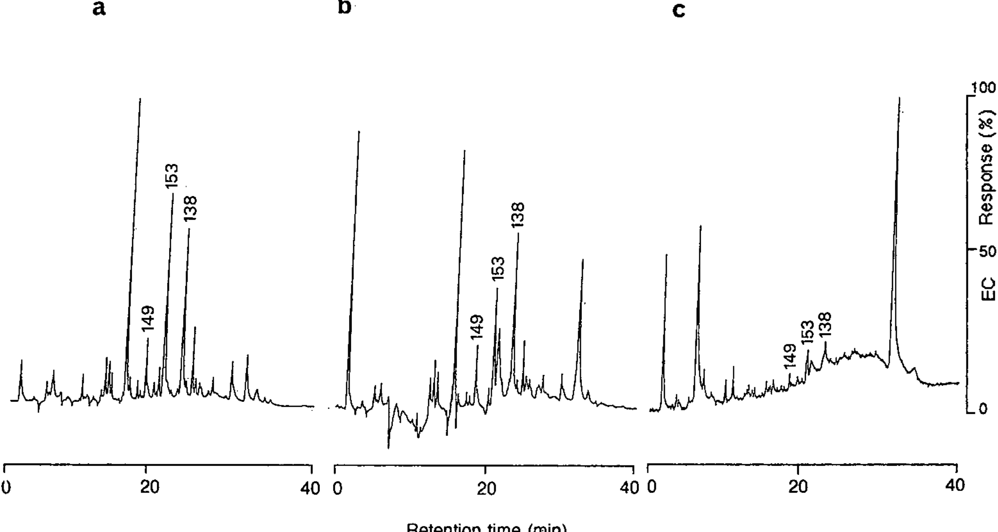

Fig. 4. HRGC–ECD: chromatogram of the blood of a harbour seal. For explanation, see legend to Fig. 2.

untreated liver samples at ng/g ΣCBs per g of lipid. Several congener peaks that appear in Fig. 3a are missing from the samples treated with acid and alkali. There is no drastic difference in the composition of marker CBs between these samples.

Concentration (ΣCBs) levels in blood and brain were 300–500 pg/g. The composition of CBs in these low-concentration samples is affected drastically by harsh treatment with akali and acid (Figs. 4 and 5). Chromic acid seems to affect the CB composition in blood severely (Fig. 4c). On the other hand, potassium hydroxide treatment affected the CB composition in seal brain (Fig. 5b).

In many cases, tissues with high CB concentration levels such as blubber and adipose tissue have been selected for analysis to characterize body levels. Measurement of CBs in blood is increasingly popular in human health programmes [24,27], and brain

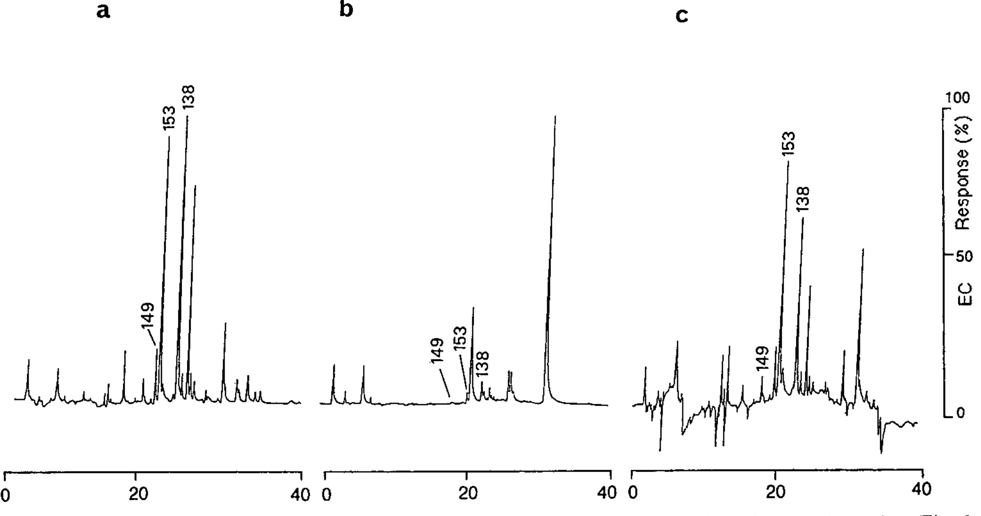

Fig. 5. HRGC–ECD: chromatogram of the brain of a harbour seal. For explanation, see legend to Fig. 2.

tissue is studied frequently as a measure of the effect of CBs on animal behaviour [8]. The high sensitivity of ECD for the detection of CBs requires only small amounts of sample (typically 2–5 g). The total amounts of CBs in such samples will be in the range obviously affected by alkali and acid treatments.

This effect may remain unnoticed in experimental protocols using spikes at levels that exceed natural concentrations (radiolabelled standards or otherwise). Moreover, standards that are added prior to or after the extraction of the sample may not represent the original integrated status in which contaminant and biological/environmental matrix exist. There are reasons to believe that the effect of saponification or acidification on CB composition could be matrix specific. For example, Van der Valk and Dao [28] noticed that degradation of CBs and HCB occurred during alkaline saponification at 90°C of an extract from sewage sludge. It was reasoned that very small particles originating from the sludge could have catalysed the degradation. Similar degradation to CBs was noticed in sediment samples in our laboratory.

There is an additional reason to believe that environmental sample matrix can trigger compositional change in CBs when treated with acids or alkali [29]. When a standard solution containing pure CB congeners (IUPAC Nos. 110, 77, 129, 126, 202 and 156, in the order of elution in a SE-54 column) at the 10-pg level was treated with chromic acid, the chemical loss was minimal. However, we noticed during heart-cuts that chromic acid introduced impurities (oxidation products?) that co-eluted with CB-126 and CB-156 (Fig. 6). This phenomenon cannot be detected in single-column (SC) GC–ECD analysis, resulting in a large overestimation of CB-126 and CB-156. Similar effects are anticipated in the use of strong oxidizing agents such as fuming sulphuric acid and concentrated sulphuric acid.

Problems in the measurement of non-ortho chlorine-substituted CB congeners

The importance of measuring aryl hydrocarbon hydroxylase (AHH)-inducing CB congeners (especially CB-77, -126 and -169) in environmental samples was realized from the mid-1980s. A charcoal enrichment method was proposed for these congeners during the 1986 Dioxin conference [18], and preliminary results on their levels in human and

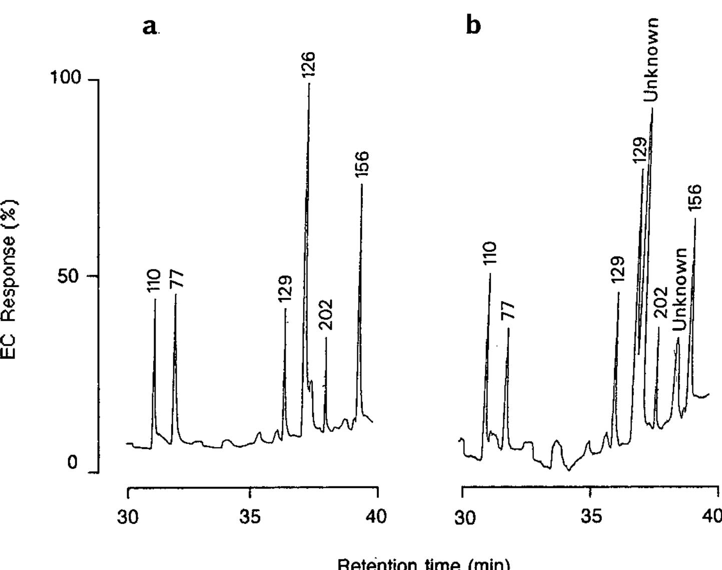

Fig. 6. MDGC–ECD chromatogram of three "heart-cuts" performed for the separation of CBs 77/110; 126/129; 202/156 and co-eluting impurities. (A) Approximately 100 pg standard mixture of toxic CBs. (b) The same after chromic acid treatment. Acid treatment introduced co-eluting impurities/byproducts, which could cause overestimations of CB-126 and -156 in single-column GC-ECD.

animal tissues were reported [30]. A more direct approach to measure these congeners with the use of MDGC–ECD was developed shortly afterwards [14]. Subsequent publications from these two groups revealed the occurrence of non-*ortho* CBs in commercial PCBs [16,31], in sediments [32], in water [20,21,33], in human tissues [34,35] and in wildlife [23,36]. A literature survey shows that at least 50 publications have been devoted to the analytical chemistry of these toxic CB congeners in the last 6 years [37]. Also, dioxin researchers are interested increasingly in these compounds, which resemble 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD) in their mode of biological action.

The analysis of these congeners is hampered by their extremely low concentrations in the presence of co-eluting congeners at much higher concentrations [16]. Only a few methods for the direct measurement of non-*ortho* CBs are known. These are MDGC–ECD [14], GC–GC–MS [38,39] and GC–NCI-MS (negative chemical ionization) [13].

The possibilities of MDGC–ECD have been described earlier in this paper. Mass spectrometric methods can also solve some fundamental problems. However, co-eluting congeners occurring at high concentrations relative to the target non-*ortho* congener can interfere through fragmentation of the parental ion even when the chlorine numbers differ from the target compound. Schmidt and Hesselberg [13] have shown recently that even NCI has this problem. Co-elution of CB-110 with CB-77 posed the greatest challenge for their methodology. In methane NCI, CB-110 produced a small amount of tetrachlorobiphenyl fragment ion (m/z 292) interfering in the quantitation of CB-77 (m/z 292 was the quantitation ion of choice for CB-77). Similar interference occurred in the determination of CB-126 by CB-129. They report that the error will be 15% for CB-126 and 70–100% for CB-77, if no correction is applied.

All other methods involving non-*ortho* CBs utilize an intermediate enrichment step before final determination by GC–ECD or GC–MS. The materials of choice for this purpose are activated charcoals [19,40], porous graphitic carbon [41], florisil [42], alumina [43] and 2(1-pyrenyl) ethyldimethylsilylated silica (PYE) [44]. These materials are used essentially to isolate the non-*ortho* CBs from the rest of the congeners before measurement.

Several brands of activated charcoals that are in current use in various methodologies were tested for this efficiency in comparison with direct measurements in MDGC–ECD [19]. CB-77, -126 and -169 were measured in Aroclors and in seal blubber using charcoal techniques and MDGC–ECD. The charcoals were generally very efficient, but they did not result in a *complete* separation of the class of CBs that was involved in the study. As a result, a small portion of *ortho* CBs was left out in the non-*ortho* fraction. This was in the range 0.032–2.2% for CB-110 and 0.0018–0.0087% for CB-178. The concentrations of these co-eluting compounds are large enough to interfere when they co-elute with CB-77 and CB-126, respectively. The latter congeners occur at <0.01% of the total CBs. In conclusion, Kannan *et al.* [19] pointed out that these congeners could not be accurately determined using SC-GC techniques with either ECD or MS detection. This was confirmed in a recent study using HRGC–HRMS (electron impact ionization) [12]. It was observed that although the carbon/silica gel column separates the co-planar CBs (non-*orthos*) from the non-planar CBs very effectively, the concentration of CB-110 in the original sample (fish and marine mammal) was often so much larger than that of CB-77 that even a small percentage of CB-110 remaining in the co-planar fraction resulted in an interference at m/z 292, the quantitation ion for CB-77. They also noticed co-elution of CB-158 (2,3,3′,4,4′,6-H_6CB), CB-185 (2,2′,3,4,5,5′,6-H_7CB) and CB-129 with CB-126 when using a DB-5 capillary column. To avoid overestimations in HRGC–HRMS, they proposed a peak area correction factor, which had to be done manually. These recent observations point out clearly that most published reports on non-*ortho* CBs without such strict quality control measures carry an element of uncertainty in them.

Several high-resolution GC columns offer satisfactory separation of these particular congeners and are used successfully for these measurements [45–47]. However, none of these columns, except SE-54 (5% phenyl methyl silicone), has been fully characterized for retention time indices for all 209 CB congeners [48]. In the absence of such data for other columns, congener-specific analysis for chlorobiphenyls would be only partial.

The need for chemical purity of solvents and

reagents used in CB analysis was discussed above. Charcoals are also reagents. They have some problems that are unique to this class. The activated charcoal can be produced from various sources such as combustion of petroleum compounds, palm oil, coconut shells and other vegetable sources. The impurity that can arise from these materials is also varied and unpredictable. Extensive cleaning of these materials is a prerequisite for their use. Five brands of charcoals that were used in an earlier study [19] were scanned by scanning electron microscopy (SEM), which revealed that the surface characteristics were very different for different charcoals (which resulted in different elution profiles for different charcoals [19]). The SEM studies also indicated that silicon, sulphur and metals such as potassium, calcium, iron and aluminium are present in these charcoals at per cent levels. It is yet to be shown whether these metals present in the charcoals can cause some catalytic degradation of CBs. This possibility may arise when acidified silica gel columns are combined with charcoal columns in automated clean-up procedures.

Quantitation of CBs: need for uniformity

It is surprising that commercial PCBs such as Aroclors, Clophens and Kanechlors are still used as standards for qualitative and quantitative analysis of CBs in environmental samples. Except in accidental spills and in continuous discharge from PCB manufacturing plants, the PCBs that reach the environment are modified by various physicochemical and biochemical mechanisms over a period of time. The irrelevance of quantifying environmental CB residues using commercial PCBs has been pointed out by various research groups [9,10]. An isomer-specific principal component analysis [49] and a linear inverse regression method [7] have demonstrated quantitatively that CB residues in environmental samples (fish, turtles [49] and water, shrimps, fish, marine mammals [7]) are by no means described adequately by Aroclors such as 1242, 1248, 1252 and 1260. An alarming situation was pointed out recently [50]. Samples with CB concentrations as high as 10 000–50 000 μg/kg were reported "undetected" in the Superfund's contract laboratory programme (CLP) because it required reports in terms of Aroclors.

It is also not uncommon that CBs in environmental samples are quantitated on the basis of isomer groups [51]. Again, only limited information can be extracted from such isomer data.

The most modern and accurate approach is to quantitate CBs in environmental matrices on the basis of individual congeners. Standards that represent selected persistent and toxic congeners can also be used, depending on the situation [36,52]. It is important to base the quantitation on a reliable high-resolution chromatographic technique, such as using SE-54 capillary columns. All the modern research on CBs clearly indicates that it is time that PCBs are quantitated and studied exclusively as individual chemical entities rather than as an uncharacterized mixture of compounds.

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Application of gas chromatography with photoionization and electron-capture detectors for field screening of semi-volatiles in soil and water

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ABSTRACT

The Resource, Conservation and Recovery Act and the Comprehensive Environmental Response, Compensation and Liability Act expanded the list of hazardous chemicals under US Environmental Protection Agency (EPA) regulation during the 1970s and 1980s. This expansion aggravated the backlog of analyses in the EPA Contract Laboratory Program (CLP), and led to the development of field screening methods for volatile organics to augment CLP. We will review field methodology, compare laboratory to field methods, and discuss the applicability of on-site analysis for semivolatiles. Portable gas chromatographs developed for the analysis of volatiles can also be used for field screening of semivolatile organics such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, pesticides and phenols. The photoionization detector, a common detector for volatiles, would have to be supplemented with an electron-capture detector to analyze the wide range of analytes described in the EPA 8000 series methods. We will describe methods using these detectors for the analysis of soil and water samples.

INTRODUCTION

The passage of the Resource, Conservation and Recovery Act (RCRA) in 1976 and the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA or Superfund) in 1980 led to an expansion of the list of hazardous chemical compounds under US Environmental Protection Agency (EPA) regulation. A variety of new analytical methods were developed for their analysis. Those analyses conducted under the Superfund program during the nineteen eighties resulted in a tripling of the contract laboratory business yet remediation at the sites still progressed slowly as a result of problems with the turnaround time at the laboratories.

Under EPA's Contract Laboratory Program (CLP), turnaround time from laboratories in the program was typically greater than 30 days. Even a 30-day turnaround was not acceptable for the many critical and timely decisions that had to be made. It soon became apparent that there was a significant need for faster, lower cost field screening methods to supplement, and in some cases replace, the laboratory analytical methods [1]. This led to the evolution of field screening and analysis methods in the 1980s by the EPA and the Field Investigation and Technical Assistance Teams. Techniques such as soil gas monitoring [2], headspace [3], and static headspace [4] were adapted for field use instead of the time-consuming purge-and-trap [5] technique used in the laboratory for the analysis of volatiles.

With good technique, field methods, though quite different from laboratory methods, can provide similar results. In Table I, we compare results from the field static headspace and purge-and-trap laboratory methods. A portable gas chromatograph with a photoionization detector was used for static head-space while the laboratory results employed a purge-and-trap with a laboratory gas chromatograph and a mass-selective detector for a

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TABLE I

COMPARISON OF FIELD STATIC HEADSPACE METHOD WITH LABORATORY PURGE-AND-TRAP FOR VOLATILES

Field gas chromatograph: HNU Model 311 with PID; laboratory gas chromatograph: HP5890 with mass-selective detection.

	Static headspace (ppb)	Purge-and-trap (ppb)
Benzene	250	500
Toluene	4300	3200
Xylenes	7800	7200

detector. The correlation coefficient between the methods was 0.99 over the range from a few to several thousand ppb (v/v). Following proper quality control procedures in the field will improve the dependability of the data and its potential use in litigation.

Considerable effort was expended on the development of analytical capabilities to support new field methods. The principal performers among these methods were *portable* field instruments such as the total volatile organics analyzer based on photoionization or flame ionization detection, and portable gas chromatographs. These field methods focused primarily on the analysis of volatile organic compounds frequently lost by volatilization or biodegradation prior to analysis by the contract laboratories. Some of the field methods developed under the Superfund program were found to be very useful and could be modified for the analysis of semivolatile organic hydrocarbons.

We will describe some modifications to EPA methods to simplify for field use and discuss the substitution of the photoionization detection (PID) and electron-capture detection (ECD) methods for the detectors in the EPA 8000 series methods for the field analysis of semivolatile organic hydrocarbons.

METHODOLOGY FOR LEVEL I AND II FIELD SCREENING

The framework of the EPA methodology involves five levels of investigative screening or analyses. The first level (level I) involves field screening with hand held analyzers (EPA protocol specifies a photoionization detector such as an HNU Model PI or HW101) and other site characterization equipment such as an oxygen meter, explosimeter, radiation survey equipment and chemical testing tubes [6]. Level I effort is designed to determine the real-time total level of contaminants present (*i.e.*, *total volatile organics*) that allows determination of the appropriate level of on-site respiratory protection and evaluation of air quality for existing or potential threats to surrounding populations [3]. It is possible to accomplish the following during level I screening: (i) identification of contamination source, (ii) monitoring soil vapor wells to determine the extent of the pollutant plume (*headspace*), (iii) measuring the total concentration profile in a borehole to determine contaminant migration in the ground water or leaching down through the soil and thus contaminating the underlying ground water and (iv) protecting the health of workers involved in the investigation and remedial work.

The two most serious threats from the volatiles involve evaporation into the air and migration away from the original source of contamination through the soil and into a source of ground water. Remediation of the ground water to EPA levels can take years. The plumes from contaminated sources can migrate long distances unless contained. The semivolatile hydrocarbons do not migrate but may have to be removed as a result of their proximity to a source of drinking water, toxicity, or other environmental concerns.

Once a level I screening identifies a contaminated area and delineates its extent, a level II screening can establish the identity of the compound(s) and relative concentrations. Previously, this was accomplished by sending samples to a laboratory for detailed analysis. EPA introduced the intermediate level II analysis in order to reduce both the times required to start remedial actions and the high costs associated with laboratory analysis and keeping trained personnel in the field waiting for results [6]. Level II involves field analysis with more sophisticated instrumentation (*i.e.*, portable gas chromatograph) to provide identification (as far as possible) of specific components present. The final three levels (levels III–V) use laboratories located "off-site" and frequently involve CLP analysis [6]. As such, we will not be concerned with these latter techniques. We are interested in rapid analysis which can be conducted in the field.

The new field methods for volatiles developed under the Superfund program were very successful and could be extended to include the semivolatiles, if some changes in detector technology and methodology are made in the process. Two potentially interesting GC detection systems for field use include PID and ECD since only carrier gas is needed for operation. The need for such field methodology for semivolatiles was obvious in a recent study at a hazardous waste site in Puerto Rico contaminated by DDT [7]. Samples were collected and sent to the laboratory on the two previous visits. We observed additional problems (areas of contamination), not previously encountered on the third visit with the "on-site" analysis by portable GC. During the three days sampling and analysis period, we collected and analyzed forty-four samples. We were finally able to clean up the site during the 1-week time period with field analysis of semivolatiles. This type of success in the field indicates both the advantage and necessity of developing field methodology for semivolatiles.

LABORATORY PROTOCOLS

When samples (containing semivolatiles) from the field site are returned to the laboratory for analysis under EPA protocol [8], an appropriate extraction method is selected for the samples. Manual or liquid–liquid extraction is used for water samples and Soxhlet extraction or ultrasonic extraction is employed for solid samples (soil or sludge). Manual extraction of water samples requires approximately 1 l of sample extracted with three 60-ml portions of methylene chloride in a 2-l separatory funnel. Formation of an unbreakable emulsion during the manual extraction forces the use of liquid–liquid extraction over a 16–24 h period. The organic layers are first dried then carefully concentrated in a Kuderna–Danish apparatus to 1 ml. If the analytical method requires a different solvent, now the solvent can be changed from methylene chloride.

Similarly, the ultrasonic extraction of solid samples (a faster process than Soxhlet extraction) requires that a large sample (on the order of 100 g) be extracted three times with solvent and the combined extracts be concentrated using a Kuderna–Danish apparatus with solvent change occurring during the concentration process.

The sample is analyzed to determine whether any further operations are needed prior to data reduction. If the initial chromatographic analysis shows that there are interfering peaks in the chromatogram, cleanup of the sample is necessary. Column chromatography on silica gel or Florasil or gel permeation chromatography will suffice. This entails a further concentration step with solvent change from the solvent used in the fractionation to the solvent desired for chromatography.

The preparation of the final analytical sample can take from 1 to 3 days for each sample submitted for analysis.

DISCUSSION OF FIELD METHODS

Field extraction of semivolatile hydrocarbons is a much simpler method than the laboratory methods described above. EPA has published a field screening methods catalog as a reference [9]. The development of field screening and analytical methods continued through the 1980s and into the 1990s thanks in large part to the Superfund Program. A procedure from that method's guide for semi-volatiles is as follows: a small sample, 10 ml of water or 800 mg of soil are mixed with 1 ml of a 1:4 mixture of water and methanol and 1 ml of hexane in a vial. The vial is shaken for 30 s and let stand for an additional 30 s to allow the layers to separate. Any emulsion that forms is easily broken by centrifuging the sample. The upper organic layer containing the extracted semivolatile hydrocarbons is injected directly into the gas chromatograph.

The field methods do not have a cleanup step; however, any observed interferences can be noted and the appropriate cleanup procedure applied to the sample undergoing laboratory analysis to provide confirmation of the field results.

One of the most widely used detectors in the laboratory is the flame ionization detector but the use of this detector in the field requires support gases (hydrogen and air) and the detector may take as long as 30 min to stabilize. Clearly, not ideal characteristics for field usage! Nitrogen–phosphorus (NPD) and flame photometric detection (FPD) have similar limitations to the flame ionization detection (FID), namely, the requirement for support gases and long warm-up times. *PID and ECD would appear to be ideal candidates for field detection for the GC analysis of semivolatiles.*

PID is the most frequently used laboratory detection method in a variety of EPA methods [5,8] for volatiles in water or soil. PID is again the most popular detection system for the field analysis of volatiles [7] as a result of its superior sensitivity to flame ionization detection (see Fig. 1 with the 50-fold improvement in sensitivity of PID; note the attenuation difference) and the lack of a support gases (hydrogen and air). Although PID has found considerable application for volatiles (a computerized literature search identified nearly 150 publications using PID for volatiles), there were few applications for the analysis of semivolatile hydrocarbons by PID. We believe that this is more indicative of the popularity of the volatiles methods than the applicability of PID for semivolatiles. Methods are described for the detection of polycyclic aromatic hydrocarbons (PAHs), nitrosamines, chlorinated pesticides, nitrogen containing pesticides, as well as nitro aromatics by PID. These compounds are the semivolatiles described in the EPA methods in Table II. The detection methods used for these meth-

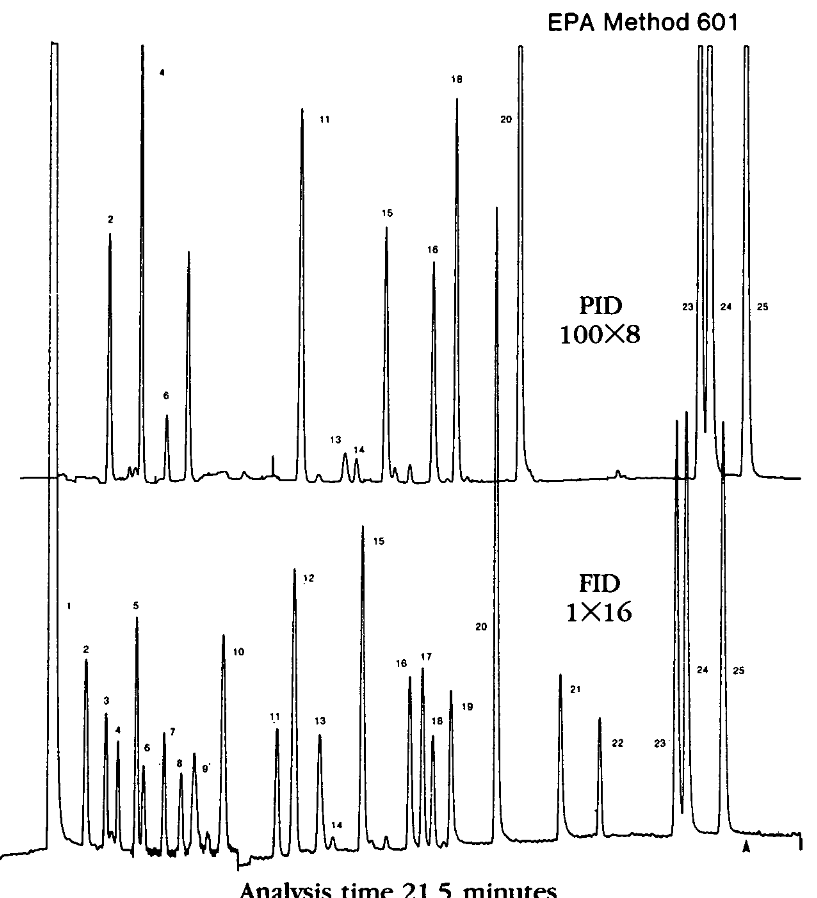

Fig. 1. Comparison of PID and FID sensitivity and selectivity for 601 priority pollutants. Conditions: HNU Model 421 GC; Quadrex Halomatics, 30 m × 0.53 mm I.D.; 3 min at 35°C then to 120°C at 8°C/min, hold at 120°C; 15 ml/min nitrogen. EPA 601 peak identification: 1 = solvent (methanol); 2 = 1,1-dichloroethene; 3 = methylene chloride; 4 = *trans*-1,2-dichloroethene; 5 = 1,1-dichloroethane; 6 = *cis*-1,2-dichloroethene; 7 = chloroform; 8 = 1,1,1-trichloroethane; 9 = carbon tetrachloride; 10 = 1,2-dichloroethane; 11 = trichloroethene; 12 = 1,2-dichloropropane; 13 = bromodichloromethane; 14 = 2-chloroethylvinylether; 15 = *trans*-1,3-dichloropropene; 16 = *cis*-1,3-dichloropropene; 17 = 1,1,2-trichloroethane; 18 = tetrachloroethene; 19 = dibromochloromethane; 20 = chlorobenzene; 21 = bromoform; 22 = 1,1,2,2-tetrachloroethane; 23 = 1,3-dichlorobenzene; 24 = 1,4-dichlorobenzene; 25 = 1,2-dichlorobenzene.

TABLE II

EPA GC METHODS (NON-GC–MS) FOR SOIL AND WATER ANALYSIS

These methods are analysis only (8000 series) and are applicable to ground water and solid waste samples. Sample preparation (extraction and cleanup) are covered by the 3500 series methods (extraction) and the 3600 series methods (cleanup). ELCD = electrolytic conductivity detector.

Method No.	Compounds	Detection
Volatiles		
8020	Volatile aromatics	PID, packed column
8021	Volatile aromatics	PID, capillary
8031	Acrylonitrile	NPD
8032	Acrylamide	ECD
8110	Haloethers	ELCD (ECD alternative)
Semivolatiles		
8040A	Phenols	FID and ECD
8060	Phthalate esters	ECD, packed column
8061	Phthalate esters	ECD, capillary
8070	Nitrosamines	NPD
8080A 8080B	Chlorinated pesticides and polychlorinated biphenyls	ECD, packed column
8081	Chlorinated pesticides and chlorinated biphenyls	ECD, capillary
8090	Nitro aromatics	ECD or FID
8100	Polycyclic aromatic hydrocarbons	FID
8120A	Chlorinated hydrocarbons	ECD, packed column
8121	Chlorinated hydrocarbons	ECD, capillary
8140	Organophosphorus pesticides	NPD or FPD, P Mode
8141	Organophosphorus pesticides	Capillary NPD or FPD
8150	Chlorinated herbicides	ECD, packed column
8151	Chlorinated herbicides	ECD, capillary
8410	Semivolatile organics	Capillary GC–Fourier transform IR

ods include ECD, FID, NPD, FPD, Fourier transform infrared and mass-selective detection.

What requirements are necessary to replace FID? The requirements would include a detector with a similar response (namely a carbon counter), a high sensitivity and a wide dynamic range. PID with a 10.2-eV lamp will not respond to small molecules such as methane, ethane–butane that have high ionization potentials (12.98–10.63 eV). For alkanes C_5 and above, PID will respond in a similar manner to FID. Langhorst [10] determined the sensitivities for nearly two hundred compounds for PID with a 10.2-eV lamp. She found that the photoionization detector was a carbon counter (on a molar basis), that the sensitivity for alkanes < alkenes < aromatics; that sensitivities for cyclic > non-cyclic and branched > non-branched; and that for substituted benzenes, ring activators increased sensitivity while ring deactivators decreased sensitivity. Driscoll [11] discussed the improved range and sensitivity for PID compared to FID. Thus, PID is a suitable replacement for FID for many environmental applications [5,11].

The EPA semivolatiles methods that employ FID (Table II) include phenols, nitroaromatics and PAHs. Langhorst [10] detects these compounds with excellent sensitivity. PAHs in asphalt and diesel engine emissions by are analyzed by Arnold [12] with capillary GC and PID (8.3 eV). Sixteen EPA priority pollutants were analyzed in less than 14 min with better detection limits than FID. PAHs in water were separated by HPLC first, then injected into a gas chromatograph with PID to obtain detection limits of 50–100 pg or 10–40 times more sensitivity than FID [13], PAHs in sediment [14], nitroaromatics [15] and phenols [16] for methods 8100,

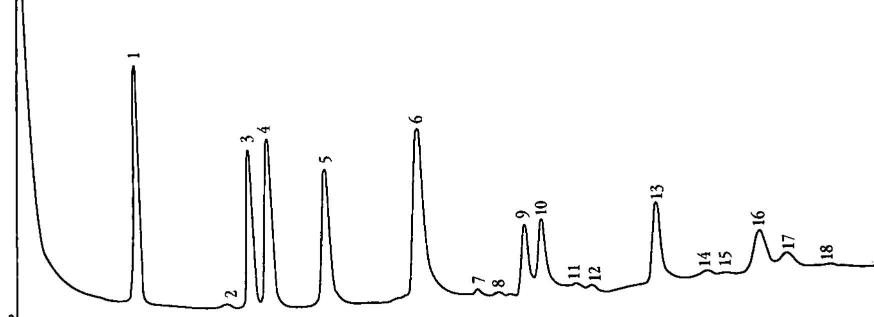

Fig. 2. Chromatograms of PAHs on portable GC. Conditions: HNU Model 311 portable gas chromatograph; NB30, 5 m × 0.53 mm capillary column (HNU); 1 min at 80°C then to 140°C at 16°C/min; hold 1 min, then to 270°C at 14°C/min for 8 min, flow 15 ml/min nitrogen through photoionization detector; 1.5 μl sample containing 20 ng/μl of each PAH; 25 min analysis time; Attennuation × 10. Peaks: 1 = naphthalene; 3 = acenaphthylene; 4 = acenaphthene; 5 = fluorene; 6 = phenathrene/anthracene; 9 = fluoranthene; 10 = pyrene; 13 = chrysene/benz[*a*]anthracene; 16 = benzo[*b,k*]fluoranthene; 17 = benzo[*a*]pyrene. Peaks not identified are impurities.

8090, and 8040A, respectively. Some PAHs analyzed on a field portable gas chromatograph with PID are shown in Fig. 2. The HNU GC311 was modified through the addition of temperature programming to enable more rapid elution of higher-molecular-mass PAHs. Nitrosamines are detected with NPD for the EPA method 8070. Meili *et al.* [17] described a GC–PID method for nitrosamines in meat with detection limits of 50–100 pg.

EPA methods 8060, 8061, 8080A and B, 8120A, 8121, 8150 and 8151 are all ECD methods. ECD is quite useful for the analysis of chlorinated compounds and has excellent selectivity. Some of these methods can be performed in the field on an isothermal GC with ECD. Temperature programming as shown in Fig. 2 would also be very useful for the analysis of pesticides to reduce analysis times and improve detection limits for the longer-eluting species. An example of a group of pesticides analyzed by field GC at a level of 0.1 μg/ml is shown in Fig. 3. Chlorinated hydrocarbons such as chlorophenols, chlorobenzenes, DDT, polychlorinated biphenyls and chlorinated pesticides [10,11,18,19] are also detected by PID. Thus, ECD and PID can be used as a complementary detection combination to detect or confirm isomers or less toxic compounds as described by Krull *et al.* [15]. Here, one would have to use a solvent that is optimized for both detectors.

The only other semivolatile methods are 8140 and 8141 for the detection of organophosphorus pesticides. PID will detect nitrogen-containing pesticides [20], nitrogen-containing hazardous pollutants [21] and phosphorus-based pesticides [19].

In summary, we have shown that PID and ECD

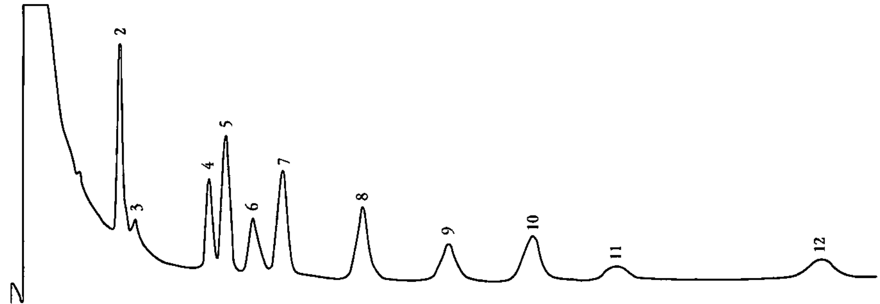

Fig. 3. Analysis of pesticides on portable GC with ECD. HNU Model 311 portable gas chromatograph; conditions: 1 μl containing 0.1 μg/ml of each pesticide; NB30 25 m × 0.32 mm (HNU), isothermal 180°C, column flow 3 ml/min argon–methane; analysis time 14 min; Attenuation × 1. Peaks: 2 = dichlorane; 4 = vinclozolin; 5 = heptachlor; 6 = dichlorfluanid; 7 = aldrin; 8 = heptachlor epoxide; 9 = α-endosulphan; 10 = dieldrin; 11 = β-endosulphan; 12 = endosulphan sulphate. Peaks not identified are impurities.

TABLE III

QUALITY CONTROL PROTOCOL FOR FIELD SAMPLING FOR DDT IN SOIL

A*n* are sample numbers; 1, 2, 3 represent replicates.

Chronological order of samples and standards					
10 ng/μl	Calibration		H1		
1 ng/μl	Standard	91.44% Yield	I1		
A1				Calibration	
A2			J1		
B1			K1		
B2			L1		
C1			L2		
C1	Duplicate	3.48% RPD	L3		
C2				Calibration	
D1			M1		
	Standard	86.53% Yield	N1		
D2			O1		
D3			M2		
	Calibration		N2		
New septa			N2	Duplicate	16.4% RPD
	Calibration			Standard	110.8% Yield
E1			P1		
E2			Z1		
E3			P2		
F1			1 ppm	Standard	93.5% Yield
F2			10 ppm	Standard	121.7% Yield
F3			Z2		
G1			Z3		
G2			Z4		
G3			NH1 (N Horizontal)		
	Standard	84.4% Yield	NH2		
Day 2			DDD 10 ppm		
			10 ppm	Standard	93.5% Yield
	Calibration				
	Calibration				

are useful alternatives for detection methods such as FID, FPD and NPD for the semivolatile compounds. One might trade off some selectivity in the process but the advantages of timely on-site analyses surely outweigh any other considerations.

IMPROVEMENT OF FIELD METHODS

Field analytical methods were determined to be most useful when the contaminants of concern were already identified, so that appropriate methods, dilutions, calibration ranges, etc. could be employed [22]. It was also found that credibility could be lent to field data by using quality control techniques similar to laboratory methods (*i.e.*, duplicates, standards run at regular intervals, etc.). Note that the quality control protocol described in Table III is very similar to laboratory protocol. A quality control protocol was maintained for the analytical results obtained in the field [6] which consisted of analysis of a standard to determine percent recovery and analyzing duplicates on sample extracts to verify analyst reproducibility. The instrument was recalibrated at the beginning of each morning and afternoon shift and at any change in condition (new septa), or shift in peak retention time. Fifteen percent of the samples were returned to the laboratory for verification. In this case, excellent agreement was obtained [6] although different methodology was used in the field and laboratory. The data are shown in Table IV.

The agreement found between the two methods

TABLE IV

COMPARISON OF FIELD *VS.* LABORATORY

Field GC: HNU Model 311 with PID; laboratory GC: HP5890 with ECD.

DDT (ppm)	
Field GC–PID	Lab GC–ECD
0.314	0.170
0.500	0.400
0.774	0.410
5.26	5.560
	6.000
10.235	12.150

was excellent with a correlation coefficient (r^2) of 0.997. Thus with good technique, equipment and quality control, level III type results can be obtained in the field while maintaining flexibility of remediation activities at the site. An example of the quality control protocol used for the field data above is given in Table III.

Field analytical equipment is currently used for on-site detection and identification of volatile organic contaminants in air, water and soil. Portable gas chromatographs such as the HNU Model 311 which are frequently used for characterization of volatile organic compounds can also be used for semivolatile organics, such as pesticides, polychlorinated biphenyls and PAHs. Temperatures of 200°C are required along with dual detector capability to be able to analyze the semivolatiles.

The lack of temperature programming can be offset in some cases by calculating the response factors of the peaks and comparing them with standards in the lab at a later time. This was used by Krull *et al.* [15] to determine nitro containing aromatics in mixtures. The PID response changed slightly with nitro substitution (factor of 3–5) while the ECD response changed by three to five orders of magnitude.

Another method for improving the identification is the retention index monitoring (RIM) system [23] which is a tool for the automatic interpretation (identification) of complex mixtures based on a unique pattern recognition algorithm for search of index peaks. Compound identification is based on two columns of different polarity. Compound libraries are available with space up to 1100 compounds. Separations are accomplished on a pair of fused-silica capillary columns; one using NB-1701 and the other an NB-54 bonded stationary phase. This can be performed in the field isothermally or with temperature programming. Both column inlets are installed into a single column injector. Identifications were made with the Micman identification software (available from HNU Systems) which compares the results on both columns to a pre-established library and then lists results only when the compound is found on both columns. Identifications were based on absolute retention time. The PID results are shown in Fig. 4a for a 10-ppm sample. The detection limit for this method was 0.1 ppm

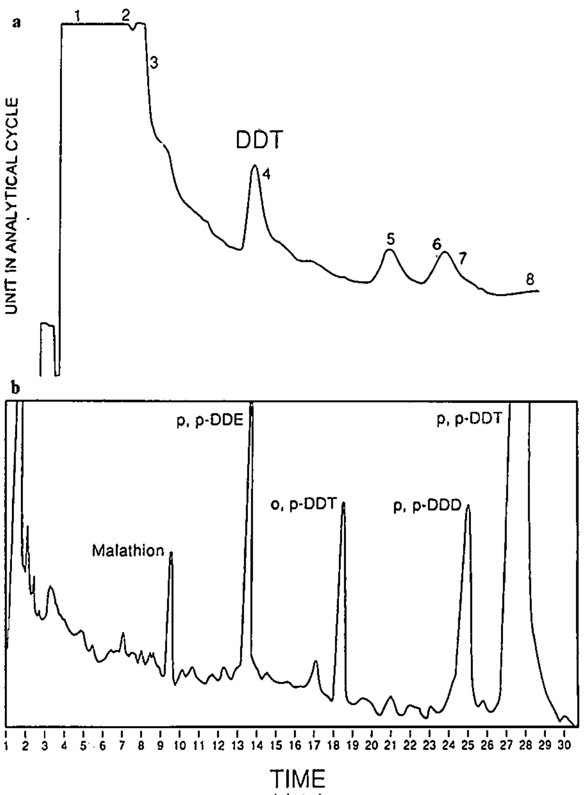

Fig. 4. PID and ECD chromatograms for DDT in soil. HNU Model 311 portable gas chromatograph; (a) PID, conditions (field): NB30 15 m × 0.32 mm capillary column (HNU), isothermal 180°C, column flow 15 ml/m in of prepurified nitrogen; attenuation × 1; analysis time 10 min. (b) ECD, conditions (laboratory): NB30 25 m × 0.32 mm capillary column (HNU), isothermal 150°C, column flow 15 ml/min of prepurified nitrogen; attenuation × 10. Peak: 4 = DDT (10.235 ppm). Peaks not identified are impurities.

and the action limit for DDT was 2 ppm so that the method had sufficient sensitivity to determine whether the soil was contaminated. ECD is a more selective detection method for this application with a detection limit two orders of magnitude lower, but for field applications, we can see that the additional sensitivity or specificity was not needed. In Fig. 4b, other chlorinated isomers of DDT were identified along with low levels of malathion which was not supposed to be present at this site. The peaks with a longer retention time than DDT do not appear to be pesticides since there was no response with ECD. They are probably hydrocarbon impurities in the solvent. The detection limit for DDT with ECD was < 1 ppb.

We have shown that the sample preparation for field methods can be simplified for semivolatiles. This will enable more rapid and "on-site" availability of tests needed for remediation. GC with a PID and ECD would appear to have sufficient sensitivity and selectivity to analyze all of the 8000 series semivolatiles for water and soil. Typical methods could be performed in the field with analysis times of between 20 and 60 min.

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Review

Planar layer chromatography in the analysis of inorganic pollutants

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ABSTRACT

This review covers salient features of planar layer liquid chromatography as applied to the analysis of inorganic pollutants present in a variety of complex matrices. The discussion is limited to classical planar chromatography (paper and thin-layer chromatography) as used in the analysis of real and synthetic environmental samples containing inorganic pollutants. The matrices from which inorganic pollutants have been isolated and analysed include: biological, food, geological, industrial, pharmaceutical, soil, water and wastewater. Eighty-nine references have been cited from the literature of the last twenty years. Over-pressured layer chromatography has not been utilized so far for the analysis of inorganic species present in natural environmental samples. *In situ* densitometry and spectrophotometry have been commonly used for the quantification of inorganic ions present in environmental samples.

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1. INTRODUCTION

Planar layer chromatography (PLC) is a liquid chromatographic technique in which the mobile phase migrates through a porous support (stationary phase) either by capillary forces or under the influence of forced flow. Depending on the mode of transport of the mobile phase, PLC can be classified as conventional (classical) PLC and forced-flow PLC (FFPLC). Many of the procedural steps (sam-

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ple spotting, development of chromatograms, detection of spots, direct scanning, etc.) and principles are identical in both techniques. High-performance thin-layer chromatography (HPTLC), an instrumentalized version of TLC with a perfectly uniform surface of thin layers, is capable of providing faster separations, reduced zone diffusion, better separation efficiency and higher sensitivity. FFPLC includes all techniques where the mobile phase migrates by a forced solvent flow, which can be achieved either by application of external pressure (over-pressured layer chromatography, OPLC) [1–3] or by centrifugal force (rotational planar chromatography, RPC) [4–6]. Electroosmosis is applied to force the mobile phase in high-speed TLC (HSTLC) [7–8]. OPLC, developed by Tyihak *et al.* [1] combines the advantages of classical TLC, HPTLC and a high-performance liquid chromatography (HPLC). Several reviews [9–12] have appeared on OPLC with emphasis on instrumentation, chamber types, development modes and applicability. More recently, Kaiser and Rieder [13,14] developed high-pressure planar liquid chromatography (HPPLC), a circular version of OPLC for achieving a better separation efficiency at higher operating pressure. According to the literature, OPLC has applications in solving various analytical problems relating to agriculture, biochemistry, food stuffs and forensic medicine. As a result, it has several applications to the analysis of a large number of organic substances, *e.g.*, dyes, amino acids, antibiotics, antioxidants, aromatic hydrocarbons, polyamines, alkaloids, aliphatic aldehydes, amines and their derivatives, doping agents, essential oils, lipids, peptides, fatty acids, steroids, flavonoid glycosides, ginsenosides, iridoid glycosides and anthraquinone aglycones [15].

It is surprising that in spite of the availability of several attractive features of OPLC, it has not been used in the analysis of inorganic samples. As far as we are aware, no work has been reported on the use of OPLC in analysing environmental samples containing inorganic pollutants.

This review is therefore restricted to outlining the current state-of-art procedures for classical layer chromatography [paper chromatography (PC) and TLC] as applied to the analysis of synthetic and real environmental samples for inorganic pollutants.

2. SAMPLE PREPARATION

A sample volume (1–10 μl) containing a sufficient amount of analyte (0.1–10 μg) is generally applied with the aid of calibrated microcapillary, syringe or micropipette (Drummond microcaps) about 2 cm above the lower edge of a precoated or laboratory-made chromatoplate or paper strip. The one-dimensional ascending technique has usually been used for the development of chromatograms in a closed chamber (cylindrical or rectangular), allowing the mobile phase (solvent) to migrate 8–10 cm from the starting line on the plate or paper strip. Multiple, two-dimensional, centrifugal and gradient development techniques have also occasionally been used.

3. DETECTION

Chemical, physical, enzymatic and radiochemical procedures have been usually used to locate the analytes on chromatoplates. Chromogenic and fluorogenic spray reagents that are capable of forming coloured products with the separated species are sprayed on the chromatoplates to detect the solute. Typical chromogenic reagents used are dithizone, dithiosemicarbazone, aluminon, dimethyl glyoxime, 1-(2-pyridylazo)-2-naphthol (PAN), arsanazo III, *m*-nitrochlorophosphonazo, alizarin and tribromochlorophosphonazo. Among the physical methods, observation under UV light has been the most preferred procedure. Selenium in food samples [16] has been detected as the 2,3-diaminonaphthalene–Se complex, which produces pink fluorescence under UV light (366 nm). An enzymatic method [17] has been used to detect $CuSO_4$, $HgCl_2$, $CdSO_4$ and $AgNO_3$ in fresh water after their separation on TLC plates. Sometimes labelled species of very low-level inorganic materials can be detected using a radioactivity detector [18,19].

4. IDENTIFICATION AND QUANTIFICATION

Compound identification in planar liquid chromatography is based on the R_F value, which is a measure of the ratio of the distance travelled by the analyte from the point of origin to the distance travelled by the solvent. Visual observation, zone elution and scanning densitometric methods have been

used for quantitative analysis. *In situ* densitometry has found some novel applications in the determination of trace amounts of inorganics present in complex matrices [16,20–29].

In spite of the extensive application of planar chromatography to analyses for inorganic and organic compounds in standard sample solutions, its use in the analysis of real environmental samples [30–46] containing inorganic polluting agents is limited. Extraction of the analyte from the sample matrix, clean-up of the extract and concentration of the analyte usually precede TLC or PC if the concentration of the analyte in a complex sample (food, biological, human, plant or environmental) is low. However, if a compound is present in sufficient purity and concentration, the sample can be applied directly.

The following sections provide a brief description of planar chromatography as used in the analysis of environmental samples.

5. APPLICATIONS

Applications have been divided into sections depending on the nature of the sample to be analysed, *viz*., biological, food stuffs, geological, industrial, pharmaceutical, soil, water and industrial wastewater and miscellaneous.

5.1. Biological samples

TLC in combination with densitometry has been applied to determine selenium in biological tissues [28]. Selenium is removed from the matrix, complexed with diaminonaphthalene and the complex is extracted into cyclohexane and analysed on CRP-Whatman HP plates. TLC separation of the selenium-containing complex permits the complete elimination of interfering fluorescent compounds. The recovery of selenium achieved with this method is 85–90%.

An accurate and highly sensitive HPTLC method for the determination of selenium in water and biological matrices has been developed by Funk *et al.* [29]. Selenium-containing biological samples (blood and serum) and environmental materials (drinking and surface water) were oxidized by a wet chemical digestion procedure, derivatized with 2,3,1-naphthoselenodiazole, extracted on an Extrelut column using cyclohexane, dried, dissolved in chloroform and analysed. The chromatographic separation was carried out on purified silica gel HPTLC plates (Merck) using chloroform containing 0.01% of butylhydroxytoluene (antioxidant) as the mobile phase. The quantitative evaluation was completed in the fluorescence mode with λ_{ex} = 365 nm and fluorescence intensity measurement at λ_{fl} = 560 nm. The proposed method includes the following advantages: comparable to spectrophotometry, polarography, gas chromatography, neutron activation analysis, X-ray fluorescence and atomic absorption spectrometric methods; high sensitivity (detection limit 250 fg of Se per spot); novel digestion procedure (wet chemical digestion) for oxidation of organic matrices; accurate quantitative preparation of biological matrix; the sample preparation step [oxidation of selenium to Se(VI) and subsequent reduction to Se(IV)] is less susceptible to systematic errors; and interfering metal ions (Fe^{3+}, Cu^{2+}) can be easily masked by addition of suitable chelate-forming reagent.

Scanning densitometry and TLC have been used for the simultaneous determination of traces of Cu^{2+}, Fe^{2+}, and Fe^{3+} in serum [23] as their complexes with 2-[(5-bromo-2-pyridinyl)azo]-5-(diethylamino)phenol. The complexes, after extraction from serum samples, were separated on TLC plates coated with silica gel G and sodium carboxymethylcellulose using butyl acetate–acetone (7:3) as the eluent. The absorbances of the separated metallic complexes were measured by densitometry.

Silica gel KSK layers (free from iron) with *n*-butanol–water (84:14) as the eluent were used for the detection of magnesium chlorate isolated from biological objects [47]. This method provides a detection limit of $\geqslant$5 ng per 100 g of biological sample.

A TLC method for the detection of metal–EDTA complexes in human faeces [48] has been reported. An anion-exchange column packed with carboxymethylcellulose was used to clean up and preconcentrate the sample.

Spectrophotometry in combination with TLC has been used to determine Cd^{2+}, Hg^{2+} and Pb^{2+} in blood and urine samples [49]. Metals were separated on silica gel layers using various organic solvents as eluents prior to their quantification.

A chromatographic system [silica gel layers, di-

chloromethane–methanol (9:1) and benzene eluents] has been identified as a sensitive method [50] for the detection of heavy metals (Zn^{2+}, Hg^{2+}, Ag^{+}, Cu^{2+}, Bi^{3+}, Co^{2+}, Ni^{2+} and Pb^{2+}) as dithizonates in biological samples (urine, blood and excrement). The sensitivity of the proposed method was 10^{-3} g/l.

A TLC method for the detection of Pb^{2+}, Cd^{2+}, Hg^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+} and Mn^{2+} present in autopsy tissues [51] has been described. The metals were separated on silica gel layers developed with carbon tetrachloride–chloroform (5:2), xylene, benzene and toluene.

A successful method for the quantitative separation of inorganic mercury and methylmercury from animal, fish and plant tissues has been developed [52]. Mercury species were determined by TLC after extraction from tissues. To achieve the highest efficiency of extraction the fish tissue was initially treated with ethanolic KOH solution whereas the plant tissue was treated with a mixture containing HNO_3 and $HClO_4$.

5.2. Food stuffs

An analytical procedure involving extraction, TLC and densitometry has been developed for the determination of bromate ion in breads and fluor dough [22] after its separation on silica gel plates developed with *n*-butanol–*n*-propanol–water (1:3:1). The limit of detection of BrO_3^- with tolidine–HCl was 0.1 μg/g in bread. Bromate was extracted from food stuffs (bread) and purified using an alumina column.

$[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ present in juice and wine samples have been detected on paper chromatograms [53]. A method has been reported [54] for the separation and identification of Fe^{3+}, Mn^{2+} and Co^{2+} present in human milk. The metals were extracted with isobutyl methyl ketone–amyl acetate (2:1), chromatographed and identified on cellulose plates. Similarly, Li^{+} and Bi^{3+} down to 0.25 and 1.5 μg, respectively, were identified and detected in spiked human milk samples [55]. The sample milk was spiked with Li_2CO_3 or $Bi(NO_3)_3$, ashed, dissolved in an appropriate solvent, applied to cellulose-coated plates developed with methanol–10 *M* HCl (6:4) and Li^{+} or Bi^{3+} were detected using an appropriate chromogenic reagent. Cheese and milk samples have been analysed for polyphosphoric acids [56]. The acids were extracted from cheese or milk samples with 25% trichloroacetic acid and separated on polyamide plates using *n*-butanol–formic acid (1:1) as developer. The separated polyphosphoric acids were identified and determined. Two-dimensional TLC and ion-exchange column chromatography have also been used for the separation and determination of ortho- and polyphosphates in soft drinks [57].

Pb^{2+} and Zn^{2+} in model food systems have been determined by TLC on Silufol plates after complexation with sodium diethyldithiocarbamate [58]. The plates were developed with benzene–chloroform (1:1) or xylene–dichloromethane (2:1). Diphenylthiocarbazone in CCl_4 was used as the detection reagent.

A TLC method for the identification of selenium in foods was developed by Moreno-Dominguez *et al.* [16] using thin layers of MN-300 cellulose powder (activated at 110°C for 30 min before use). A spectrofluorimetric procedure was used for the determination of selenium in different foods of animal and vegetable origin and blood samples. The procedure involves the digestion of the food sample, formation and extraction of selenium–2,3-diaminonaphthalene (DNA) complex with cyclohexane, fluorimetric determination and confirmation of the presence of selenium by TLC. After carrying out the fluorimetric determination, the cyclohexane phase containing the selenium–2,3-diaminonaphthalene complex was concentrated nearly to dryness and the residue was dissolved in 0.5 ml of cyclohexane. This solution was spotted on the chromatoplate and chromatographed along with a standard selenium sample using ethanol–25% ammonia solution (70:30) as the mobile phase. The selenium–2,3-diaminonaphthalene complex produces pink fluorescence on exposure of chromatogram to UV radiation (360 nm).

Silicon in edible oils has been separated [59] on silica gel layers using light petroleum–diethyl ether (98:2) as the mobile phase. Rhodamine B was used as the detection reagent.

5.3. Geological samples

A novel method for the analysis of rocks for rare earth elements was developed by Ryabukhin *et al.*

[60]. Rare earth metals, after preconcentration by circular TLC on Fixion 50-X8, were determined by neutron activation analysis. The determination limits ranged from 0.05 to 10 μg/g for 10–30-mg samples. Another attractive method for the simultaneous determination of light rare earths in monazite sand by densitometry on thin-layer chromatograms using diisopropyl ether–diethyl ether–bis(2-ethylhexyl) phosphate–nitric acid (8:8:0.4:0.07) as eluent was reported by Chinese workers [20]. The R_F values for lanthanum, cerium, praseodymium, neodymium, samarium and yttrium on silica gel H mixed with 1% carboxymethylcellulose binder containing 4% ammonium nitrate layers were 0.13, 0.39, 0.55, 0.69, 0.90 and 0.98, respectively, showing good separation possibilities. The densitometric calibration graphs were linear in the range 0.015–0.60 μg of individual rare earth metals. The limits of detection for lanthanum, cerium, praseodymium, neodymium and samarium were in the range 9–12 ng.

TLC has been used for the determination and separation of rare earth metals in ores, rocks and irradiated nuclear fuels [61] using diethyl ether–bis (2-ethylhexyl) phosphate–nitric acid (100:1:3.5) as the mobile phase. Another TLC method [62] includes the use of silica gel as stationary phase and diisopropyl ether–tetrahydrofuran–tributyl phosphate–nitric acid (10:6:1:1) as the mobile phase for the determination of lanthanum, cerium, praseodymium, neodymium and samarium in monazite sand.

Paper electrophoresis has been used for the separation and determination of Al^{3+}, Ti^{4+} and Fe^{3+} in bauxite [63] using lactic acid as carrier electrolyte. The bands were eluted and the metals were determined spectrophotometrically at 510 nm (Al^{3+}), 400 nm (Ti^{4+}) and 510 nm (Fe^{3+}).

A simple paper chromatographic procedure for the determination of microgram amounts of germanium and gallium [64] in different raw mineral materials has been reported. A TLC method [65] involving the use of alumina layers and mixed aqueous–organic solvent systems as mobile phase has been used for the analysis of minerals consisting of Mo^{6+}, Au^{3+}, Sb^{3+}, Hg^{2+}, Cd^{2+}, Bi^{3+}, Mn^{2+}, Pb^{2+}, UO_2^{2+}, Cr^{3+}, Ti^{4+}, etc.

5.4. Industrial samples

TLC coupled with densitometry has been applied to the determination of Co^{2+} in white wine samples [25] in the concentration range 2.5–4.5 μg l^{-1}. The process involves the fixation of Co^{2+} as the 1-(2-pyridylazo)-2-naphthol complex on a membrane filter followed by direct determination of the reflection absorbance of the complex by densitometry.

A method involving circular TLC and spectrophotometry for the determination of 0.01–1.0% lanthanum and yttrium in molybdenum-based alloys has been described [66]. Cellulose layers treated with 0.2 M trioctylamine in toluene were used as the stationary phase. Hydrochloric acid at various concentrations was used as the developer. TLC has been used for the determination of Cu in Al alloys following the sampling of the investigated material by anodic dissolution [67]. The chromatograms were developed with acetone–HCl–H_2O (70:15:15) and 1-(2-pyridylazo)-2-naphthol reagent was used for detection.

The radiochemical purity of $Na^{131}I$ solution has been evaluated [68] by TLC on silica gel layers developed with acetone. Perchlorates in explosive residue have also been detected on paper strips and TLC plates [69].

A combination of paper electrophoresis, TLC and densitometry has been proposed for the determination of anionic species [21]. Diphenylamine solution (0.2% in H_2SO_4) with which most of the anions produce blue products was used for detection. Anions were chromatographed on Silufol 254 plates and developed with propanol–ammonia solution (2:1). The method was successfully applied to the densitometric determination of NO_3^- and Fe $(CN)_6^{3-}$ in molasses.

A TLC method has been reported for the rapid detection of copper, iron and manganese ions in cotton materials [70]. The separated metal ions on microcellulose plates developed with acetone–HCl–H_2O (8:1:2) were detected with rubeanic acid followed by exposure to ammonia vapour. TLC in combination with spectrophotometry has also been used for the determination of traces of manganese in textile materials.

5.5. Pharmaceutical products

Spectrophotometry in combination with TLC has been used for the determination of Fe^{2+} in pharmaceuticals [71]. Fe^{2+} was separated from oth-

er cations present in pharmaceutical formulations on microcrystalline cellulose plates using propanol–4 *M* HCl–HNO_3–acetic acid–chloroform (30:5:5:5:10) as developer. 1,10-Phenanthroline was used as the colour reagent. A TLC system consisting of silica gel G as stationary phase and chloroform–acetone–concentrated HNO_3 (5:4:1) as eluent has been used for the identification of mercury salts (chloride, nitrate, cyanide, sulphate and sulphide) in homeopathic drugs [72].

Buchbauer and Vasold [73] developed a TLC method for the separation and identification of inorganic impurities (cationic and anionic) in drugs of the Austrian Pharmacopoeia. Aqueous solutions (10%) of drugs were spotted on cellulose-coated micro-plates and developed with methanol–HCl (8:1) for cations or with methanol–*n*-butanol–water (2:1:1) for anions. Inorganic impurities as coloured spots were detected by spraying the developed plates with sixteen spray reagents.

Aqueous–organic solvent systems have been used as mobile phases with silica gel layers for the determination of radioactive impurities in pharmaceuticals [74] and the radiochemical purity of $Na_2{}^{51}CrO_4$ injections [75]. A photodensitometric method [76] for the determination of manganese in pharmaceuticals, vitamins, etc., as a manganese–1-(2-pyridylazo)-2-naphthol complex with preliminary TLC separation on silica gel layers using pyridine–methyl isobutyl ketone–choroform (20:4:1) as mobile phase has been reported.

5.6. Soil, water and industrial wastewater samples

A rapid TLC method for the analysis of industrial and wastewaters for total heavy metal content (Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+} and Cu^{2+}) was developed by Volynets et al. [77]. Heavy metals as coloured diethyldithiocarbamates were concentrated during the chromatographic process on plates in the shape of irregular strips as shown in Fig. 1, coated with Silufol, and determined semi-quantitatively directly on the plates on the basis of their colour intensities. The method was successfully applied to the semi-quantitative determination (mg l^{-1}) of nickel and copper in electroplating wastewater.

An interesting method involving the use of TLC in combination with spectrophotometry has been reported [27] for the determination of boron at ng

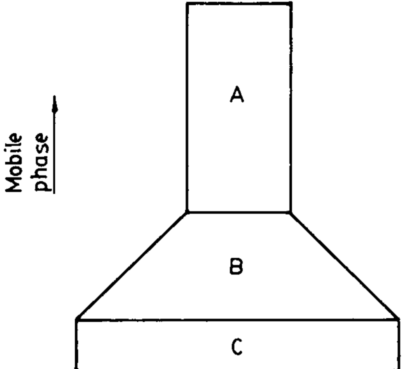

Fig. 1. Irregular strips used for concentrating diethyldithiocarbamates of heavy metals during the chromatographic process. A = Separation zone; B = concentration zone; C = sample application zone.

levels in soil and water samples. A water sample (25 ml) was concentrated by evaporation to 0.5 ml and acidified to pH 1. Aliquots (5–25 μl) of the sample were applied on MN 300 cellulose layers. The plates were developed with butanone–water–ethylene glycol (85:13:2). Boron (R_F = 0.43) was detected with azomethine (1 g in 100 ml of 1% ascorbic acid) spray reagent. After 1 h the sample zone was scanned at 400 nm. The calibration graphs were linear for 50–450 ng of boron.

A combined spectrophotometric–TLC method for microgram determination and recovery of Hg^{2+} from river and industrial wastewater was developed by Ajmal *et al.* [78]. Hg^{2+} (R_F = 0.85) was separated from Pb^{2+}, Ni^{2+}, Hg^{+} and Cu^{2+} on silica gel layers impregnated with 2% oxalic acid using ethyl acetate–acetone–formic acid–water (8:7:4:1) as the mobile phase. Hg^{2+} was detected with dithizone; the area corresponding to Hg^{2+} was scraped off the working plate, and the complex was extracted with carbon tetrachloride and determined spectrophotometrically. The calibration graph obtained under optimum experimental conditions was linear over the concentration range 5–15 μg per 10 ml of Hg^{2+}.

HPTLC and *in situ* densitometry have been successfully used to detect, separate and determine inorganic mercury and some organomercury species at the nanogram level as dithizonates in tap and sea waters [79]. Detection and semi-quantitative determination of Pb^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and Cu^{2+} in

industrial wastewater has been accomplished on silica gel and cellulose plates [80].

An attempt has been made to use a chelating cellulose sorbent with azopyrocatechol groups for the separation and determination of heavy metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Cr^{6+}, Fe^{2+}, V^{4+} and V^{5+}) by TLC [81]. The metals were determined according to the colour reaction in the sorbent zone. Heavy metals react with the sorbent phase to produce coloured products at pH 1–6. The detection limit of the elements in the zone is in the range 0.05–2.0 μg. A mixture of 1-butanol, acetone, glacial acetic acid, 5% ammonia solution and water (7:5:3:3:2) was used to develop the chromatogram. A brief outline of the procedure as used for the determination of heavy metals is given below.

Sample solution containing 20–400 mg/l of the ions being determined is adjusted to pH 1 by adding HCl and an aliquot (2–5 μl) is applied to the chromatoplate. The coloured zone that appears is dried and the plate is developed with the chosen mobile phase keeping the ascent to 10 cm (development time 15–45 min). After complete drying of the developed plate, the content of the elements in the zone is evaluated by comparing the obtained chromatogram with the standards.

The method was applied to the analysis of waste waters from an electroplating process before their treatment to determine the contents of heavy metal ions in the range 20–400 mg/l. The results were comparable to those obtained by atomic emission spectrometry.

Reversed-phase paper chromatography using triphenylphosphine oxide as the stationary phase and organic complexing agents (sodium malonate, acetate and succinate) as the mobile phase has been applied to the separation and determination of As^{3+}, Sb^{3+} and Bi^{3+} in water and alloy samples [82].

A simple and portable bacterial enzymatic paper chromatographic procedure has been developed for the determination of Cu^{2+} and Hg_2^{2+} in water samples [83].

Hg^{2+}, Zn^{2+}, Cu^{2+} and Pb^{2+} present in water and aquatic plants have been detected on silica gel layers [84]. Aquatic plants were mineralized in concentrated H_2SO_4, HNO_3 and H_2O_2 and extracted in water. The extract was treated with chloroform containing dithizone and the resulting extract was chromatographed on silica gel plates. The detection limit of metals was in the range 0.5–5.0 μg.

A simple PC and micro-TLC method for the separation and detection of some heavy metals ($HgCl_2$, $CuSO_4$, $CdSO_4$, $AgNO_3$, etc.) in fresh water has been reported [17]. Precipitation and evaporation techniques were adopted to concentrate the samples. The preconcentrated samples were dissolved in citric acid and separated by PC and micro-TLC using 0.1 *M* NaCl solvent. The separated heavy metals were detected by horse liver acetone powder succinate dehydrogenase inhibition using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride–sodium succinate–N-methylphenazonium methosulphate mixture as the chromogenic reagent. PC was found to be most suitable for the separation of $CuSO_4$–$HgCl_2$ and $CdSO_4$–$HgCl_2$ mixtures whereas micro-TLC was suited to the separation of $CuSO_4$–$CdSO_4$ mixture.

5.7. Miscellaneous

TLC has found an interesting application in the analysis of cosmic dust particles containing Fe^{3+} and Co^{2+} [85]. Thin-layer plates coated with silica gel KSK mixed with starch (5%, w/w) were spotted with solutions containing 15–20 μg of material and developed with acetone or acetone–3 *M* HCl (99:1). The semi-quantitative determination of Fe^{3+} and Co^{2+} on the basis of spot size and colour intensity or by reflectance densitometry was carried out.

A method involving qualitative TLC followed by quantitative PC has been developed to determine Pb^{2+} in airborne dust [86]. TLC was performed on silica gel layers using dioxane–1.5 *M* HCl–butanol–acetylacetone (50:20:50:0.5) and dioxane–1.5 *M* HCl–acetylacetone (50:20:0.5) as mobile phases.

Rare earth elements (REEs) and other fission products in freshly irradiated nuclear fuels have been determined after their enrichment and separation by TLC [87,88]. REEs were quantitatively separated from all other fission products by two-dimensional TLC using diisopropyl ether–tetrahydrofuran–HNO_3 (100:80:4) and diethyl ether–bis (2-ethylhexyl) phosphate–HNO_3 (100:4:2) as developer. After separation, REEs were determined by γ-spectroscopy.

NO_3^- in feeds has been determined after its separation on alumina G plates [89] developed with

0.05 M NaOH–acetone (3:17). After TLC, the nitrate-containing zone was scraped off and treated with 3,4-xylenol in sulphuric acid medium. The reaction product was extracted with light petroleum. The organic layer was subsequently shaken with aqueous alkali. The aqueous layer was separated and the absorbance of this layer was measured at 430 nm.

6. CONCLUSIONS

From the above survey it is clear that planar layer chromatography (specially TLC) has been a dependable and useful analytical tool for the determination of inorganic pollutants after their separation. However, these studies have been mostly restricted to heavy metals and therefore efforts are required to utilize TLC by coupling it with sensitive instrumental techniques for the determination and identification of other inorganic species in environmental samples. In addition, forced-flow planar liquid chromatographic techniques should be developed for utilization in the analysis of inorganic species present in both environmental and non-environmental samples. OPLC, a real planar version of HPLC, has special advantages as a planar system with great prospects for the future.

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CHROMSYMP. 2686

New surface-modified sorbent layer for the analysis of toxic metals in seawater and industrial wastewater

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ABSTRACT

Thorium nitrate-impregnated silica gel layers were utilized for the separation of zinc, cadmium, thallium and mercury from seawater and industrial wastewater. Some common pesticides, namely malathion, carbaryl, carbofuran and bavistin, do not impair the separation and detection of the above-mentioned toxic metals in seawater and industrial wastewater. Detection limits of these toxic metals are reported.

INTRODUCTION

In recent years, thin-layer chromatography (TLC) has proved to be a convenient technique for the analysis of environmental samples containing inorganic ions. It has been successfully applied for the determination of heavy metals in water and aquatic plants [1], analysis of wastewater for total heavy metal contents [2], characterization of hazardous wastes [3] and quantification of toxic metals in industrial sewage [4]. An enzymatic method has been reported [5] for the detection of copper, mercury, cadmium and silver salts in fresh water after their separation on TLC plates.

In our earlier communication [6,7] we showed that silica gel layers impregnated with inorganic salt solutions are more selective for cations and can provide better separation possibilities than untreated silica gel layers. In continuation, we have now developed a new sorbent phase for TLC analysis of heavy metals, by impregnating silica gel with 0.1% aqueous thorium nitrate solution. The resulting sorbent phase was examined for its possible use in the analysis of seawater and industrial wastewater.

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EXPERIMENTAL

Apparatus

A TLC applicator (Toshniwal, India), electrical centrifuge (Remi, India) and pH meter Model LI-10T (Elico, India) were used.

Reagents

Silica gel G (E. Merck, India), thorium nitrate (Riedel De Haen, Germany) and sodium formate (S.D. Fine Chemicals, India) were used. All other reagents were also of analytical grade.

Test solutions

The test solutions (1%) were chloride or nitrate salts of cadmium, mercury, thallium and zinc. Double-distilled water having a specific conductivity (K) of $1.5 \cdot 10^{-6}$ ohm^{-1} cm^{-1} at 25°C was used to prepare the test solutions.

Alcoholic solutions (1%) of malathion, carbaryl, carbofuran, bavistin, 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid were used.

Detection

The following detection systems were used: (i) dithizone (0.1%) in carbon tetrachloride for Zn^{2+} and Cd^{2+}; (ii) hydrogen sulphide gas for Tl^{+} and Hg^{2+}.

Mobile phase

A 1.0 *M* aqueous solution of sodium formate (pH 7.65) was used.

Stationary phase

The stationary phase was silica gel impregnated with a 0.1% aqueous solution of thorium nitrate.

Preparation of TLC plates

Impregnated TLC plates were prepared by mixing silica gel G with an aqueous solution of 0.1% thorium nitrate in a 1:3 (w/v) ratio. The resultant slurry was mechanically shaken for 10 min, after which it was coated onto glass plates with the help of a TLC applicator to give a layer of 0.25 mm thickness. The plates were air-dried at room temperature and then activated by heating at 100°C for 1 h. After activation the plates were kept in an air-tight chamber until use.

Preparation of spiked water

A 100-ml aliquot of distilled water was spiked with 250 mg each of zinc, cadmium, thallium and mercury salts. The pH of this solution was adjusted to 3 using dilute hydrochloric acid. Hydrogen sulphide gas was passed through the solution till the metal ions present in this solution were completely precipitated. But it was found that zinc could not be precipitated at pH 3. The precipitate was washed with distilled water, centrifuged and dissolved in the minimum possible volume of concentrated nitric acid followed by dilution to 100 ml, using distilled water.

Preparation of spiked industrial wastewater and seawater

A 100-ml volume of industrial wastewater (collected from Link Lock Industries, Aligarh, India) or seawater (collected from Anjuna beach of the Arabian Sea, Goa, India) was spiked with 333 mg each of zinc, cadmium and mercury salts to give a 1% solution. The pH values of the resultant spiked industrial wastewater and seawater were 1.5 and 8.3, respectively.

Procedure

About 0.01 ml of the spiked test solutions (distilled water, industrial wastewater and seawater) was spotted separately on the activated TLC plates. The spots were air-dried and the chromatograms were developed allowing the solvent to ascend up to 10 cm from the point of application in all cases. After development, the chromatograms were air-dried at room temperature and the Tl^{+} and Hg^{2+} spots were visualized by exposing the TLC plate to hydrogen sulphide gas. On exposure to hydrogen sulphide gas, the regions containing Tl^{+} and Hg^{2+} turn to blackish brown. When dithizone solution is sprayed onto the TLC plate, Cd^{2+}-containing zones turn yellowish orange, whereas Zn^{2+}-containing zones turn reddish pink. R_L (R_F of the leading front) and R_T (R_F of the trailing front) values for the detected spots were determined. The R_F [$R_F = (R_L + R_T)/2$] and ΔR_F (ΔR_F = mean R_F on plain silica gel for a particular metal ion minus mean R_F on impregnated silica gel for the same metal ion) values for the metal ions were calculated. The standard deviation in R_F values was also calculated using the formula

$$\text{S.D.} = \sqrt{\frac{\sum (x_i - x)^2}{n}}$$

where x_i is the individual R_F value, x is the mean R_F value and n is the number of observations made for each metal ion.

To study the effect of pesticides on the separation of metal ions present in spiked water, 0.01 ml of spiked distilled water was spotted on the chromatogram followed by the spotting of 0.02 ml of pesticide solution on the same spot. The plates were developed in 1.0 *M* sodium formate, dried and the spots were detected as described above. The R_F values of the separated metal ions were determined and compared with those obtained in the absence of pesticides.

The limits of detection of the cations were determined by spotting 0.01 ml of cation solutions of various concentrations on the chromatoplates, which were developed in sodium formate, and the spots were visualized using the appropriate detector. This was repeated with successive reduction of the concentration of the cation solution until no detection was possible. This method was carried out three times for each cation. The amount of salt just detectable in the solution was taken as the detection limit.

TABLE I

hR_F ($R_F \times 100$), STANDARD DEVIATION IN R_F, ΔR_F AND DETECTION LIMITS OF METAL IONS PRESENT IN STANDARD SAMPLE SOLUTIONS, CHROMATOGRAPHED ON 0.1% THORIUM NITRATE-IMPREGNATED SILICA LAYERS, USING 1.0 *M* SODIUM FORMATE AS ELUENT

Sample No.	Metal ion	hR_F	S.D. in R_F	ΔR_F	Detection limit (μg)
1	Zn^{2+}	11	0.0047	+0.76	0.23
2	Cd^{2+}	79	0.0124	+0.15	6.13
3	Tl^{+}	56	0.0141	+0.15	7.68
4	Hg^{2+}	93	0.0047	+0.04	7.40

RESULTS AND DISCUSSION

When silica gel is mixed with an aqueous solution of thorium nitrate, cation exchange takes place at the surface of hydrated silica gel to give a sorbent phase of altered selectivity.

$$Th^{4+} + 4(\text{–SiOH}) \rightleftharpoons Th(OSi^{-})_4 + 4H^{+}$$

Dugger *et al.* [8] determined some thermodynamic parameters for several such ion-exchange reactions to establish the cation-exchange process.

The positive ΔR_F values (Table I) show that the impregnated silica layers are more selective than the plain silica layers.

Thin layers prepared from impregnated silica gel were of good quality, providing well formed and compact spots for the cations chromatographed. The development time for a 10-cm run was 15 min. The hR_F and ΔR_F values reported are the averages of triplicate observations, hence they are reproducible.

The results of this study are summarized in Tables I and II. It is clear from Table I that the developed chromatographic system is well suited for microgram separation of Zn^{2+}, Cd^{2+}, Tl^{+} and Hg^{2+} from their synthetic mixture.

Depending upon the results shown in Table I an attempt was made to establish the applicability of the proposed method in the analysis of spiked water containing zinc, cadmium, thallium and mercury. Three of the matal ions were identified and separated (Table II). Zinc could not be detected because at pH 3 zinc is not precipitated by hydrogen sulphide, and remains in the supernatant liquid. The presence of pesticides in this sample did not show any considerable effect either on the separation or on the R_F values of metal ions, as evident from Table II.

As good results were obtained in the analysis of spiked water (Fig. 1), we made use of this newly developed sorbent phase in the analysis of real environmental samples, namely industrial wastewater and seawater. It is evident from Table II that all the spiked metal ions were readily detectable and separable from these environmental samples.

From the results of this study we anticipate that these metal ions can be easily identified and detected

TABLE II

Zn–Cd–Hg–Tl MIXTURE SEPARATED, CHROMATOGRAPHED ON 0.1% THORIUM NITRATE-IMPREGNATED SILICA LAYERS USING 1.0 *M* SODIUM FORMATE AS ELUENT

NS = Not spiked; ND = not detected.

Metal ion	Spiked industrial wastewater		Spiked seawater		Spiked distilled water		Spiked distilled water in the presence of pesticide	
	hR_F	S.D. in R_F	hR_F	S.D. in R_F	hR_F	S.D. in R_F	hR_F	S.D. in R_F
Zn^{2+}	11	0.0124	10	0.0094	ND	ND	ND	ND
Cd^{2+}	81	0.0124	79	0.0124	81	0.0124	83	0.0170
Hg^{2+}	90	0.0094	96	0.0094	93	0.0047	95	0.0124
Tl^{+}	NS	NS	NS	NS	36	0.0286	52	0.0356

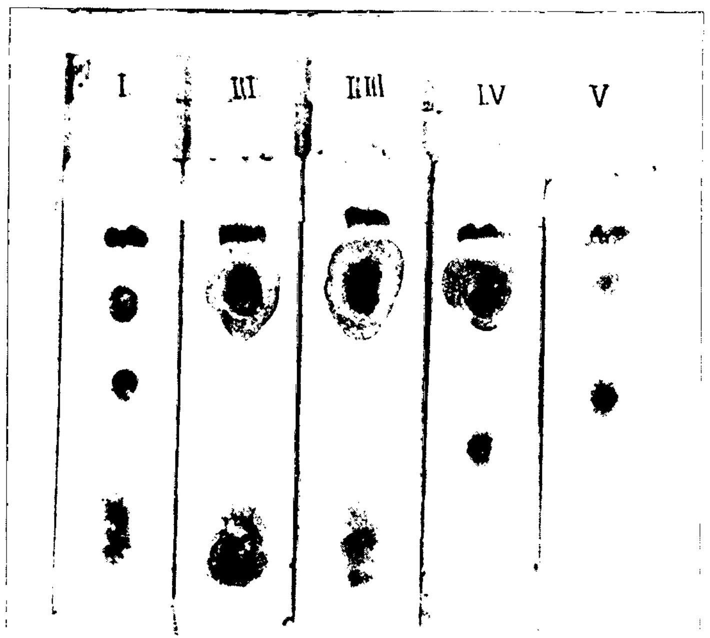

Fig. 1. Separation of a mixture of Zn, Cd, Hg and Tl. (I) Separation in standard sample; (II) separation in spiked industrial wastewater; (III) separation in spiked seawater; (IV) separation in spiked distilled water; (V) separation in spiked distilled water in the presence of pesticides.

if present in river water, using the proposed chromatographic system.

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CHROMSYMP. 2660

Determination of size and element composition distributions of complex colloids by sedimentation field-flow fractionation–inductively coupled plasma mass spectrometry

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ABSTRACT

Sedimentation field-flow fractionation (SdFFF) and inductively coupled plasma mass spectrometry (ICP-MS) have been directly combined and the resulting SdFFF–ICP-MS instrument can be used to produce element based size distributions of colloidal samples. Using appropriate tracer elements the size distributions of specific components can be picked out from a complex mixture. Changes in chemical composition of mixtures as a function of particle size can be readily monitored by plotting appropriate element atomic ratio distributions. These applications have been illustrated using data obtained with samples of the clay minerals kaolinite and illite and a natural suspended particulate matter from the Darling River (Australia).

INTRODUCTION

Colloid particles, which are often formally defined as being within the diameter range 1 nm to 1 μm, are extremely important in many industrial, biological and environmental processes. Consequently many methods have been developed in order to physically and chemically characterise these materials. Despite this, the sizing of polydisperse samples in the colloid range remains a difficult task with no single method proving to be totally adequate.

In analysing heterogeneous samples there are distinct advantages in using a technique that incorporates a preliminary separation step so that relatively monodisperse fractions are presented to the detection system. This is achieved with many methods based on transport properties, such as disc centrifugation. The problem is overcome in single particle counters (*e.g.*, Coulter counter, HIAC size analyser) by simply passing very dilute suspensions through the detector although the lower size for such methods is limited to about 0.5 μm by detector sensitivity. Elution methods such as field-flow fractionation (FFF) and hydrodynamic chromatogra-

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phy have the additional advantages that narrow size range fractions can be collected at points across the distribution for subsequent observation and analysis. In the case of FFF this approach has been used quite extensively to establish if separation has occurred [1], confirm the calculated particle diameter [2], investigate particle morphology and mineralogy [3], determine particle density [4,5] and to study particle aggregation phenomena [6].

A particular focus in our research work has been the role of suspended colloids on the transport and fate of pollutants in natural waters [7]. These colloids are important due to their potentially high adsorption capacity and the fact that they generally remain suspended in fresh waters but aggregate and settle in saline waters such as during estuarine mixing. We have developed methods for applying sedimentation FFF (SdFFF) to size aquatic colloids [8]. In addition the high-resolution separations achieved have enabled detailed characterization of the size fractions using scanning electron microscopy, energy dispersive analysis of X-ray emissions [9], X-ray diffraction and inductively coupled plasma-emission spectrometry [3]. Beckett *et al.* [10] have introduced a methodology for investigating the adsorption behaviour of pollutants onto aquatic colloids. In these experiments SdFFF was used to separate the colloid particles after adsorption of a radiotracer labelled pollutant. The amount of adsorbent on the particles was determined by analysis of the eluent with a scintillation counter. Using the data it is possible to calculate the adsorption density distribution which gives the amount of pollutant adsorbed per unit particle surface area as a function of particle size.

A limiting factor with these analyses is the small amount of sample that can be processed in an FFF separation run. This means that only very sensitive analytical methods will be of use in characterizing the separated particle fractions. A preliminary set of experiments has been conducted using inductively coupled plasma mass spectrometry (ICP-MS) to analyse for selected elements [11]. A range of colloid samples, including a pigment, the minerals alumina and goethite and a river suspended particulate matter, were first separated by SdFFF and fractions of the eluent were collected and analysed by ICP-MS. This batch mode approach of combining SdFFF and ICP-MS has demonstrated that ICP-MS has a high enough sensitivity to detect even minor elements (*e.g.*, Mg) in the particles and that adsorption experiments with trace metal pollutants are feasible.

In this paper we report on the first experiments in which SdFFF and ICP-MS were directly coupled. The potential of SdFFF–ICP-MS is illustrated by the analysis of some clay minerals and suspended colloids from the Darling River (Australia). Ion response and element molar ratio distributions can be plotted which reflect the changes in mineralogy across the particle size distribution of the sample.

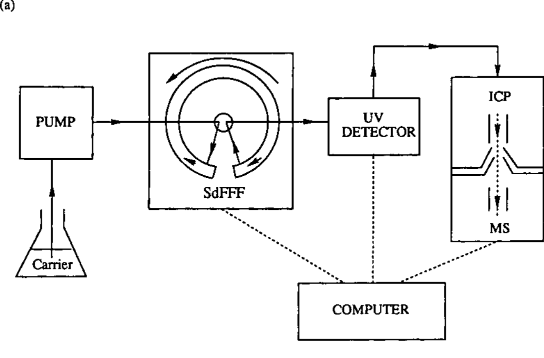
THEORY

Field-flow fractionation is a set of elution based separation and sizing methods which have been gaining wider use in recent years for characterizing a range of particles and macromolecules [12]. FFF is analogous to chromatography except that the separation mechanism is based on the physical interaction of sample particles with an applied field and the subsequent migration down the channel caused by the carrier fluid. In SdFFF the field is created by centrifugal acceleration as the thin unpacked channel is inserted inside a centrifuge basket (see Fig. 1). This causes sample components to accumulate at the outer wall of the channel where they form an equilibrium cloud whose mean thickness l depends on the buoyant mass of the particles (*i.e.*, size and density) and the field strength applied. Carrier flow in the thin flat channel is laminar with the linear fluid velocity being zero at the channel walls and increasing with distance away from each wall, thus approaching a maximum at the centre of the channel. Particles with larger effective mass will have more compressed sample clouds (smaller l) and will thus be swept down the channel by the carrier flow at a lower average velocity than smaller particles. The theory which describes this elution process has been described in detail elsewhere [8,12] and only the major equations that will be used here are summarised as follows.

For normal-mode FFF the retention ratio R, which is obtained from the measured elution volume V_r and channel void volume V^0, is related to the retention parameter λ by

$$R = \frac{V^0}{V_r} = 6\lambda \left(\coth \frac{1}{2\lambda} - 2\lambda \right) \quad (1)$$

(a)



(b)

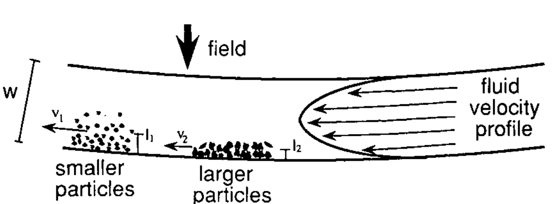


Fig. 1. Schematic representation of (a) the SdFFF–ICP-MS apparatus and (b) the SdFFF separation mechanism occurring in the channel.

where $\lambda = l/w$, with w being the channel thickness. In SdFFF runs at constant field strength the equivalent spherical particle diameter d can be calculated from λ provided the difference in density between the particle and carrier liquid $\Delta\rho$ is known

$$d = \sqrt[3]{\frac{6kT}{\pi\omega^2 rw\Delta\rho\lambda}} \tag{2}$$

where k is the Boltzmann constant, T the absolute temperature, ω the centrifuge speed (radians s^{-1}) and r the centrifuge radius.

Samples consisting of a broad size distribution require high field strengths for the resolution of the smaller particles. However, this may result in excessively long retention times for larger particles. To overcome this problem the field decay programming strategy of Williams and Giddings [13] was employed. This involved applying an initial constant speed ω_0 for a period t_1 after which the speed decayed according to the power equation

$$\omega = \omega_0 \left(\frac{t_1 - t_a}{t - t_a}\right)^4 \tag{3}$$

where t is the run time and t_a is a constant that controls the decay rate. If suitable values for ω_0, t_1 and t_a are chosen the fractionating power F_d [14] of the separation can be optimised to achieve a constant desired level of resolution across the entire size distribution.

The raw data obtained from a SdFFF instrument is usually a plot of detector signal *versus* elution volume (or time) and is referred to as a fractogram (*cf.*, chromatogram). The equivalent spherical particle diameter corresponding to a given elution volume can be computed employing eqns. 1 and 2 and assuming the run is made up of a large number of constant field increments stepping down from ω_0 as the field decays.

If the detector signal is proportional to the mass concentration of the sample in the eluent dm'_i/dV_i then the fractogram may be converted into a particle size distribution by plotting d on the x-axis and dm'_i/dd_i on the y-axis [10] where

$$\frac{dm'_i}{dd_i} \approx \frac{dm'_i}{dV_i} \cdot \frac{\delta V_i}{\delta d_i} \propto \text{detector signal} \cdot \frac{\delta V_i}{\delta d_i} \tag{4}$$

with m'_i being the mass of sample eluted up to point i of the digitised fractogram and δd_i is the change in d due to a very small increment in elution volume δV_i at point i. In most studies a UV detector is used to monitor the sample concentration although it is recognised that there will be some perturbation from the simple relationship between the measured absorbance level and eluent concentration due to the dependence of the signal on the particle size as the light attenuation is mostly due to scattering rather than absorption.

The SdFFF eluent was fed into an ICP-MS instrument which generates an ion current I_E for each element E of interest and I_E will be proportional to the mass concentration of the element (*i.e.*, dm'_{Ei}/dV_i). Thus in a similar manner the element based fractograms (*i.e.*, dm'_{Ei}/dV_i *versus* V_i) can be converted into element based size distributions (*i.e.*, dm'_{Ei}/dd_i *versus* d_i) [11] using

$$\frac{dm'_{Ei}}{dd_i} = \frac{dm'_{Ei}}{dV_i} \cdot \frac{\delta V_i}{\delta d_i} \propto I_{Ei} \frac{\delta V_i}{\delta d_i} \tag{5}$$

Furthermore data for different elements can be combined to give atom (or molar) ratio based size distributions which indicate very clearly any change in chemical composition across the size range of the sample.

EXPERIMENTAL

Sample preparation

RM30 is a sodium-saturated dithionite-treated illite with particle size less than 1 μm in diameter [15]. Purvis School Mine kaolinite was cut at approximately 0.5 μm by centrifugation to give two subsamples for analysis. The clay minerals were provided by Dr. D. Eberl from US Geological Survey, Denver, CO, USA. The solid concentration of clay dispersions injected into the SdFFF was about 5% (m/m).

The Darling River suspended particulate matter (SPM) was concentrated from raw river water by coagulation [8]. The method involved filtering the water through a Whatman GFC filter to remove large particulates and macroscopic debris, and then $CaCl_2$ was added to a concentration of 0.03 *M*. The solution was stirred then allowed to settle for 24 h during which time the aggregates settled. The supernatant was then syphoned from the vessel. Dionized water (Millipore Milli-Q) was added, the solution was stirred again, the allowed to settle as above. The procedure was repeated three times to wash the $CaCl_2$ from the suspension. The suspension was then sonicated to disaggregate and redisperse the sample. The density of both the clays and riverine SPM was assumed to be 2.5 g/ml. The final solids concentration in the suspension was about 1.2% (m/m).

SdFFF–ICP-MS apparatus

The SdFFF instrument used was the Model S101 fractionator from FFFractionation Inc. (Salt Lake City, UT, USA) and was similar to that described previously [11]. The channel void volume given by manufacturer was 4.93 ml. The channel width of 0.0283 cm was calculated using elution times from runs using polystyrene latex standards. The UV detector used was a Dionex UV–Vis detector operated at 254 nm. A Perkin-Elmer Series 100 pump was used to deliver carrier at 2.00 ml/min. The FFFractionation control and data acquisition program controlled and recorded the centrifuge speed, and recorded the UV signal.

The eluent from the SdFFF was delivered directly into a modified Sciex Elan Model 250 ICP-MS instrument operating with argon gas and equipped with a Babington-type pneumatic nebuliser. Analyte standards used included aluminium, magnesium, iron, strontium and rubidium at 100 μg/l, silicon dioxide at 500 μg/l, and calcium at 1000 μg/l. Internal standards used were rhodium, praseodymium and bismuth at 100 μg/l. ICP-MS data were collected by Perkin-Elmer software and this data analysed using FFFractionation and EXCEL spread sheet software. Standards were used to establish the specific sensitivity for each element and hence to determine the concentration of each element in the eluent from

$$\frac{dm'_{Ei}}{dV_i} = I_{Ei}(\text{sample}) \cdot \frac{\text{element concentration (standard)}}{I_E(\text{standard})} \quad (6)$$

The carrier was an aqueous solution of 1.00 g/l (0.0013 *M*) sodium pyrophosphate ($Na_4P_2O_7 \cdot$

TABLE I

SUMMARY OF SdFFF RUN CONDITIONS USED FOR EACH SAMPLE

Sample	Kaolinite 1 (<0.5 μm)	Kaolinite 2 (>0.5 μm)	Illite (RM30)	Clay mixture	Darling River SPM
Initial field (ω_0 RPM)	1500	150	1000	1000	1000
Holding field (ω_H RPM)	10	10	10	10	10
Time lag (t_l min)	7.28	12.95	8.057	8.057	10
Decay constant (t_a min)	−58.24	−103.6	−64.46	−64:46	−80
Fractionating power (F_d)	3	3	3	3	3.4

$10H_2O$, J. T. Baker) made up in water purified by a Barnsted NANOpure II system. The internal standards (Rh, Pr, Bi) were also added to the carrier. The sample injection volume for all the experiments using clay suspensions was 10 μl and for the Darling River SPM was 20 μl.

FFF run parameters

The field for each experiment was decayed according to the power program (eqn. 3) using the parameters listed in Table I. The FFF run parameters were chosen to yield a fractionating power F_d for the separation of about 3 [14,16]. A computer program written by P. S. Williams (University of Utah, Salt Lake City, UT, USA) was utilised in this calculation.

RESULTS AND DISCUSSION

Clay mineral samples

The fractograms generated for the two kaolinite samples (1 and 2) using the UV detector and the Al ion current from the ICP-MS are given in Fig. 2a and b. The normalised (total area = 1) particle size distributions computed from the fractograms utilising eqns. 4 and 5 are plotted in Fig. 2c and d. Although the <0.5 μm sample (kaolinite 1) has little material (5%) greater than the intended cutoff diameter of 0.5 μm the >0.5 μm sample (kaolinite 2) contained about 80% of material less than the nominal cutoff. This indicates that prior fractionation by centrifugation had provided a cut at about 0.35 μm but with some overlap in the distributions of the two subsamples.

The UV trace in the fractogram and consequently in the particle size distribution is shifted slightly to larger elution volume (and hence diameter) compared to the Al element curves. This is more pronounced with the smaller kaolinite 1 sample (<0.5 μm) and may be due to the dependence of the light scattering (and hence the intensity attenuation in the UV detector) on the particle size thereby causing a down weighting of the concentration of smaller particles in each sample.

The UV detector and Al ion current fractograms and particle size ditributions for the illite sample are given in Fig. 3. The shift in the UV detector response curves relative to those obtained by element analysis with ICP-MS are much more pronounced than for the kaolinite samples. This may be due to differences in the particle shape for the two minerals as scanning electron microscopy revealed that the illite consisted of very thin platelets and has a higher calculated aspect ratio than the kaolinite particles.

The element ion current data for the illite SdFFF run was converted to concentration (μg/l) using the

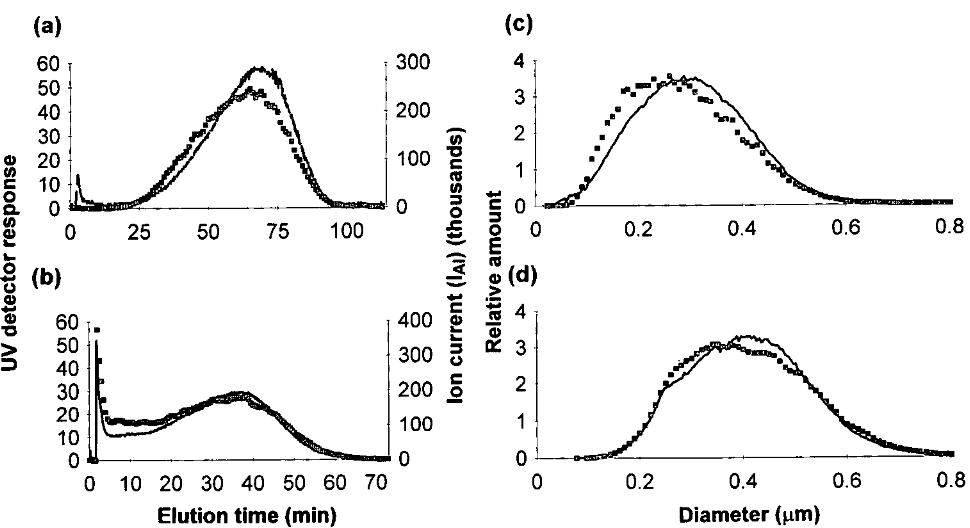


Fig. 2. Fractograms showing the SdFFF separations of Purvis School Mine kaolinite using the UV detector response (line) and Al ion current (squares) from the ICP-MS. (a) Kaolinite 1 (<0.5 μm), (b) kaolinite 2 (>0.5 μm). The corresponding size distributions calculated using the fractograms in (a) and (b) are also given. (c) Kaolinite 1 (<0.5 μm) and (d) kaolinite 2 (>0.5 μm).

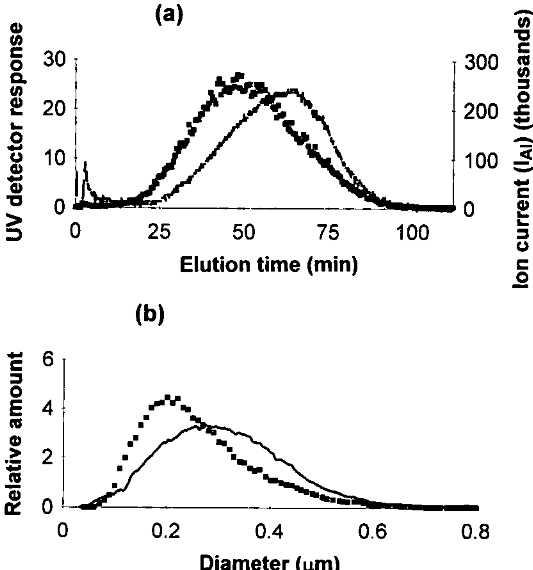


Fig. 3. (a) Fractograms showing the SdFFF separations of RM30 illite using the UV detector response (line) and Al ion current from the ICP-MS. (b) The calculated mass and Al-based size distributions obtained using the fractograms in (a). Line: UV response; squares: Al response.

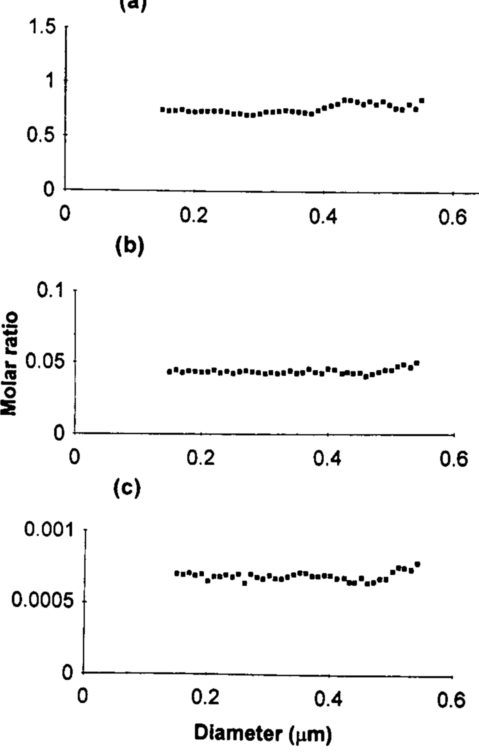


Fig. 4. Atomic ratio distributions (*i.e.*, element mol ratio *versus* particle diameter) for the illite sample (a) Si:Al, (b) Mg:Al and (c) Rb:Al.

element standard solutions (eqn. 6) and then the atomic ratio of selected elements compared to Al in the sample particles was calculated. The atomic ratios for Si:Al, Mg:Al and Rb:Al are plotted against particle size in Fig. 4. These atomic ratios are very constant across the entire size distribution showing that this illite sample has a uniform composition. The low Rb:Al atomic ratio of about $7 \cdot 10^{-4}$ shows that even trace elements can be detected in the solid samples.

The fact that Rb was present in the illite but was not detected in kaolinite suggests that suitable marker elements may be used to monitor the size distributions of certain components in a mixture. This is illustrated by the element based particle size distributions given in Fig. 5 which were obtained from fractograms of the individual illite and kaolinite 2 clays as well as a mixture of the two minerals. Thus Fig. 5a shows plots of the Al frequency function (*i.e.*, $dm'_{Al\,i}/dd_i$ from eqn. 5) for illite and kaolinite 2 and the Rb frequency function (*i.e.*, $dm'_{Rb\,i}/dd_i$) for illite. Each of these size distributions have been normalised to give a total area of 0.5 as the mixture contained equal masses of the two clays. Fig. 5b gives the Al- and Rb-based particle size distributions for the mixture with the area under the Al curve being 1 since it reflects the total amount of illite and kaolinite whereas the area under the Rb curve is 0.5 as it represents only the illite component. By comparison with Fig. 5a it can be seen that the size distribution of illite has been accurately picked out from the clay mixture by monitoring the Rb ion response with ICP-MS. Also plotted in Fig. 5b is the sum of the Al curves for the individual illite and kaolinite 2 runs from Fig. 5a. This agreed quite well with the Al-based size distribution of the mixture despite the implied assumption that the Al content of both clays is the same (*cf.*, actual Al concentration of 20.9% for kaolinite and 17.5% for illite [15]).

Darling River SPM sample

Fig. 6a shows the fractograms for a suspended particulate matter sample collected from the Darling River (Australia) at Wentworth, N.S.W. just above

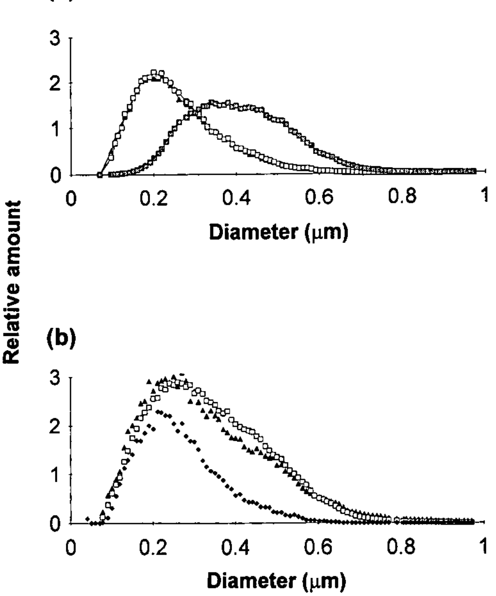
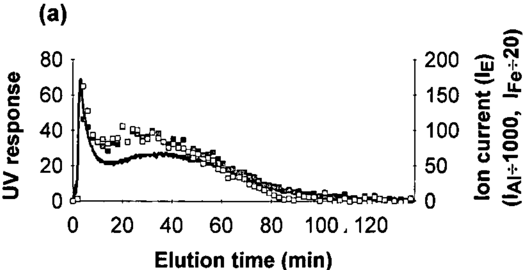


Fig. 5. (a) Individual clay separations: Al-based size distributions for illite (open squares) and kaolinite (>0.5 μm) (grey sqaures) and Rb-based size distribution for illite (triangles). All distributions normalized to have total area of 0.5. (b) Al- (▲) and Rb- (◆) based size distributions for a mixture of equal masses of the illite and kaolinite (>0.5 μm) clays. (Al area normalised to 1, Rb area normalised to 0.5). A summation of the Al responses for the individual clay curves is also shown (□).



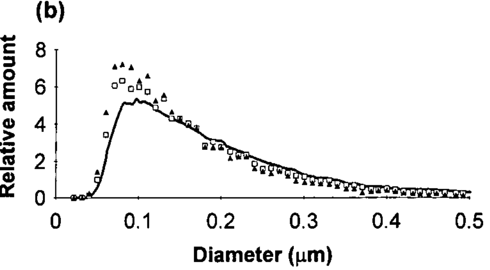


Fig. 6. (a) Fractograms showing the SdFFF separations of the Darling River SPM sample using the UV detector response and Al and Fe ion currents from ICP-MS. (b) The corresponding mass and element-based size distributions obtained using the fractograms in (a). Line: UV response; black squares or triangles: Al response; open squares: Fe response.

its confluence with the Murray River. The UV detector response as well as the Al and Fe ion currents measured by ICP-MS are plotted. The corresponding particle size distributions have been computed and are given in Fig. 6b. There appears to be general agreement between the UV and element based size distributions indicating that the light scattering shift in the UV-based size distribution, so apparent in the illite clay data, is not significant with this SPM sample.

Some knowledge of the mineralogical composition of the Darling River SPM has been attained with X-ray powder diffractometry and elemental analysis on the bulk sample [17]. The major minerals present are illite 15%, kaolin 22%, smectite 48% and quartz 15%. More detailed information on the size ranges of the mineral phases should be feasible using SdFFF–ICP-MS. An excellent method for examining these trends in mineralogy across the size distribution of a sample is to plot the relevant element atomic ratios as a function of the equivalent spherical diameter as illustrated by the graphs in Fig. 7.

The Si:Al atomic ratio plotted in Fig. 7a shows an almost constant ratio for the smaller particles (0.08–0.25 μm) followed by a pronounced increase which results in a doubling of the atomic ratio over the size range from 0.25 to 0.45 μm. A feasible explanation for this trend in Si:Al atomic ratio may be an increase in the proportion of silica (SiO_2) compared to the clay minerals as the size increases. Alternatively it could be caused by a change in the relative amounts of kaolin, illite and smectite minerals as particle size increases, since smectite in particular has a much higher Si:Al ratio. At present we have no independent evidence for the exact mineralogical changes occurring across the size range in these samples but this could be provided by more detailed characterization (*e.g.*, diffraction measurements) of the fractions produced by SdFFF [3].

The Mg:Al atom ratio (Fig. 7b) decreases significantly with increased particle size. Since Mg is probably present as isomorphous replacement ions in the clay lattice or in interlayer cation exchange

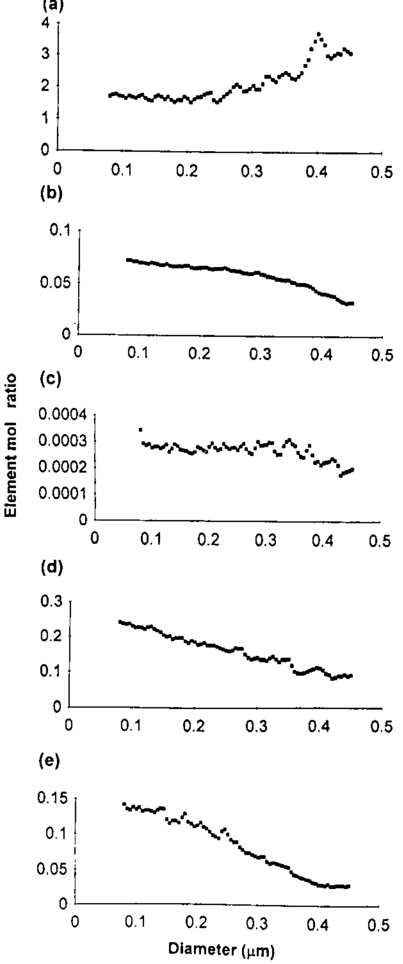


Fig. 7. Atomic ratio distributions (*i.e.*, element mol ratio *versus* particle diameter) for the Darling River SPM sample. (a) Si:Al, (b) Mg:Al, (c) Rb:Al, (d) Fe:Al and (e) Fe:Si.

positions this trend suggests some change in clay mineralogy is occurring with particle size. Furthermore the most likely explanation would be a decrease in illite and smectite (as opposed to a possible increase suggested above) as one would expect silica or kaolin to have the lowest Mg content of the minerals present. This supports the hypothesis that the proportion of silica increases substantially with particle size thus resulting in an over all increase in the Si:Al ratio rather than the alternative explanation of an increase in the smectite and illite clays with particle size.

In contrast the Rb:Al ratio is almost constant over the whole size range (Fig. 7c). Thus Rb and Mg are not present in the same proportion in all of the minerals present which is not unexpected as Rb is more likely to be a replacement for K or Na in clays.

The Fe:Al mole ratio plotted in Fig. 7d shows a small but steady decrease over the entire size range (0.08–0.45 μm). A decrease in Fe content as particle size increases would be anticipated if a significant amount of iron were in the form of surface coatings of hydrous iron oxides. Minerals with high Fe:Al ratios such as illite or smectite may also contribute to the trend in Fe content if they are present in larger amounts in the smaller size ranges. All the element atomic ratios with Si show substantial downward trend with increase in particle size. For example the Fe:Si ratio shown in Fig. 7c decreases by a factor of 3 over the range 0.08 to 0.45 μm. The most likely explanation is the dilution of the element concentrations by the increasing amount of silica rather than the alternative proposition of an increase in the smectite and illite clays with particle size.

CONCLUSIONS

This study has demonstrated that SdFFF can be interfaced directly with ICP-MS to produce element composition data across the size distribution of colloidal samples. Data collected with the clay minerals koalinite and illite have shown that both major and minor elements can be detected. If a suitable tracer element is present then the size distribution of one component can be picked out from a mixture. For complex samples changes in composition as a function of particle size can be conveniently monitored using plots of atomic ratio *versus* particle size for appropriate elements. This approach was illustrated with data for a suspended particulate matter sample collected from the Darling River (Australia).

Previous work on SdFFF and ICP-MS, in the rather more laborious fraction collection and sample analysis batch mode of operation, has demonstrated that SdFFF–ICP-MS will be a valuable method for studying adsorption behaviour [11]. This could include generating adsorption density distributions which show the trend in amount adsorbed per unit particle surface area as a function of particle diameter [10]. Future work should expand the range

of elements analysed as well as check for sample matrix and particle size effects on the raw ion currents produced by the ICP-MS.

The size range covered by the current SdFFF–ICP-MS instrument is about 0.05–2 μm although it has been proposed that this could be expanded by using other subtechniques of FFF [18]. Thus flow FFF could be used for very fine colloids (<0.05 μm) and even macromolecules down to about 1000 dalton in molecular mass. The hyperlayer or steric mode of FFF operation could be used for larger silt sized particles (>2 μm), however, in this range the effect of particle size on the degree of atomization and ionization of the elements by the ICP torch would need to be carefully tested.

The FFF–ICP-MS method shows great potential for the detailed physical and chemical characterisation of complex mixtures.

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The use of tradenames does not imply endorsement by the US Geological Survey.

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Biocatalysts in Organic Synthesis

by **J. Halgaš**, Comenius University, Bratislava, Czechoslovakia

Studies in Organic Chemistry, Volume 46

The fundamental problem in modern organic synthesis is the selectivity of preparative organic reactions. This book reflects the recent growth of interest in the use of biocatalysts to attain high chemo-, regio- and particularly, stereoselectivity. Enantiomerically pure compounds are required as building blocks for the synthesis of many new agrochemicals, drugs, or as bioorganic models and probes.

The first two chapters are devoted to a brief description of basic properties of various forms of biocatalysts: free and immobilized enzymes, free and immobilized microbial cells and other biopreparations, e.g., monoclonal antibodies. The third chapter deals with different levels of selectivity of biocatalyzed reactions. Attention is paid mainly to the differentiation of enantiomers, enantiotopic groups and faces. The remaining six chapters cover particular types of organic reactions and some 939 references from recent original papers are given. These include substitution reactions, eliminations and additions, synthesis and hydrolysis of esters and amides, oxidations and reductions.

Audience

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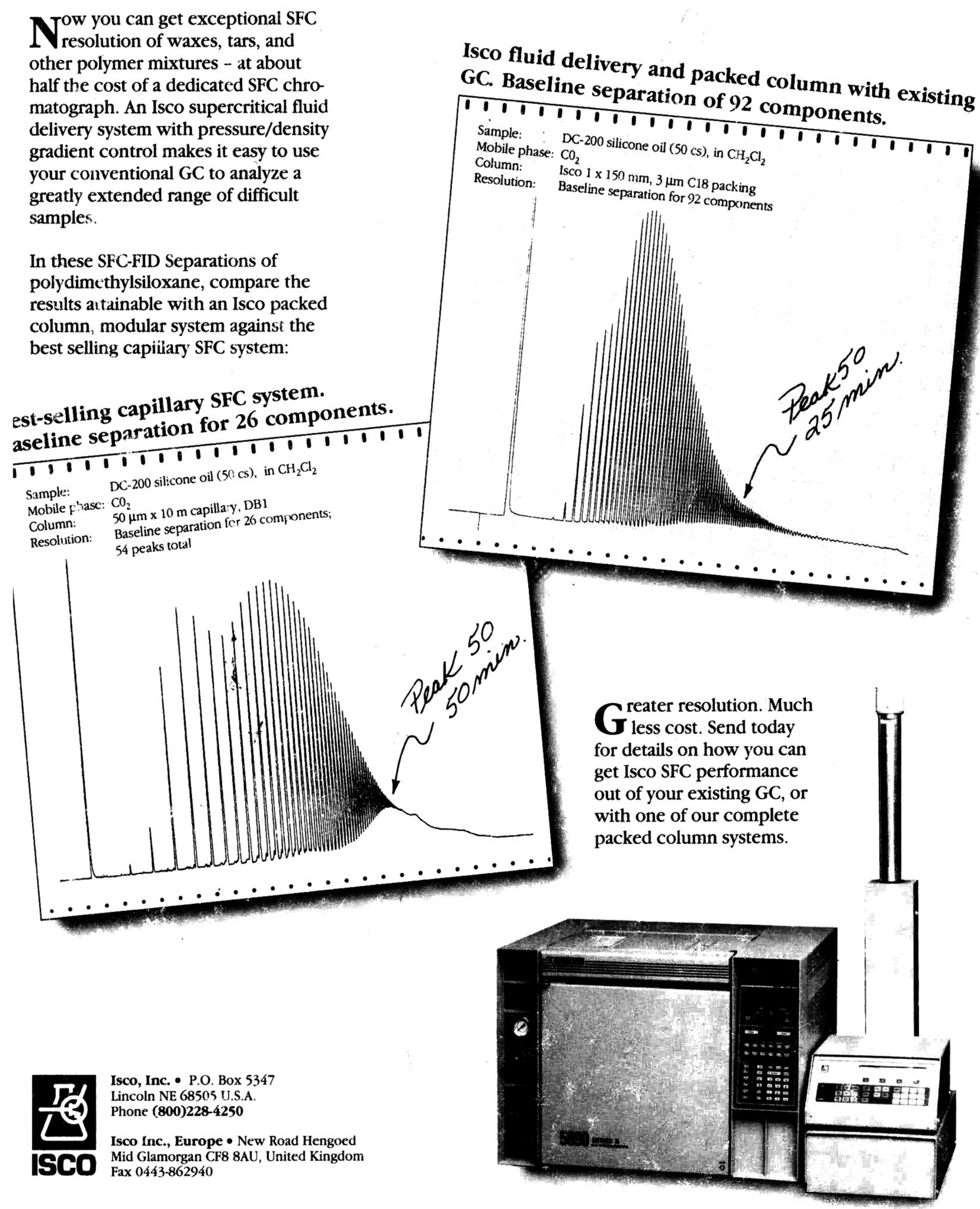

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