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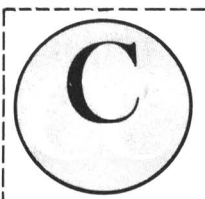

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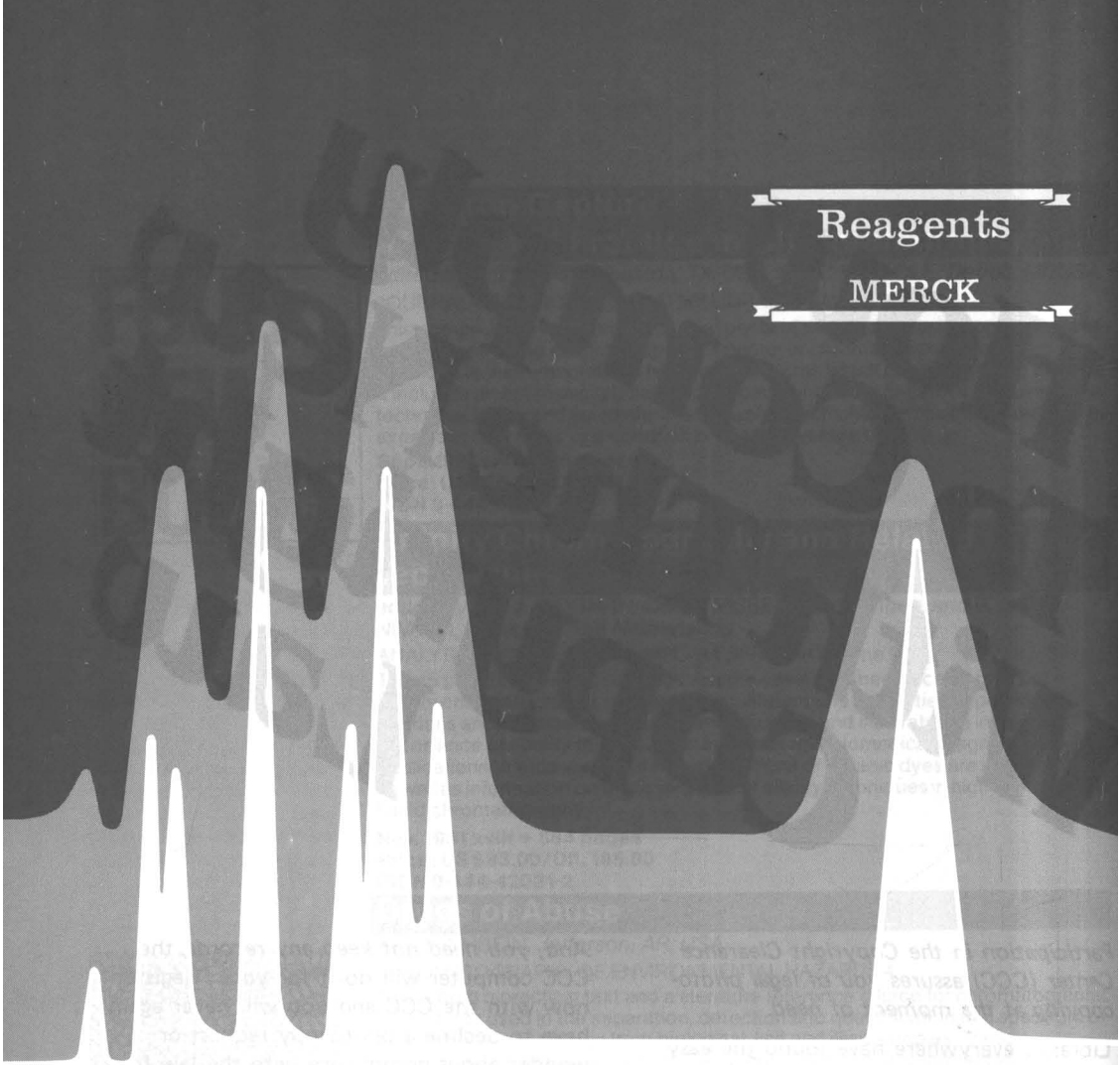

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CHREV. 148

GAS–LIQUID CHROMATOGRAPHIC RETENTION INDICES OF 1318 SUBSTANCES OF TOXICOLOGICAL INTEREST ON SE-30 OR OV-1 STATIONARY PHASE*

ROBERT E. ARDREY and ANTHONY C. MOFFAT*
Home Office Central Research Establishment, Aldermaston, Reading, Berkshire RG7 4PN (Great Britain)
(Received May 11th, 1981)

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1. INTRODUCTION

Gas–liquid chromatography (GLC) is one of the most widely used techniques, either alone or in combination with mass spectrometry, for the separation and identification of drugs, their metabolites and other endogenous materials present in solvent extracts of biological samples submitted for toxicological analysis. Many different stationary phases have been used and retention data have been expressed in a variety of ways such as retention times, retention times relative to arbitrary internal standards and retention indices. It has been proposed[1] that the low polarity dimethylsilicone stationary phases such as SE-30 and OV-1 should be used as the preferred liquid phases for the identification of drugs and that the most reproducible and useful GLC retention parameter is the retention index. As an aid to analysts wishing to standardise on these stationary phases, retention index data for either SE-30 or OV-1 have previously been provided for 480 drugs (see ref. 1) and 296 non-drug substances likely to be encountered in toxicological analyses (see ref. 2). A wealth of retention data using SE-30 and similar stationary phases is available both in the published literature and in private collections and some of this data has now been used in this compilation comprising 1318 compounds. These include not only drugs, but plasticizers, antioxidants, stabilisers, etc., so that the data provide a comprehensive list of retention indices of materials likely to be encountered in a wide variety of medicinal, toxicological and pharmacological analyses.

Data have been extracted from thirty-six sources and the multiplicity of data for a particular compound enables an estimate of the reproducibility of the retention index to be made. The determination of a mean value for the retention index of a

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compound has in many cases highlighted retention index values which differed greatly from the mean and these have been noted in the compilations.

2. COMPILATION OF DATA

This work lists retention index values from 1318 compounds on SE-30 or OV-1 stationary phases, being compiled from 4586 separate retention index values taken from thirty-six sources. These sources included the scientific literature and colleagues who generously donated their data[1–36]. Where the source gave a retention index it was used, otherwise retention times or relative retention times were converted to retention indices using appropriate calibration curves[1]. The names of the compounds used in this compilation were taken from Martindale[37] whenever possible or the Merck Index[38] or, for the economic poisons, the Nanogen Index[39]. For each compound the mean of all retention index values extracted from the sources was calculated. Values differing by more than ± 50 retention index units were then extracted and the mean recalculated. This procedure was repeated until all of the values used to calculate the mean retention index were within this ± 50 retention index unit window.

3. RESULTS AND DISCUSSION

Table 1 gives the retention index data for 1318 substances in alphabetical order of compound name. The contents of each column are: *RI*: mean retention index, 9999 indicates that no material was detected under the conditions used by the respective authors; (*n*): the number of values used to obtain the mean retention index; − +: these two columns show the range of the (*n*) retention index values about the mean; >50: any values which differed by more than 50 retention index units from the mean and which were extracted during the calculation of the mean, are shown in this column. If a single reference contained multiple determinations in this category they are listed separately; [Ref]: the origin of the value cited in the previous column; Refs.: the origins of the (*n*) values used to calculate the mean retention index. Where a single reference contained multiple determinations it has only been cited once.

The retention index quoted for many of the compounds is that obtained from a single value quoted from one source and therefore the reliability and reproducibility of that value cannot be estimated. However, many are the mean of retention indices from several sources. These mean values enable an estimate to be made of both the reproducibility of measurement of the retention index of a particular compound and also the reliability with which a retention index was determined by the original author. For example, the retention index of barbitone in Table 1 is 1497, which is the mean of 20 determinations, the range of reported values being from 17 retention index units below this value (1480) to 33 above (1530). Thus, the measurement of the retention index of barbitone is very reproducible. Only one reported value differed from the mean by more than 50 retention index units, this being a value of 1560 reported in ref. 12. It is therefore likely that, on this occasion, these authors used conditions of measurement which were different to those of the other authors. On the other hand, the range of reported values for chlorpromazine (retention index 2486, mean 21 determinations) is much greater than for barbitone being from 46 retention index units below the mean (2440) to 49 retention index units above (2535) and five

TABLE 1

RETENTION INDICES OF 1318 COMPOUNDS, USING SE-30 OR OV-1 AS THE STATIONARY PHASE, IN ALPHABETICAL ORDER OF COMPOUND

RI = Mean retention index from (*n*) values all of which were within ±50 retention index units of the mean; 9999 = no peak observed; + − = range of values about the mean; >50 = retention indices greater than 50 retention index units from the mean; [Ref.] = source of the value in the preceding column; Refs. = sources of the retention indices used to calculate the mean value.

Compound name	*RI*	*(n)*	−	+	*>50*	*Ref.*	*Refs.*
Acepifylline	1000	(1)					7
Acepromazine	2694	(5)	28	41	2850	[14]	4, 10, 11, 15, 27
7-Acetamidoclonazepam	3263	(2)	8	7			32, 35
7-Acetamidoflunitrazepam	3115	(1)					32
7-Acetamidonitrazepam	3205	(1)					32
Acetanilide	1358	(1)					10
Acetophenazine	9999	(2)					1, 10
Acetylcarbromal	1215	(2)	15	16	1505	[35]	7, 10
Acetylcodeine	2510	(2)	15	15			14, 35
Acetylcysteine	1547	(1)					10
Acetyldihydrocodeine	2455	(1)					11
Acetylindole	1487	(1)					10
Acetylmethylamphetamine	1630	(1)					15
Acetyl-β-phenethylamine	1524	(1)					33
Acetylprocaine	2340	(1)					15
Acetylsalicylic acid (aspirin)	1309	(6)	49	31	1400	[12]	5, 10, 14, 16, 17
					1410	[12]	35
Acetyltributyl citrate	2253	(1)					2
Acetyltriethyl citrate	1730	(1)					2
Acetyl-D-tryptophan	2184	(1)					10
Acetyl-L-tryptophan	2058	(1)					10
Aconitine					2280	[5]	
					2670	[11]	
					9999	[10]	
Adiphenine	2186	(12)	26	29	2250	[23]	5, 10, 12, 13, 15
Ajmaline	2705	(1)					35
Aldrin	1943	(5)	28	27			5, 7, 13
Aletamine	1293	(5)	16	27			3, 10, 11, 22, 29
Allobarbitone	1606	(11)	20	9			6, 7, 9, 10–12, 16, 18, 19, 26, 35
Allobarbitone metabolite	1785	(1)					35
Allopurinol	882	(1)					10
5-Allyl-5-butylbarbituric acid	1698	(1)					18
N-Allyl-3-hydroxymorphinan	2370	(1)					21
5-Allyl-5-(2-hydroxypropyl)-barbitone	1837	(3)	22	33			12
5-Allyl-5-phenylbarbituric acid					2003	[10]	
					2106	[10]	
Allylprodine	1929	(1)					30
Alphacetylmethadol	2187	(3)	7	13			11, 15, 20
Alphameprodine	1850	(1)					11
Alphamethadol	2180	(1)					11
Alphaprodine	1792	(4)	42	33	1895	[7]	10, 11, 20, 30
Alprenolol	1760	(2)	5	5	1840	[14]	12
Alverine	2142	(2)	2	3			7, 15

(Continued on p. 198)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
Amantadine	1257	(3)	7	13			7, 11, 35
Ambucetamide	2235	(2)	5	5			11
Ametazole	1390	(1)					1
Amethocaine	2219	(15)	29	26	2280	[14]	5, 7, 9–12
					2285	[11]	13, 15, 25, 28
Ametryne	1775	(2)	0	0			11
Amidephrine	9999	(1)					11
Amidopyrine	1903	(19)	25	32	2000	[28]	4, 5, 7, 9, 10, 12, 13, 15, 16, 25, 26, 35
Aminacrine	2240	(1)					7
p-Aminobenzoic acid	1547	(1)					10
2-Amino-5-chlorobenzophenone	2039	(6)	39	41	2125	[36]	7, 35, 36
2-Amino-5-chlorodiphenylamine	2078	(2)	3	3			36
2-Amino-5-chloro-2′-fluoro-benzophenone					1980	[7]	
					2045	[36]	
					2090	[36]	
2-Amino-5-chloro-3-hydroxy-benzophenone	2390	(2)	10	10			36
2-Amino-2′-chloro--5-nitrobenzophenone	2516	(4)	46	34			7, 35, 36
7-Aminoclonazepam	2900	(2)	5	5			32, 35
2-Amino-5,2′-dichlorobenzophenone					2120	[7]	
					2195	[36]	
					2240	[36]	
					2480	[36]	
7-Aminoflunitrazepam	2723	(2)	3	2			32
5-Amino-2′-fluoro-2-methyl-aminobenzophenone	2703	(2)	3	2			36
2-Amino-2′-fluoro-5-nitro-benzophenone	2363	(2)	18	17			36
Aminoglutethimide	2227	(1)					10
7-Amino-3-hydroxyclonazepam	2890	(1)					35
7-Aminonitrazepam	2828	(2)	43	42			32
2-Amino-5-nitrobenzophenone	2388	(4)	38	47	2475	[36]	7, 35, 36
Aminonitrothiazole	9999	(1)					11
7-Aminonorflunitrazepam	2825	(1)					32
Aminoparathion	1885	(1)					35
p-Aminophenol	1265	(1)					10
Aminophylline					1970	[12]	
					1970	[12]	
					2083	[10]	
5-Aminoquinoline	1598	(1)					2
p-Aminosalicylic acid	1309	(1)					10
Aminotriazole	9999	(1)					11
Amisometradine	2021	(1)					10
Amitriptyline	2196	(25)	46	34			4–7, 9–16, 17, 25, 27, 28, 30, 35
Amodiaquine	9999	(1)					10
Amolanone	2226	(2)	10	9			10, 11
Amotriphene	9999	(1)					11
Amphetamine	1123	(17)	38	32	1090	[7]	3, 5–10
					1190	[13]	11, 13, 14, 16, 17
					1260	[13]	22, 25, 29, 35
					1265	[12]	

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	*−*	*+*	*>50*	*[Ref.]*	*Refs.*
Amprotropine	2038	(1)					11
Ampyrone	1950	(1)					5
iso-Amylamine	1046	(1)					10
Amylobarbitone	1718	(25)	18	47			5–7, 9–16, 17–19, 24, 26, 35
Amylocaine	1600	(8)	38	25			4, 10, 12, 14, 34
Androsterone	2488	(2)	8	7			7, 11
Anethole	1284	(1)					2
Anhalonidine	1825	(1)					15
Anhalonine	1850	(1)					15
Anilazine	2010	(1)					2
Anileridine	2850	(4)	5	15			5, 7, 10, 15
Aniline	1158	(1)					10
Anisaldehyde	1246	(2)	19	19			10
Anisindione	2273	(2)	13	13			7, 10
Antazoline	2328	(9)	28	32			4, 5, 7, 9–11, 14, 15, 25
Anthracene	1754	(2)	43	42			2, 10
9,10-Anthracenedicarbonitrile	2288	(1)					2
Apoatropine	2050	(1)					11
Apomorphine	2530	(1)					11
Aprobarbitone	1622	(12)	22	33			7, 9–11, 15, 16, 18, 19, 26, 33, 35
Apronal					1231	[10]	
					1578	[19]	
Atrazine	1655	(3)	25	50			2, 11
Atropine	2199	(21)	49	46	2048	[34]	5–11
					2145	[12]	12–15, 17
					2250	[14]	22, 28
Azacyclonol	2243	(5)	33	22	2377	[23]	5, 10, 11, 14, 15
Azaperone	2705	(1)					11
Azapetine	1939	(8)	14	28			7, 11, 12, 15
Azatadine	2415	(1)					11
Azinphos-methyl	2430	(1)					2
Azobenzene	1556	(1)					2
Bamipine	2211	(4)	21	14			12
Barbitone	1497	(20)	17	33	1560	[12]	5–7, 9–14,16,18,19, 24, 26, 35
BBO (2,5-di(4-biphenyloxazole)	3710	(1)					2
BBOT (2,5-bis(5′-*tert.*-butyl-	2745	(1)					2
benzoxazoyl(2′))thiophene)	3750	(1)					2
Beclamide	1480	(1)					11
	1678	(1)					11
Bemegride	1373	(2)	34	37			9, 10, 14
Benactyzine	2248	(13)	28	32			5, 7, 10, 12, 13, 15
Benethamine	1798	(3)	8	7			11, 15
Benorylate	1840	(2)	10	10			11
Benperidol					1471	[35]	
					2975	[11]	
Benzamine	1816	(3)	21	17			14, 15, 30
α-Benzenehexachloride	1690	(1)					2
β-Benzenehexachloride	1710	(1)					2

(Continued on p. 200)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
δ-Benzenehexachloride	1755	(1)					2
γ-Benzenehexachloride	1715	(1)					7
Benzenesulphonamide	1525	(1)					10
Benzethidine	2695	(2)	15	15			7, 11
Benzhexol	2219	(11)	34	41	2275 2280	[11] [13]	5, 7, 10, 12, 13, 28
2-Benzhydroxymethyl-2-imidazoline	2210	(4)	15	15			12
Benzocaine	1555	(9)	25	45			1, 4, 7, 8, 10, 14
Benzoctamine	2082	(3)	12	23			11, 35
Benzoic acid	1180	(2)	11	10			10, 35
Benzonatate	9999	(1)					11
Benzophenone	1610	(2)	0	1			2, 33
Benzoylecgonine	2570	(1)					35
Benzphentermine	1760	(1)					25
Benzphetamine	1855	(8)	16	47			3, 7, 9–11, 14, 15, 29
Benzthiazide	2680	(1)					7
Benztropine	2314	(6)	24	41			7, 10, 11, 14, 15, 28
Benzydamine	2368	(5)	33	42			12, 14
Benzyl alcohol	1046	(1)					2
Benzylamine	995	(2)	10	10			10, 22
N-Benzylamphetamine	1784	(1)					29
Benzyl benzoate	1738	(1)					2
Benzylbutyl phthalate	2290	(1)					8
1-Benzyl-3-ethyl-6,7-dimethoxyisoquinoline	2488	(2)	3	2			12
Benzylmorphine	3015	(1)					11
α-Benzylphenethylamine	1800	(1)					15
Berberine	2070	(1)					11
Betahistine	1235	(1)					11
Betameprodine	1823	(1)					11
Betaprodine	1790	(1)					11
Bethanidine	1925	(1)					7
Bibenzonium bromide	1923	(2)	3	2			12
Biperiden	2266	(8)	41	34	2205	[12]	7, 11, 12, 14, 25, 35
Biphenyl	1389	(1)					2
4,4′-Bipyridyl dihydrate	1507	(1)					2
Bisacodyl	2820	(1)					7
Bisnortilidine	1825	(1)					35
Brallobarbitone	1858	(5)	16	7			9, 16, 18, 26, 35
Brallobarbitone metabolite (5-(2-oxopropyl)-5-(2-propenyl)barbituric acid)	1795	(1)					35
Brallobarbitone metabolite (5-(2-hydroxypropyl)-5-(2-propenyl)barbituric acid)	1835	(1)					35
Brallobarbitone metabolite (5-(2-bromo-2-propenyl)-5-acetonylbarbituric acid)	2040	(1)					35
Brallobarbitone metabolite (5-(2-bromo-2-propenyl)-5-(2-hydroxypropyl)barbituric acid)	2135	(1)					35

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Brocresine	9999	(1)					11
Bromazepam	2663	(4)	17	37	2762	[9]	16, 32, 35
Bromazepam metabolite (3-hydroxybromazepam)	2500	(1)					35
Bromazepam metabolite (2-(2-amino-5-bromo-benzoyl)pyridine)	2243	(3)	48	32			35, 36
Bromazepam decomposition product	2255	(1)					35
Bromdiphenhydramine	2155	(10)	15	25			5, 7, 8, 10, 11, 14, 15, 28
Bromhexine					2295	[12]	
					2295	[12]	
					2330	[12]	
					2340	[12]	
					2380	[11]	
					2385	[11]	
1-Bromodecane	1326	(1)					2
Bromodiacetylurea	1567	(3)	17	13			12, 13
Bromodimethoxyamphetamine	1813	(2)	18	17			14, 15
Bromonaphthalene	1434	(1)					2
Bromo STP					1868	[30]	
					2195	[7]	
Brompheniramine	2096	(11)	26	34			4, 7, 10, 11, 12, 14, 15, 28
Bromvaletone					1212	[10]	
					1510	[35]	
Broxaldine	2686	(2)	4	4			11
Brucine	3280	(2)	0	0	3560	[14]	5, 7
Buclizine	3286	(2)	1	1	3365	[11]	5, 10
Bufotenine	2030	(1)					10
Bufylline	2301	(1)					30
Bulan	2310	(1)					2
Bunamidine	9999	(1)					11
Buphenine	2314	(6)	29	36	2384	[10]	1, 4, 7, 11, 14, 25
					2613	[10]	
	1270	(1)					1
Bupivacaine	2273	(3)	33	27	2360	[14]	7, 11, 28
Butacaine	2457	(4)	27	20			7, 10, 15, 34
Butalamine	2490	(1)					11
Butalbital	1668	(12)	28	27			7, 9–12, 15, 16, 18, 19, 26, 35
Butalbital metabolite	1940	(1)					35
Butallylonal	1995	(6)	15	30			7, 9, 10, 16, 26, 35
Butanilicaine	2025	(1)					11
Butethamate	1754	(2)	4	4			8, 11
Butethamine	2061	(3)	11	14			1, 10, 11
Butobarbitone	1665	(12)	20	20			6, 7, 10–12, 14, 16–19, 35
Butoxyethyl nicotinate	1640	(1)					11
Butriptyline	2181	(4)	26	19			7, 11, 15
Butylamine	909	(1)					10
Butyl aminobenzoate	1742	(3)	7	8			7, 10, 15
N-2-Butylamphetamine	1365	(1)					29

(Continued on p. 202)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
N-*n*-Butylamphetamine	1422	(1)					29
Butylated hydroxyanisole	1462	(1)					2
Butylated hydroxytoluene	1490	(1)					2
Butylbenzyl phthalate	2327	(1)					2
Butylbenzyl sebacate	1585	(1)					2
	2130	(1)					2
	2520	(1)					2
Butylisodecyl phthalate	1950	(1)					2
Butyl PBD	3342	(1)					2
Butylsextyl phthalate	1940	(1)					2
	2235	(1)					2
Butyl stearate	2157	(1)					2
	2362	(1)					2
n-Butyric acid	1309	(1)					10
Cadaverine	1035	(1)					2
Caffeine	1810	(30)	30	25	1945	[28]	4–17, 22, 24, 25, 31, 35
Calusterone	2750	(1)					7
Camphor	1137	(2)	0	1			2, 10
Cannabidiol	2383	(2)	18	17	2190	[11]	15, 35
Cannabinol	2520	(3)	45	35			11, 15, 35
Cantharidin	1490	(1)					5
Capric acid	1473	(1)					10
n-Caproic acid	1249	(1)					10
	1399	(1)					10
Captan	2000	(1)					2
Captodiame	2774	(7)	39	36			5, 9–11, 15, 23, 25
Caramiphen	1971	(4)	6	4			11, 12
Carbamazepine	2290	(1)					7
Carbaryl	1490	(1)					5
Carbazole	1784	(1)					2
Carbetapentane	2232	(6)	32	32			1, 10, 12
Carbimazole	1678	(1)					11
Carbinoxamine	2080	(12)	28	30			8, 10–12, 14, 15, 34
Carbophenothion	2255	(1)					2
Carbromal	1513	(5)	13	12	1192 1580	[10] [12]	5, 7, 16, 35
Carbromal metabolite [2-bromo-2-ethyl-(butyric acid) amide]	1205	(1)					35
Carbromal metabolite [2-bromo-2-ethyl-3-hydroxy-(butyric acid)]	1340	(1)					35
Carbromal metabolite (diethylacetylcarbamide)	1380	(1)					35
Carisoprodol	1830	(7)	35	25	2135	[23]	5, 7, 10, 12, 15
Carminic acid	1507	(1)					10
	1702	(1)					10
Carphenazine	3590	(1)					5
CDEC (2-chloroallyldiethyl-dithiocarbamic acid)	1685	(1)					2
Chlophedianol	2080	(7)	20	15			7, 11, 12, 15
Chloral hydrate	9999	(1)					7

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Chloramphenicol	2310	(1)					7
Chlorbenside	2045	(2)	5	5			2, 5
Chlorcyclizine	2225	(19)	50	30			4, 5, 7, 10–15, 23
Chlordane	2020	(1)					5
Chlordecone	2240	(1)					2
Chlordiazepoxide					2190	[25]	
	2453	(6)	28	22	2300	[1]	1, 11, 12
	2530	(9)	30	50	2660	[32]	1, 6, 7, 10, 14, 16, 28, 32, 35
	2799	(5)	19	16	2893	[9]	1, 7, 13
Chlormethiazole	1230	(3)	9	5			9, 16, 35
Chlormethiazole metabolite (dechlorchlormethiazole)	1185	(1)					35
Chlormethiazole metabolite (2-hydroxychlormethiazole)	1365	(1)					35
Chlormezanone	2238	(4)	18	17	2150	[12]	7, 10, 11, 28
					2165	[12]	
					2335	[23]	
o-Chlorobenzylidenemalonitrile	1516	(1)					2
5-Chloro-2-cyclopropylmethyl-aminobenzophenone	2407	(3)	37	43			36
2′-Chloro-2,5-diaminobenzophenone	2330	(1)					35
5-Chloro-2-diethylaminoethyl-amino-2′-fluorobenzophenone	2558	(2)	3	2			26
5-Chloro-2-fluoro-2-hydroxy-ethylaminobenzophenone	2472	(3)	47	28			36
5-Chloro-2-methylamino-diphenylamine	2250	(2)	20	20			36
Chloroprocaine	2229	(4)	39	46	2347	[30]	10, 11, 29
Chloropyrilene	2133	(6)	8	20	2058	[11]	1, 10, 12
Chloroquine	2590	(4)	15	20	2660	[14]	4, 5, 7, 10
Chlorothiazide					1720	[33]	
					9999	[7]	
Chlorothymol	1486	(2)	21	19			10
Chlorphenesin	1677	(4)	17	18	2113	[10]	7, 10, 11
Chlorpheniramine	2002	(20)	22	28			4–15, 28
Chlorphenoxamine	2072	(4)	41	23			7, 10, 11, 15
Chlorphentermine	1342	(12)	32	27			3, 5, 7, 9–11, 14, 16, 22, 25, 29, 35
2-Chloroprocaine	2290	(1)					28
Chlorproethazine	2617	(1)					27
Chlorproguanil	1621	(1)					30
Chlorpromazine	2486	(21)	46	49	2400	[12]	4–7, 10–17
					2410	[12]	
					2415	[28]	23, 25, 27, 30, 35
					2553	[9]	
					2685	[13]	
Chlorpropamide	1791	(4)	48	39	1720	[33]	6, 7, 10, 11
Chlorprothiazide	2510	(1)					7
Chlorprothixene	2487	(9)	37	38	2400	[12]	7, 10–12, 15, 25
					2410	[12]	27
					2542	[9]	

(Continued on p. 204)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
Chlorthalidone	2145	(1)					7
Chlorzoxazone	1728	(7)	17	17			10–12, 15
5-α-Cholestane	2852	(1)					2
Cholesterol	3086	(6)	26	29	3008	[2]	6, 7, 10, 11, 16, 35
					3020	[17]	
Chromonar	2820	(1)					11
Cinchocaine	2701	(15)	41	39			5, 7, 9–15
Cinchonidine	2598	(6)	24	27			4, 5, 7, 10, 11, 14
Cinchonine	2583	(7)	9	22	2644	[10]	4, 5, 7, 10, 11, 14, 15
Cinchophen	9999	(1)					11
Cinnamylcocaine	2495	(1)					15
Cinnarizine	3065	(1)					11
Citral	1272	(1)					2
Citroflex A4	2224	(1)					2
Clemastine	2415	(3)	5	5			7, 11
Clemizole	2675	(4)	15	15	2585	[12]	10, 11, 14, 34
					2590	[12]	
Clioxanide	9999	(1)					11
Clobazam					2645	[32]	
					2660	[32]	
					2777	[30]	
Clobutinol	1784	(4)	9	16			12
Clofazimine	9999	(1)					12
Clofibrate	1549	(3)	44	36			7, 10, 15
Clomiphene	2930	(1)					7
Clomipramine	2406	(7)	26	19	2335	[12]	7, 11, 12, 30, 35
					2345	[12]	
Clonazepam	2885	(3)	25	35	2965	[32]	7, 14, 35
Clonazepam metabolite [7-(2-chlorophenyl)-1,3-dihydro-7-amino-2H-1,4-benzodiazepin-2-one]	2850	(1)					35
Clonazepam metabolite [7-acetamino-5-(*o*-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one]	3105	(2)	20	20			35
Clonidine	2248	(1)					30
Clopenthixol	2274	(1)					35
Cloponone	2011	(2)	4	4			11
Clorazepate	2457	(3)	8	18	2655	[32]	12, 15
					2760	[14]	
Clorgyline	1883	(2)	8	7			11
Clorprenaline	1604	(2)	34	33			7, 30
Clotrimazole	2100	(1)					7
Clozapine					2915	[35]	
					3011	[9]	
Cocaethylene	2330	(1)					15
Cocaine	2187	(24)	27	50	2240	[14]	4–10
					2245	[28]	11–13, 15–17, 25, 31, 35
Codeine	2376	(24)	41	37	2290	[12]	4–7, 9–11
					2295	[12]	12, 14–17, 20–22, 25, 28, 31, 35

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Colchicine					2380	[7]	
					2490	[11]	
					3340	[34]	
					3466	[10]	
Coniine	9999	(1)					7
Cotarnine	1808	(4)	28	42			5, 7, 11, 15
Cotinine	1678	(2)	8	7			4, 35
Cropropamide	1738	(2)	2	3			9, 30
Crotamiton	1550	(1)					7
Crotethamide	1688	(2)	4	4			9, 30
Cyclandelate	1903	(4)	13	7			7, 10, 15
Cyclazocine					2065	[11]	
					2070	[11]	
					2195	[21]	
					2342	[30]	
Cyclizine	2020	(19)	20	30			4, 5, 8–15, 17
Cyclobarbitone	1963	(19)	23	25			7, 9–12, 14–16, 18, 19, 24, 26, 35
Cyclobarbitone metabolite (hydroxycyclobarbitone)	2170	(1)					35
Cyclobarbitone metabolite (3′-ketocyclobarbitone)	2190	(1)					35
Cyclododecanone	1524	(1)					2
Cyclohexylamine	917	(2)	15	15			10, 22
Cyclohexylisooctyl phthalate	2446	(1)					2
	2532	(1)					2
Cyclohexyltridecyl phthalate	2518	(1)					2
Cyclomethycaine					2225	[7]	
					2227	[10]	
					2515	[28]	
					2972	[30]	
Cyclopentamine	1087	(9)	14	22	1510	[12]	1, 3, 7, 8, 10, 11, 22
					1515	[12]	23
Cyclopentobarbitone	1862	(3)	4	3			10, 18, 19
Cyclopentolate	2020	(2)	10	10			7, 11
2-Cyclopropylmethylamino-5-chlorobenzophenone	2385	(1)					35
Cycrimine	2114	(6)	24	26			7, 12, 15
Cyheptamide	2265	(1)					15
Cypenamine	1345	(1)					3
Cyproheptadine	2366	(5)	16	34			7, 10, 11, 14, 15
2,4-D Butyl ester (2,4-dichlorophenoxyacetic acid)	1840	(1)					2
2,4-D Isobutyl ester	1805	(1)					2
2,4-D Isopropyl ester	1700	(1)					2
2,4-D Methyl ester	1605	(1)					2
Dapsone	2880	(2)	20	21			5, 10
DCPA (dimethyl-2,3,5,6-tetra-chloroterephthalate)	1960	(1)					2
DDA methyl ester (2,2-bis-(4-chlorophenyl)acetic acid)	2085	(1)					2

(Continued on p. 206)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
o,p'-DDE	2070	(1)					2
p,p'-DDE (1,1-dichloro-2,2-bis-(4-chlorophenyl)ethylene)	2130	(1)					2
Deanol	2539	(1)					10
Deanol acetamidobenzoate	2227	(1)					10
Debrisoquine	9999	(1)					7
Decoquinate	9999	(1)					11
Demeton S-methyl	1628	(1)					2
Demoxepam	2529	(4)	49	46			11, 15, 28, 32
Deptropine	2615	(1)					11
N1-Desalkylflurazepam	2471	(5)	41	39			7, 28, 32, 35
N1-Desalkyl-3-hydroxyflurazepam	2240	(1)					35
Desipramine	2242	(15)	46	39	2170	[12]	1, 4, 6, 7, 10–12, 14, 15, 27, 28, 30, 35
Desmethylchlordiazepoxide					2885	[32]	
					2930	[32]	
					3100	[7]	
Desmethylchlorpromazine	2480	(1)					4
Desmethyldiazepam	2496	(7)	16	14			6, 7, 11, 14, 17, 28, 35
Desmethylmedazepam					2275	[35]	
					2410	[28]	
Desmetryne	1795	(2)	5	5			11
Desomorphine	2295	(1)					11
Dexamethasone	2970	(1)					10
Dexbrompheniramine	2075	(1)					10
Dexoxadrol	2340	(1)					4
Dexpanthelol	1807	(2)	2	3			11
Dextromethorphan	2140	(6)	15	32			6, 7, 10, 11, 15, 28
Dextromoramide	2940	(2)	0	0			11, 14
Dextropropoxyphene	2188	(21)	33	32	1687	[11]	5–7, 10–12
					1940	[11]	13, 14, 16, 17, 20
					1964	[9]	25, 28, 35
					2074	[12]	
Dextropropoxyphene metabolite	2375	(1)					10
Dextropropoxyphene metabolite	2551	(1)					10
Dextrorphan	2397	(1)					30
Diallyl phthalate	1712	(2)	14	13			2, 8
2,5-Diaminobenzophenone	2198	(2)	28	27	2300	[36]	36
2,5-Diamino-2'-chlorobenzophenone	2345	(2)	10	10			36
2,5-Diamino-2'-fluorobenzophenone	2188	(2)	38	37			36
Diamorphine	2614	(17)	49	33	2505	[12]	4–7, 10, 11, 13
					2510	[12]	14–16, 20, 35
					2510	[25]	
					2692	[9]	
Diampromide	2434	(1)					30
	1734	(1)					30
	1859	(1)					30
	2079	(1)					30
	2232	(1)					30
Diamyl phthalate	2127	(2)	13	13			2, 8

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Diazepam	2425	(14)	20	35	2335	[12]	6, 7, 10–12, 14
					2345	[12]	15–17, 25, 28
					2490	[32]	34, 35
					2505	[9]	
					2510	[32]	
Diazinon	1758	(3)	13	11	1485	[12]	2, 7, 10
					1495	[12]	
Diazoxide	9999	(1)					11
Dibenzepin	2443	(7)	38	27	2505	[9]	10–12, 14, 27, 35
Dibenzyl phthalate	2690	(1)					2
Dibenzyl sebacate	2135	(1)					2
m-Dibromobenzene	1197	(1)					2
o-Dibromobenzene	1221	(1)					2
p-Dibromobenzene	1193	(1)					2
p-Dibutoxyethoxyethyl adipate	1285	(1)					2
Di(butoxyethyl) phthalate	2850	(1)					2
Di(butoxyethyl) sebacate	2700	(1)					2
Dibutyl adipate	1695	(2)	35	35			2, 8
N,N-Di-*n*-butylamphetamine	1689	(1)					29
Dibutyl maleate	1505	(1)					2
Dibutyl phthalate	1913	(3)	25	15			2, 8, 10
Dibutyl sebacate	2137	(2)	0	1			2, 8
Dibutyl terephthalate	2066	(1)					2
Dichlone	1760	(1)					2
Dichloroaniline	1323	(1)					10
p-Dichlorobenzene	1023	(1)					10
Dichlorophen	2140	(1)					7
Dichlorophenazone	1855	(1)					10
Diclofenac	2271	(1)					30
Dicophane (*o,p'*-isomer)	2218	(2)	2	2			2, 10
Dicophane (*p,p'*-isomer)	2299	(5)	9	10			2, 5, 7, 10, 11
Dicyclohexyl adipate	2282	(1)					2
Dicyclohexyl oxalate	1880	(1)					2
Dicyclohexyl phthalate	2461	(1)					2
Dicyclomine	1708	(2)	8	7			11, 15
	2097	(8)	48	18			1, 7, 10, 12, 14
Didesmethylchlorpromazine	2480	(1)					4
Dieldrin	2110	(7)	10	35	2170	[13]	2, 5, 12, 13
					2175	[11]	
					2215	[13]	
Diethadione	1490	(2)	5	5			12
Diethazine	2377	(6)	8	18			7–9, 11, 15, 27
Di(ethoxyethyl) adipate	1924	(2)	44	44			2, 8
Di(ethoxyethyl) phthalate	2103	(2)	33	32			2, 8
Di(ethoxyethyl) sebacate	2270	(1)					2
Diethyl adipate	1344	(2)	6	5			2, 8
Diethylaminoethyl diphenyl-propionate	2292	(1)					30
N,N-Diethylamphetamine	1371	(1)					29
Di-(2-ethylhexyl) adipate	2381	(1)					2
Di-(2-ethylhexyl) isophthalate	2730	(1)					2

(Continued on p. 208)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*Ref.*	*Refs.*
Di-(2-ethylhexyl) phthalate	2507	(1)					2
Di-(2-ethylhexyl) sebacate	2792	(1)					2
Diethyl maleate	1081	(1)					2
Diethyl-*p*-nitrophenyl phosphate	1880	(1)					35
Diethyl phthalate	1564	(2)	4	4			2, 8
Diethylpropion	1486	(11)	43	41	1402	[25]	3, 5, 7, 9–11, 13
					1550	[12]	14, 22, 29
					1555	[12]	
					1555	[12]	
Diethyl sebacate	1745	(2)	0	1			2, 8
Diethylstilboestrol	2366	(1)					10
Diethylthiambutene	2008	(2)	8	9			7, 11
N,N-Diethyl-*m*-toluamide	1583	(2)	12	12	1490	[11]	2, 11
Diethyltryptamine	1910	(4)	10	15	1800	[28]	7, 10, 15
					1993	[11]	
Digitalin	1902	(1)					10
Digitoxin	1902	(1)					10
Diheptyl phthalate	2500	(1)					2
Dihexyverine	2315	(1)					7
9,10-Dihydroanthracene	1662	(1)					2
Dihydrocodeine	2363	(7)	23	17	2290	[12]	7, 10–12, 14, 15
					2295	[12]	
					2417	[9]	
					2660	[25]	
Dihydrodesoxymorphine	2232	(1)					10
Dihydroergotamine	2315	(3)	5	5	2385	[13]	5, 7, 13
Dihydromorphine	2451	(4)	11	19			7, 11, 15, 20
Diisobutyl adipate	1660	(1)					2
Diisobutyl phthalate	1853	(2)	11	10	1930	[35]	2, 8
Diisobutyl terephthalate	1972	(1)					2
Diisodecyl adipate	2745	(1)					2
Diisodecyl phthalate	2511	(1)					2
Diisooctyl adipate	2444	(1)					8
Diisooctyl phthalate	2525	(1)					8
Diisopropyl phthalate	1633	(1)					8
Diloxanide	2420	(2)	15	15			11
Dimefline	2555	(2)	5	5			12
Dimenhydrinate	1844	(1)					10
Dimethindene	2258	(4)	38	22	2342	[10]	5, 7, 11, 15
Dimethisoquin	2030	(1)					7
Dimethoate	1725	(3)	5	10			2, 12
Dimethocaine	2108	(2)	13	12			11
Dimethothiazine	3078	(2)	18	17			7, 27
Dimethoxanate	2029	(2)	0	1	2160	[10]	1, 8
Dimethoxyethyl phthalate	1980	(1)					2
3,4-Dimethoxyphenethylamine	1551	(2)	11	11			5, 10
Dimethrin	1210	(1)					5
Dimethyl adipate	1213	(2)	10	10			2, 8
p-Dimethylaminobenzaldehyde	1528	(1)					2
N,N-Dimethylamphetamine	1236	(2)	6	7			3, 29
N,N-Dimethylaniline	1080	(3)	5	4	1010	[9]	5, 10, 22
Dimethyl isophthalate	1488	(1)					2

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Dimethylmescaline	1700	(1)					15
α,α-Dimethyl-β-methylsuccinamide	1195	(1)					2
N,N-Dimethylphenethylamine	1159	(3)	1	1			5, 10, 22
Dimethyl phthalate	1406	(3)	26	28			2, 7, 8
Dimethyl POPOP (POPOP = 1,4-bis-(5-phenyloxazolyl-2)benzene)	3618	(1)					2
2,4,-Dimethylquinoline	1446	(1)					2
2,7-Dimethylquinoline	1425	(1)					2
Dimethyl sebacate	1634	(2)	12	13			2, 8
Dimethyl terephthalate	1475	(1)					2
Dimethylthiambutene	1885	(1)					2
Dimethyltryptamine					1745	[28]	
					1885	[11]	
Dimetridazole	1353	(1)					11
Dimophebumine	2300	(1)					11
4,6-Dinitro-*o*-cresol	1617	(1)					10
2,4-Dinitrophenol	1471	(2)	36	35			10
Dinonyl adipate	2484	(1)					2
Dinonyl phthalate	2649	(1)					2
Dioctyl adipate	2383	(1)					2
Dioctyl phthalate	2515	(2)	5	4			2, 35
Dioctyl sebacate	2782	(1)					2
Di-(*n*-decyl) adipate	2905	(1)					2
Dioxadrol	2323	(2)	3	2			15
Dioxaphetyl butyrate	2491	(1)					30
Dioxyamidopyrine	2018	(2)	3	2			11
Dioxyline	2895	(1)					10
Diperodon	2370	(1)					7
Diphenadione	2934	(1)					10
Diphenamid	1970	(2)	5	5			12
Diphenazoline	2275	(2)	5	5			11
Diphenhydramine	1873	(23)	23	47			4, 5, 7–17, 25, 28, 35
Diphenidol	2384	(3)	6	6			7, 10, 15
Diphenoxalate	2443	(1)					30
Diphenyl adipate	2397	(1)					2
Diphenylamine	1601	(2)	18	18	1520	[25]	9, 29
Diphenyl mercury	2397	(1)					2
Diphenyl phthalate	2550	(1)					2
Diphenylpyraline	2099	(8)	19	22			1, 7, 8, 10, 11, 15, 23
Dipipanone	2474	(4)	7	6			4, 11, 17, 34
Diprophylline					1285	[25]	
					2470	[7]	
Dipropyl adipate	1545	(1)					2
N,N-Di-*n*-propylamphetamine	1526	(1)					29
Dipropyl phthalate	1746	(2)	3	2			2, 8
Dipyramidole	1640	(1)					10
Dipyrone	1983	(6)	14	12	1916	[10]	9, 10, 12
Disextyl maleate	2116	(2)	0	0			2, 12
Disulfiram	2141	(2)	14	14			10, 35
Dofamium chloride	1676	(2)	9	9			11
	1898	(2)	8	7			11
	1974	(2)	9	8			11

(Continued on p. 210)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
Domiphen	2310	(1)					15
Dopamine	2175	(1)					11
Dothiepin	2380	(1)					11
Doxapram	2906	(3)	29	44	3014	[30]	7, 10, 11
Doxepin	2217	(7)	12	13	2340	[14]	7, 10, 11, 15, 16, 28, 35
Doxylamine	1906	(7)	21	14	1968	[10]	11, 12, 15
Droperidol					3430	[10]	
					9999	[7]	
Dropropizine	2112	(2)	7	8			11
Dyclonine	1678	(2)	38	37			11, 15
Dyrene	2010	(1)					2
Ecgonine	9999	(1)					7
Ectylurea	1396	(6)	46	44			5, 10, 12, 13
Embramine	2185	(1)					11
Emepronium bromide	1973	(2)	8	7			11
Emetine	2505	(1)					11
Emylcamate	1105	(1)					10
Enallylpropymal	1561	(2)	2	1			10, 18
Endosulphan I	2085	(1)					2
Endosulphan II	2175	(1)					2
Endrin	2183	(2)	3	2			2, 7
Ephedrine	1363	(17)	30	42	1440	[12]	3, 5–10
					1445	[12]	11, 13, 14, 16, 22, 25, 31, 35
Ergocristine	2495	(1)					10
Ergocryptine	2184	(1)					10
Ergosterol	1714	(1)					10
Ergotamine	2366	(1)					10
Etafedrine	1519	(7)	44	21	1460	[11]	3, 11, 12, 30
Etenzamide	1542	(1)					11
Ethacrynic acid	9999	(1)					10
Ethamivan					1740	[25]	
					1894	[9]	
					1930	[14]	
Ethanolamine	780	(1)					2
Ethchlorvynol	1023	(3)	13	7			5, 7, 10
Ethinamate	1363	(6)	5	7			5–7, 10, 16, 35
Ethinyloestradiol	2719	(1)					10
Ethion	2220	(1)					2
Ethionamide	1756	(5)	36	9			7, 12
Ethoheptazine	1857	(10)	13	30			4, 7, 8, 10, 11, 14, 15, 34
Ethomoxane	1975	(3)	15	18			11, 15
Ethopropazine	2357	(7)	17	33	2270	[14]	1, 7, 10, 11, 15, 23, 27
Ethosuximide	1206	(4)	16	14			6, 7, 10, 35
Ethosuximide metabolite (3-hydroxyethosuximide)	1320	(1)					35
Ethosuximide metabolite (1-hydroxyethyl-ethosuximide)	1370	(1)					35
Ethosuximide metabolite (3-ketoethosuximide)	1385	(1)					35
Ethotoin	1800	(4)	15	10	1875	[7]	7, 10, 12, 15
					1915	[9]	

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Ethoxazene	9999	(1)					11
Ethoxyquin	2800	(1)					5
Ethoxzolamide	2578	(1)					10
Ethylallylbarbitone	1555	(1)					26
3-Ethylamino-3-phenylnorcamphane	1570	(1)					25
N-Ethylamphetamine	1228	(7)	18	22			3, 5, 9, 10, 22, 25, 29
Ethylan	2175	(1)					2
Ethyl benzoate	1227	(1)					2
N-Ethylbenzylamine	1031	(2)	8	7	1120	[5]	10, 22
N-Ethyl-*p*-chloroamphetamine	1434	(1)					29
Ethylene glycol	771	(1)					10
Ethylisobutrazine	2455	(1)					4
Ethylmethylthiambutene	1943	(1)					11
Ethylmorphine	2411	(19)	36	39	2335	[12]	1, 5, 7, 9–12
					2335	[12]	13–15, 20, 25
					2505	[11]	28, 31
Ethyl oleate	2175	(1)					2
Ethyl parathion	1953	(3)	8	7			12, 35
5-Ethyl-5-phenylhydantoin					1795	[12]	
					1810	[12]	
					1913	[26]	
5-Ethyl-5-*p*-tolylbarbituric acid	2085	(1)					2
Ethynodiol diacetate	2445	(1)					10
	2779	(1)					10
Etilefrine					1685	[35]	
					9999	[11]	
Etisazole	1668	(2)	33	32			11
Etodroxizine	3175	(1)					35
Etomidate	2008	(1)					30
Etoxeridine	2325	(1)					11
Etryptamine	1848	(2)	8	7			4, 15
Eucatropine	2026	(3)	16	11			7, 11
Eugenol	1368	(1)					2
Famprofazone	3059	(1)					30
Fencamfamin	1677	(5)	22	20			9, 11, 29, 31
Fenethylline	2830	(5)	35	46			9, 15, 16, 25, 35
Fenfluramine	1222	(7)	10	14			3, 6, 7, 10, 11, 14, 29
Fenmetramide					1765	[4]	
					1915	[28]	
Fentanyl	2650	(3)	25	20	2720	[15]	11, 12
					2779	[9]	
Flavoxate	9999	(1)					11
Flufenamic acid	1950	(3)	20	40			11, 14
Flunitrazepam	2645	(3)	35	35	2535	[28]	32, 35
Flunitrazepam decomposition product	2410	(1)					35
Fluorene	1580	(1)					2
Fluorenone	1705	(1)					2
2′-Fluoro-2-methylamino-5-nitrobenzophenone	2430	(3)	45	40			36

(Continued on p. 212)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
Flupenthixol	9999	(1)					11
Fluphenazine	3065	(4)	20	20			5, 7, 16, 35
Flupromazine	2211	(15)	21	39			4, 7, 10, 12, 13, 15, 23, 27
Flurazepam	2785	(10)	39	23	2555	[28]	6, 7, 10, 11, 14, 15
					2858	[9]	17, 32, 35
Fluspirilene	1017	(1)					35
Folpet	2015	(1)					2
Fosazepam	2610	(2)	5	5			32
Furethidine	2637	(1)					30
Galantamine	2285	(3)	15	25			12
Gallamine					2625	[11]	
					9999	[10]	
Gelsemine	2850	(1)					11
Gentisic acid	2352	(1)					10
Geraniol	1192	(1)					10
Gitalin	9999	(1)					10
Glutethimide	1836	(24)	36	34	2165	[23]	4–7, 9–17, 19, 24, 26, 35
Glutethimide metabolite (hydroxyglutethimide)	1875	(1)					35
Glyceryl dibenzoate	2442	(1)					2
Glycopyrrolate	2120	(1)					10
Griseofulvin	2700	(1)					7
Guaiacol	1092	(1)					10
Guaiphenesin	1650	(1)					35
Guanethidine	9999	(1)					1
Haloperidol					2810	[12]	
					2835	[12]	
					2905	[7]	
					2965	[10]	
					2980	[35]	
					3020	[14]	
					3149	[30]	
Halopyramine	2234	(1)					30
Halquinol	1743	(2)	8	7			11
Harman	1952	(7)	32	48			2, 7, 12, 14
Harmine	2291	(3)	11	22			1, 10, 11
Heptabarbitone	2058	(11)	33	48			6, 7, 9, 10, 12, 15, 16, 18, 26, 35
Heptabarbitone metabolite [5-ethyl-5-(3-oxo-1-cyclohepten-1-yl)-barbituric acid]	2320	(1)					35
Heptabarbitone metabolite [5-ethyl-5-(3-hydroxy-1-cyclohepten-1-yl)-barbituric acid]	2275	(1)					35
Heptachlor	1880	(2)	10	10			2, 7
Heptachlor epoxide	2015	(1)					2
Heptaminol	1118	(2)	22	22	1030	[25]	9, 11
Hexachlorophane	2807	(3)	12	18			7, 10, 11

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*Ref.*	*Refs.*
Hexamine	1210	(2)	6	5			10, 11
Hexethal	1858	(3)	23	20			10, 18, 19
Hexobarbitone	1857	(19)	37	33			6, 7, 9–12, 14–16, 18, 19, 24, 26, 35
Hexobarbitone metabolite [1,5-dimethyl-5-(3-hydroxy-1-cyclohexen-1-yl)barbituric acid]	2080	(1)					35
Hexobarbitone metabolite [1,5-dimethyl-5-(3-oxo-1-cyclohexen-1-yl)-barbituric acid]	2050	(1)					35
Hexobendine	9999	(1)					11
Hexoestrol	2402	(1)					10
Hexylcaine	1965	(3)	10	8			11, 15
Hexylresorcinol	1777	(1)					10
Hippuric acid					1390	[35]	
					1735	[10]	
Histamine	1497	(2)	4	3			7, 10
Homarylamine	1519	(1)					9
Homatropine	2072	(13)	32	48			5, 7, 9–13
Hordenine					1485	[12]	
					1490	[12]	
					1490	[12]	
					1495	[12]	
					2815	[7]	
Howflex GBP	1947	(1)					2
Hydrallazine	1528	(2)	3	2			7, 10
Hydrastine	2988	(3)	13	27	2500	[11]	5, 13
Hydrastinine	1590	(1)					5
Hydrochlorothiazide	9999	(2)					7, 10
Hydrocodone	2440	(9)	29	25	2325	[12]	7, 9–11, 15, 20
					2340	[12]	25, 35
					2340	[12]	
					2365	[4]	
					2380	[12]	
					2380	[12]	
					2390	[12]	
					2500	[11]	
Hydromorphone	2467	(7)	12	13	2580	[14]	7, 10, 11, 15, 20, 21
					2585	[11]	25
Hydroquinidine	2810	(2)	10	10			7, 11
Hydroquinine	1450	(1)					7
Hydroquinone	1220	(1)					7
Hydroxyamphetamine					1320	[7]	
					2763	[10]	
Hydroxyamylobarbitone	1632	(1)					10
4-Hydroxyantipyrene	1874	(1)					10
Hydroxybenzoic acid	1565	(1)					35
3-Hydroxybromazepam	2555	(2)	15	15			32
1-Hydroxychlordene	1955	(1)					2

(Continued on p. 214)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Hydroxychloroquine	2872	(2)	12	11			5, 10
Hydroxyephedrine	1682	(1)					9
N1-Hydroxyethylflurazepam	2688	(4)	38	42			7, 32, 35
β-Hydroxyethyltheophylline	2090	(1)					25
1-Hydroxyharman	1920	(1)					2
	2015	(1)					2
	2290	(1)					2
2-Hydroxyiminodibenzyl	2265	(1)					35
3-Hydroxymorphinan	2237	(2)	7	6			20, 21
Hydroxypentobarbitone	1915	(1)					35
Hydroxypethidine	2045	(1)					11
Hydroxyphenamate	1724	(4)	34	21			5, 10, 11
4-Hydroxyphenobarbitone	2415	(1)					35
p-Hydroxyphenylpyruvic acid					1347	[10]	
					1365	[10]	
					1835	[35]	
3-Hydroxyprazepam	2860	(1)					32
β-Hydroxypropyltheophylline	2090	(1)					25
Hydroxyzine	2849	(5)	14	21	2470	[25]	5, 9–11, 13
					2750	[12]	
					2760	[12]	
					2760	[13]	
					2980	[7]	
Hymecromone	2003	(2)	28	28			10
Hyoscine	2303	(14)	38	48	2240	[12]	5, 7, 9–12
					2245	[12]	13, 14, 25
					2365	[11]	
Hyoscyamine	2192	(15)	32	33			5, 7, 10–13, 15, 28
Ibogaine	2872	(2)	29	28	9999	[11]	7, 22
Ibomal	1883	(6)	17	8			9, 10, 16, 18, 19, 35
Ibomal metabolite [5-(1-methylethyl)-5-(2-oxopropyl)barbituric acid]	1770	(1)					35
Imidazole	1095	(1)					2
Imidocarb	9999	(1)					11
Iminodibenzyl	1920	(1)					35
Imipramine	2223	(23)	37	47	2165	[12]	4–7, 9–11
					2285	[13]	12–16
					2295	[13]	17, 23, 25, 27, 28, 30, 35
Indene	1062	(1)					2
Indole	1276	(1)					2
Indomethacin	2685	(2)	5	5			10, 14
Iprindole	2335	(2)	5	5			11, 15
Iproniazid	1593	(11)	23	37	1700	[5]	1, 7, 8, 10, 12, 14
					2060	[26]	
					2065	[23]	
Isatin	1712	(1)					2
Isoaminile	1830	(1)					11
Isobutyl aminobenzoate	1795	(1)					30
Isobutylcyclohexyl phthalate	1868	(1)					2
	2159	(1)					2
	2453	(1)					2

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
3-Isobutyl-1-methylxanthine	2150	(1)					2
Isocarboxazid	1949	(11)	19	25			5, 8, 10, 12–14
Isometamidium	9999	(1)					11
Isomethadone	2128	(2)	3	2			4, 15
Isometheptene	1052	(5)	22	26			3, 10, 11, 22, 29
Isoniazid	1670	(4)	40	15	1515	[12]	5, 16, 30, 35
					1525	[12]	
					1525	[12]	
					1582	[10]	
Isooctylhydrocupreine	3103	(1)					10
Isoprenaline	1730	(1)					10
Isopropamide					2025	[12]	
					2058	[10]	
					2383	[10]	
					2430	[35]	
N-Isopropylamphetamine	1257	(1)					29
Isopropylhexedrine	1140	(1)					3
N-Isopropyl-α-methyl-β-phenethylamine	1247	(2)	6	6			10, 22
Isopyrin	2024	(4)	14	16			12
Isoquinoline	1426	(1)					10
Isothipendyl	2267	(7)	7	23			1, 4, 10, 11, 15, 27
Isoxsuprine	2300	(3)	10	15	2398	[10]	7, 12
Ketamine	1843	(5)	20	17			7, 10, 11, 15, 28
Ketobemidone	2035	(3)	25	20			7, 11, 20
Ketobisnortilidine	2030	(1)					35
Kojic acid	1443	(1)					10
Lachesine	1852	(1)					11
Laudanine	2695	(1)					15
Laudanosine	2660	(1)					15
Lauric acid	1600	(1)					10
Lecithin	1945	(1)					10
	2147	(1)					10
Leptazol	1552	(9)	29	45	1475	[25]	1, 9–12, 14
					1490	[3]	15, 25, 29
					1705	[12]	
					1705	[12]	
Leucinocaine	2240	(1)					30
	2315	(1)					30
Levallorphan	2359	(7)	19	16			7, 9–11, 20, 34
Levamisole	1928	(2)	3	2			11
Levomethorphan					2130	[20]	
					2155	[30]	
					2230	[7]	
					2255	[11]	
Levomoramide	2980	(1)					30
Levophenacylmorphan	3202	(1)					30
	2406	(1)					30
Levopropoxyphene	2185	(1)					7

(Continued on p. 216)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*Ref.*	*Refs.*
Levorphanol	2234	(6)	9	12			7, 9–11, 20, 31
Lidoflazine	9999	(1)					11
Lignocaine	1870	(24)	40	25			4–16, 25, 28, 35
Limonene	1053	(1)					2
Linalol	1100	(1)					10
Lindane	1745	(4)	15	12			2, 10, 12
cis-Linoleic acid	1309	(1)					10
	2179	(1)					10
Linolenic acid	2178	(1)					10
Lobeline					1780	[5]	
					2085	[12]	
Lophophorine	1850	(1)					15
Lorazepam	2402	(8)	37	38	2325	[12]	7, 11, 12, 14, 25
					2330	[12]	32, 35
					2478	[9]	
					2515	[32]	
Loxapine	2530	(2)	50	50			15, 28
Lysergide	3445	(1)					5
Mafenide	9999	(1)					11
Malathion	1917	(4)	17	18			2, 10, 12
Mandelic acid	1487	(1)					10
Maprotiline	2356	(2)	31	30			7, 9
Mazindol	2355	(1)					15
Mebenazine	1240	(1)					11
Mebeverine	9999	(1)					11
Mebhydrolin	2465	(1)					11
Meboral	1894	(1)					10
Mebutamate	1889	(7)	24	36	1965	[13]	5, 10, 12, 13
Meclofenamic acid	2420	(1)					14
Meclofenoxate	1770	(2)	20	20			11, 14
Mecloqualone	2255	(1)					15
Meclozine	3033	(7)	28	17	2515	[12]	5, 7, 10, 13, 14, 23
Meconic acid	1466	(1)					10
Medazepam	2226	(11)	46	49	2285	[32]	9–12, 15
					2350	[32]	25, 28, 36
Mefenamic acid	2201	(2)	29	29	2480	[14]	10, 11
Melitracene	2268	(2)	48	48			9, 25
Mepenzolate	2327	(3)	17	29			10, 12
Mephenesin	1568	(7)	28	37			6, 7, 10, 12, 15
Mephenoxalone	2155	(3)	35	29			5, 10, 15
Mephentermine	1239	(10)	29	21	1295	[12]	3, 5, 7, 10, 11, 13
					1305	[12]	14, 22, 29
					1370	[12]	
					1375	[12]	
Mepivacaine	2071	(15)	41	29			1, 7, 8, 10–12, 14, 15, 28, 35
Meprobamate	1796	(17)	16	34	1850	[11]	4–7, 9, 10, 12
					2250	[14]	13, 14, 16, 26, 35
Mepyramine	2220	(12)	35	30			4, 6, 7, 10–12, 14, 15, 34
2-Mercaptobenzothiazole	1936	(1)					2
Mercumallylic acid	2074	(2)	25	24			10

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
Mescaline	1688	(9)	28	12	1815	[11]	5–7, 10, 11, 14–16, 35
Mesoridazine	9999	(1)					11
Mestranol	2612	(1)					10
Metabutethamine	1988	(1)					15
Metabutoxycaine	2226	(2)	1	1			7, 10
Metamfepyramon	1372	(1)					9
Metaraminol	9999	(1)					7
Metaxalone	2163	(3)	18	21			7, 10, 15
Metazocine	1920	(1)					20
Metformin	9999	(1)					7
Methadone	2148	(29)	23	33			4–17, 20, 25, 28, 35
Methadone metabolite	2021	(2)	10	9			4, 10
Methallatal	1736	(1)					10
Methallibure	9999	(1)					11
Methandienone	2672	(1)					10
Methaphenilene	1966	(3)	23	22			10, 11
Methapyrilene	1981	(22)	19	29			4–16, 28, 35
Methaqualone	2125	(22)	35	25	2190	[17]	4, 6–11
					2190	[35]	12, 14–16, 24
					2235	[28]	26, 34, 35
Methaqualone metabolite [3-(*o*-tolyl)-4(3)-quinazolin]	2170	(1)					35
Methaqualone metabolite [4-oxo-3-(*o*-tolyl)-3,4-dihydro-2-quinazolincarbaldehyde]	2240	(1)					35
Methaqualone metabolite [2-hydroxymethyl-3-(*o*-tolyl)-4(3)quinazoline]	2360	(1)					35
Methaqualone metabolite [4-oxo-3-(*o*-tolyl)-3,4-dihydro-2-carboxyquinazoline]	2400	(1)					35
Methaqualone metabolite [3-(2′-hydroxymethylphenyl)-2-methyl-4(3H)-quinazoline]	2410	(1)					35
Methaqualone metabolite [3-(3′-hydroxy-2′-methylphenyl)-2-methyl-4(3H)-quinazoline]	2490	(1)					35
Methaqualone metabolite [3-(4′-hydroxy-2′-methylphenyl)-2-methyl-4(3H)quinazoline]	2520	(1)					35
Methaqualone metabolite [3-(4′-hydroxy-5′-methoxy-2′-methylphenyl)-4(3H)-quinazoline]	2560	(1)					35
Methaqualone metabolite [6-hydroxy-3-(3′-methylphenyl)-4(3H)quinazoline]	2525	(1)					35
Metharbitone	1472	(6)	32	43			10–12, 18, 19
Methazolamide	2187	(3)	27	37			11, 15

(Continued on p. 218)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Methdilazine	2467	(3)	12	10			4, 10, 11
Methimazole	1550	(1)					11
Methiomeprazine	2685	(1)					27
Methixene	2461	(7)	31	39			10–12, 15, 27
Methocarbamol					1507	[10]	
					2485	[23]	
Methohexitone	1766	(4)	11	5			6, 10, 15, 18
Methoin	1791	(14)	41	17			5–10, 12, 13, 15, 26
Methoprotryne	2098	(2)	23	22			11
Methotrimeprazine	2514	(10)	29	36	2440	[12]	1, 4, 7, 10–12
					2445	[12]	15, 27, 35
					2588	[9]	
Methoxamine	1726	(2)	1	1			10, 11
Methoxsalen	1980	(1)					7
Methoxyamphetamine	1385	(1)					7
p-Methoxybenzophenone	1874	(1)					10
Methoxychlor	2417	(9)	12	23	2345	[12]	2, 5, 7, 10, 13
					2355	[12]	
3-Methoxymorphinan	2146	(2)	18	17			20, 21
Methoxyphenamine	1361	(5)	20	25	1455	[12]	7, 10, 11, 22, 29
					1470	[12]	
					1470	[12]	
Methoxypromazine	2542	(4)	42	38			5, 10, 15, 23
Methstyridone	1885	(1)					15
Methsuximide	1622	(9)	32	38			5, 7, 12, 13, 15, 33
Methyclothiazide	9999	(1)					7
2-Methylamino-5-chlorobenzophenone	2107	(6)	37	48			7, 35, 36
Methylaminomethylheptane	1000	(1)					3
Methylamphetamine	1176	(17)	41	31	1335	[12]	3, 5–11
					1340	[12]	13, 14, 16, 22
					1340	[12]	25, 29, 35
					1345	[12]	
Methylanhalonidine	1800	(1)					15
Methyl anthranilate	1343	(1)					2
Methylbenzylamine	1078	(2)	43	42			33, 35
N-Methyl-N-*n*-butylamphetamine	1486	(1)					29
Methyl caprylate	1130	(1)					2
Methyl decanoate	1305	(1)					2
Methyldesorphine	2305	(1)					11
Methyldihydromorphine	2380	(1)					11
N-Methyl-2,3-dihydroxymorphinan	2500	(1)					21
4-Methyl-2,5-dimethoxyamphetamine	1635	(1)					15
Methylenedioxyamphetamine	1472	(6)	15	23			5–7, 11, 22, 29
3,4-Methylenedioxy-2-methoxyamphetamine	1647	(1)					10
3,4-Methylenedioxy-4-methoxyamphetamine	1702	(1)					10
3,4-Methylenedioxy-5-methoxyamphetamine	1705	(1)					10
3,4-Methylenedioxyphentermine	1646	(1)					29

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
N-Methylephedrine	1400	(5)	31	23			3, 10, 11, 22, 29
Methylergometrine	9999	(1)					10
Methyl hippurate	1675	(1)					35
Methyl *p*-hydroxybenzoate	1419	(1)					2
Methyl linoleate	2100	(1)					2
N-Methylmescaline	1700	(1)					15
N-Methyl-3,4-methylenedioxyamphetamine	1585	(1)					15
Methyl myristate	1715	(2)	5	5			33, 35
2-Methylnaphthalene	1313	(1)					2
Methyl nicotinate	1100	(1)					7
Methylnitrazepam	2570	(1)					28
Methyl nonanoate	1215	(1)					2
Methyl oleate	2086	(1)					2
Methyl palmitate	1889	(2)	22	21			2, 35
Methyl parathion	1851	(4)	6	9			2, 12, 35
α-Methylphenethylhydrazine					1400	[10]	
					2042	[23]	
Methyl phenidate	1737	(18)	46	43	1810	[14]	3, 5–7, 9–13, 15, 25, 31
Methylphenobarbitone	1891	(22)	39	29	1770	[14]	5, 6, 9, 11–13
					1988	[26]	15, 16, 18, 19, 24, 35
5-Methyl-5-phenylhydantoin	1866	(1)					2
5-(*p*-Methylphenyl)-5-phenylhydantoin	2584	(2)	37	36			2, 10
N-Methyl-N-*n*-propylamphetamine	1396	(1)					29
Methyl salicylate	1193	(3)	13	12			6, 7, 10
Methyl stearate	2115	(2)	0	1			2, 35
Methyltestosterone	2643	(2)	8	7			7, 11
5-Methyltryptamine	1795	(1)					11
α-Methyltryptamine	1740	(1)					11
N-Methyltryptamine	1770	(1)					11
Methyprylon	1529	(10)	29	29			5–7, 9–11, 15, 26, 35
Methysergide	3089	(1)					10
Metoclopramide	2630	(1)					7
Metofoline	2635	(1)					16
Metopon	2459	(3)	7	6	2380	[11]	10, 15, 20
Metronidazole	1592	(3)	32	33			7, 10, 11
Metyrapone	1860	(1)					7
Mevinphos	1450	(1)					2
Miconazole	2980	(1)					7
Mirex	2470	(1)					2
Modaline	1420	(1)					3
Molindone	2465	(1)					28
3-Monoacetylmorphine	2490	(1)					15
6-Monoacetylmorphine	2537	(6)	22	28	2402	[10]	6, 7, 11, 15, 20, 35
Monuron	1100	(1)					7
Morantel	9999	(1)					11
Morazone	1718	(2)	13	12			11
Morinamide	1906	(6)	16	14			11, 12
Morpheridine	2535	(1)					11

(Continued on p. 220)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Morphine	2454	(20)	44	41	2335	[12]	4–7, 9–11
					2340	[12]	13, 15–17, 20
					2380	[12]	21, 22, 25, 31, 35
					2390	[12]	
					2400	[14]	
					2510	[11]	
Morpholine	810	(1)					2
Myristic acid	1754	(2)	6	6			7, 35
Naftazone	1448	(1)					11
Nalorphine	2577	(9)	37	33	2643	[10]	5, 11, 13, 14, 16
					2680	[11]	20, 35
Naloxone	2640	(3)	0	0			7, 11, 20
Naphazoline	2057	(12)	47	43	1990	[25]	1, 7–12, 14, 15
Naphthalene	1186	(1)					2
1-Naphthonitrile	1489	(1)					2
2-Naphthyl acetate	1585	(1)					2
α-Naphthylamine	1531	(1)					9
Narcobarbital	1814	(2)	1	1			9, 26
Nealbarbitone	1720	(1)					18
Neocinchophen	2495	(1)					15
Neopine	2395	(2)	0	0			15, 22
Neostigmine bromide	1770	(3)	5	5	1850	[12]	12, 15
					1860	[12]	
Nialamide					1500	[1]	
					9999	[10]	
Nicametate	1608	(2)	8	7			11
Nicergoline	9999	(1)					11
Nicocodine	3408	(1)					30
Nicodicodine	3327	(1)					30
Nicotinamide	1459	(2)	16	16			2, 10
Nicotine	1348	(20)	43	37	1415	[12]	3, 5–9, 11
					1420	[12]	12–14, 16, 17
					1430	[11]	22, 25, 35
Nicotinic acid	1335	(1)					7
Nicotinic acid amide	1390	(1)					35
Nicotinyl alcohol	1215	(3)	15	31	1100	[7]	1, 8
					1119	[10]	
Nicoumalone	1779	(4)	4	1	1875	[10]	10, 12
Nifenazone	1608	(1)					11
Nifuratel	2590	(1)					11
Nifuroxime	2380	(1)					11
Nifursol	9999	(1)					11
Nikethamide	1525	(16)	25	35	1760	[25]	3, 5, 7–14, 22, 29
Nimorazole	1803	(2)	8	7			11
Nitrazepam	2750	(8)	45	30	1467	[25]	11, 12, 14–16
					2674	[35]	17, 35
					2690	[12]	
					2830	[32]	
					2913	[10]	
p-Nitromethylamphetamine	1658	(1)					10
Nitroxynil	1754	(2)	6	6			11

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	*−*	*+*	*>50*	*[Ref.]*	*Refs.*
Nomifensine	2122	(1)					35
Noracymethadol	2231	(1)					30
	2081	(1)					30
Noramidopyrine	1710	(1)					25
Norbormide	2050	(1)					11
Norclobazam					2695	[32]	
					2815	[32]	
Norcodeine	2388	(2)	13	12			20, 21
Nordiazepam	2570	(2)	15	15			32
Norethisterone	2625	(1)					10
Norethynodrel	2551	(1)					10
Norfenfluramine	1133	(2)	3	3			3, 29
Norflunitrazepam	2740	(2)	5	5			32
Norharman					1920	[7]	
					2005	[2]	
					2100	[14]	
Norlevorphanol	2244	(2)	0	1			21, 30
Normethadone	2091	(7)	11	14			4, 7, 9, 12
Normorphine	2438	(1)					20
Norpethidine	1745	(4)	5	5			4, 5, 14, 20
Norpipanone	2488	(1)					30
Norpropoxyphene	2395	(3)	30	45			7, 14, 28
Norpropoxyphene amide					2472	[14]	
					2580	[28]	
Norpropyphenazone	1780	(1)					35
Norpseudoephedrine	1302	(3)	24	17	1362	[11]	3, 25, 29
Nortilidate	1835	(1)					35
Nortriptyline	2210	(16)	40	30			6, 7, 10–12, 14, 15, 17, 27, 28, 30, 34, 35
Noscapine	3120	(2)	20	19	3240	[14]	7, 10
Noxiptyline	2267	(3)	12	8			11, 35
Noxythiolin	2370	(2)	10	10			11
Nystatin	1945	(1)					10
Obidoxime bromide	9999	(1)					11
Octacaine	1893	(2)	3	2			11
Octamylamine	1303	(1)					29
Octaphonium chloride	2013	(2)	8	7			11
Octyldecyl adipate	2540	(1)					2
	2745	(1)					2
	2940	(1)					2
4-*tert.*-Octyl-2-methylcyclohexyl acetate	1611	(1)					2
Oestradiol	2659	(1)					10
Oestriol	2970	(1)					10
Oestrone	2612	(1)					10
Orciprenaline	9999	(1)					7
Orphenadrine	1936	(16)	16	17	1875	[10]	4, 7, 8, 10–12, 14, 15, 28, 34
Orthocaine	1655	(1)					7
Oxanamide	1249	(1)					10

(Continued on p. 222)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
Oxazepam	2336	(12)	26	44	2260	[12]	6, 7, 10–12, 14
					2270	[12]	15, 17, 25, 32, 35
					2415	[28]	
					2425	[32]	
					2430	[16]	
Oxethazaine	2525	(1)					7
Oxomemazine	2725	(2)	5	5			15, 27
Oxprenolol	1870	(3)	30	40			11, 14
Oxybuprocaine	2471	(1)					30
Oxyclozanide	9999	(1)					11
Oxycodone	2524	(3)	23	26	2425	[7]	10, 20, 35
					2450	[25]	
Oxymetazoline	2168	(5)	23	32			7, 10, 11, 15, 35
Oxymetholone	2835	(1)					7
Oxymorphone	2532	(5)	12	13			7, 10, 11, 15, 20
Oxypertine	2355	(1)					11
Oxyphenbutazone					1630	[7]	
					9999	[10]	
Oxyphencyclimine	2550	(3)	10	21			7, 10, 14
	1661	(1)					10
Padimate	1964	(2)	4	4			11
Palmitic acid	1973	(4)	14	18			7, 10, 35
Panidazole	2065	(2)	0	0			11
Papaverine	2825	(11)	40	37	2770	[12]	7, 9–12, 14
					2900	[11]	15, 22, 25, 34, 35
Paracetamol	1687	(11)	27	28	1760	[6]	5, 10, 12, 14, 17, 33
					1760	[7]	35
					1780	[13]	
Paramethadione					1120	[10]	
					9999	[7]	
Parathion	1942	(5)	7	13			2, 10, 12, 35
Parbendazole	9999	(1)					11
Pargyline	1214	(7)	14	18			3, 5, 7, 10, 11, 22, 29
Pecazine	2524	(13)	39	36	2445	[12]	5, 10–12, 13, 15
					2445	[12]	23, 27
Pellotine	1825	(1)					15
Pemoline					2080	[30]	
					2240	[31]	
Penfluridol	3380	(1)					35
Pentachlorophenol	1749	(2)	9	8			1, 10
Pentapiperide	9999	(1)					11
Pentaquin	2555	(2)	15	15			7, 11
Pentazocine	2275	(20)	35	46			4–7, 9–16, 20, 28, 35
Pentifylline	2195	(1)					11
Pentobarbitone	1740	(26)	25	40			1, 5–7, 9–19, 24, 26, 35
Pentorex	1256	(1)					29
Perazine					2760	[27]	
					2930	[35]	
Perhexiline	2153	(2)	3	2			11
Perhydrophentermine	1167	(1)					29
Pericyazine					3230	[7]	
					3340	[14]	

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Perphenazine	2207	(3)	7	13			5, 7, 13
Perthane	2175	(1)					2
Pethidine	1751	(25)	26	28			4–17, 20, 25, 35
Pethidine intermediate A	1652	(1)					30
Pethidine intermediate B	1759	(2)	9	19			20, 30
Phenacaine	2617	(2)	27	27			7, 10
Phenacemide	1473	(1)					10
Phenacetin	1675	(21)	45	30	1755	[13]	4, 5, 7, 9–17, 24, 26, 35
Phenadoxone	2522	(3)	12	28			4, 7, 11
Phenampromide	2014	(1)					30
Phenanthrene	1786	(1)					10
Phenatine	2089	(2)	6	6			11
Phenazine	1703	(1)					2
Phenazocine	2684	(5)	34	36			4, 10, 11, 14, 20
Phenazone	1848	(8)	16	39			5, 7, 9, 10, 15, 16, 25, 35
Phenazone metabolite (1-phenyl-4-hydroxy-2,3-dimethyl-3-pyrazolin-5-one)	2730	(1)					35
Phenazopyridine	2245	(5)	35	25	2345	[7]	12, 15
					2346	[10]	
Phenbutrazate	2670	(1)					11
Phencyclidine	1904	(10)	34	28	1755	[28]	5–7, 9–11, 14, 15, 35
Phendimetrazine	1444	(13)	34	48	1555	[12]	3, 5, 7, 9–12
					1560	[12]	13, 22, 25, 29, 31
Phenelzine	1335	(3)	5	5	2135	[23]	1, 10, 14
Phenetamine	2213	(2)	8	7			12
α-Phenethylamine	1031	(4)	21	19			2, 5, 10, 22
β-Phenethylamine	1111	(7)	21	9			2, 5, 7, 10, 14, 22, 35
Pheneturide	1465	(1)					11
Phenglutarimide	2321	(1)					30
Phenindamine	2167	(9)	17	38			1, 4, 8, 10, 11, 15, 28
Phenindione	2055	(1)					7
Pheniprazine	1400	(1)					10
Pheniramine	1804	(11)	49	36	1969	[10]	4, 7, 8, 11, 14, 15, 28,34, 35
Phenmetrazine	1431	(16)	38	37	1495	[12]	3, 5–7, 9–11
					1495	[12]	13, 14, 16, 22, 25
					1495	[12]	29, 31, 35
Phenobarbitone	1957	(23)	27	33	2010	[13]	5–7, 9–12
					2034	[15]	13–17
					2065	[11]	18, 24, 26, 36
Phenol	981	(1)					10
Phenoperidine	2872	(1)					30
Phenothiazine	2024	(4)	14	11			7, 10, 11, 13
Phenoxybenzamine	2233	(4)	8	12			7, 10, 11, 15
Phenoxypropazine	1468	(3)	3	5			3, 7, 11
Phenprobamate	1520	(1)					11
Phensuximide	1634	(4)	14	16			6, 7, 10, 15
Phentermine	1147	(10)	18	26	1365	[12]	3, 5, 7, 9–11, 14
					1367	[12]	22, 25, 29

(Continued on p. 224)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*Ref.*	*Refs.*
Phentolamine	9999	(1)					7
Phenylalanine	2205	(1)					7
Phenylbutazone	2365	(10)	25	32	2230	[12]	6, 7, 9–12
					2235	[12]	14, 17, 25
Phenylephrine					1660	[7]	
					2158	[34]	
					9999	[10]	
α-Phenyl-α-ethylglutaconamide	1842	(1)					10
α-Phenylglutarimide	1782	(1)					10
Phenylmethylbarbituric acid	1880	(2)	5	5			15, 18
Phenylpropanolamine	1313	(8)	27	45	1375	[8]	3, 5, 7, 10, 14, 22
					1415	[1]	
Phenylpropanone	1110	(2)	3	2			10, 22
1-Phenylpropylamine	1128	(3)	3	2			5, 10, 22
3-Phenylpropylamine	1205	(1)					35
Phenylpropylmethylamine	1176	(2)	1	1			10, 22
1-Phenyl-3-pyrazolidinone	1957	(1)					10
Phenyltoloxamine	1938	(13)	18	22	2010	[5]	7, 10–13
					2055	[13]	15, 28
Phenyramidol	2006	(6)	31	32	2485	[11]	1, 8, 10, 15
Phenytoin	2330	(7)	15	14	2270	[12]	5, 9, 15–17, 35
					2270	[12]	
					2400	[14]	
					2460	[6]	
					2486	[10]	
Pholcodine					2381	[10]	
					3005	[12]	
					3030	[12]	
					3170	[14]	
Phorate	1675	(1)					2
Phthivazid	9999	(1)					11
Physostigmine	1804	(5)	24	10			5, 7, 10, 11
	2035	(1)					11
	2190	(3)	15	15			11, 12
Phytomenadione	3287	(1)					10
Picloxydine	9999	(1)					11
Picrotoxin					2205	[5]	
					2280	[10]	
					2356	[10]	
					2464	[10]	
					2545	[10]	
Pilocarpine	2014	(6)	13	26	2220	[28]	10, 12, 15
Piminodine					2020	[11]	
					2310	[11]	
					2885	[20]	
					2949	[9]	
Pimozide	9999	(2)					7, 11
Pindolol	2260	(1)					14
Pipamazine	3260	(1)					1
Pipamperone	3070	(1)					35
Pipazethate	2037	(1)					11
Piperacetazine	9999	(2)					10, 11

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Piperazine	813	(2)	23	22			2, 22
Piperidine	790	(1)					2
Piperidolate	2318	(7)	28	17			1, 10–12
Piperilate	2464	(1)					10
Piperocaine	1980	(8)	25	29			1, 8, 10, 15
Piperonal	1305	(1)					7
Piperoxan	1840	(2)	10	10			11, 15
Pipethanate					2205	[23]	
					2475	[11]	
Pipobroman	1811	(3)	16	17			10, 11
					2030	[11]	
					2050	[11]	
					2200	[10]	
Pipradrol	2145	(16)	50	50			5, 10–15, 23, 31
Piracetam	1640	(1)					35
Pivalylbenzhydrazine	1613	(1)					10
Pizotifen	2375	(1)					11
Polythiazide	2380	(1)					7
POPOP (1,4-bis(5-phenyl-oxazolyl-2)benzene)	3525	(1)					2
PPO (2,5-diphenyloxazole)	2050	(1)					2
Practolol	9999	(1)					11
Prajmalium bitartrate					2925	[35]	
					9999	[11]	
Pramoxine	2281	(5)	31	24			1, 7, 10, 11, 15
Prazepam	2641	(8)	31	14	2710	[9]	2, 7, 11, 15, 16
					2715	[32]	17, 25, 35
Prenylamine	2557	(3)	7	13			7, 11, 15
Prilocaine	1825	(6)	25	15			4, 7, 10, 14, 15
Primaquine	2314	(4)	8	6	2105	[5]	1, 7, 10
Primidone	2247	(13)	42	28	2165	[12]	4, 5, 7, 11–13
					2170	[12]	15–17, 35
Probarbital	1567	(7)	17	28			5, 10, 11, 15, 18, 19
Probenecid	2336	(2)	16	16			1, 10
Procainamide	2248	(3)	28	25			7, 10, 11
Procaine	2018	(20)	23	32	2105	[28]	4, 5, 7–15, 25
Procarbazine	1990	(2)	10	11			10
Prochlorperazine	2954	(4)	27	16	3120	[7]	10, 14, 23, 27
Procyclidine	2156	(13)	41	19	2220	[14]	1, 7–12, 35
Profadol	1748	(2)	8	7			11
Progesterone	2793	(1)					10
Proheptazine	1590	(1)					11
	1920	(1)					11
	1937	(1)					11
Prolan	2250	(1)					2
Prolintane	1634	(11)	49	21	1520	[25]	9, 11, 12, 14, 15, 29, 31
Promazine	2316	(17)	41	44			4, 5, 7, 9–11, 13–15, 23, 27, 28, 35
Promethazine	2259	(20)	39	41	2205	[12]	4, 5, 7, 10–12
					2328	[9]	13–15, 23, 25, 27, 28
Prometryne	1853	(2)	8	7			11

(Continued on p. 226)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Promoxolane	1252	(1)					10
Propanidid	2433	(2)	8	7			12
Propantheline bromide					2180	[12]	
					2205	[12]	
					2215	[12]	
					2349	[10]	
Properidine	1750	(1)					11
Propiomazine	2738	(5)	13	17			1, 11, 14, 15, 27
Propionylpromazine	2800	(1)					27
Propoxycaine	2335	(1)					11
Propranolol	2157	(5)	7	9			7, 10, 11, 14, 15
N-Propylamphetamine	1324	(3)	6	6			5, 22, 29
Propylhexedrine	1169	(7)	9	16			3, 5, 10, 11, 14, 22, 29
Propyl *p*-hydroxybenzoate	1567	(1)					2
Propyliodone	9999	(1)					10
5-Propyl-5-isobutylbarbituric acid	1693	(1)					10
N-Propyl-α-methyl-β-phenethylamine	1318	(2)	29	29			10
Propyl myristate	1859	(1)					10
Propylparaben	1620	(2)	5	5			15, 35
Propyphenazone	1925	(1)					35
Proquamezine	2434	(3)	6	11			7, 11, 27
Prothipendyl	2339	(6)	19	34			1, 10, 11, 15, 27, 35
Protokylol	1487	(1)					10
Protoveratrine A	2465	(1)					7
Protoveratrine B	2465	(1)					7
Protriptyline	2261	(5)	31	39	2340	[15]	7, 11, 14, 28, 30
Proxymetacaine	2323	(2)	13	12			7, 15
Proxyphylline	2103	(1)					9
Pseudococaine	2180	(1)					15
Pseudoephedrine	1354	(8)	37	44	1418	[22]	3, 7, 9–11, 14, 25, 31
Pseudomorphine	2770	(1)					11
Psilocin	1980	(1)					7
Psilocybin	2059	(1)					30
Putrescine	930	(1)					2
Pyrantel	9999	(1)					10
Pyrathiazine	2536	(4)	16	18			4, 10, 15, 23
Pyrazinamide	1250	(1)					7
Pyrene	1983	(1)					2
Pyridbenzamine	1980	(1)					2
Pyridostigmine bromide	1515	(1)					12
Pyridoxamine	2000	(1)					5
Pyrimethamine	2138	(3)	4	2			5, 7, 10
Pyrithyldione	1557	(3)	27	18			12, 35
Pyrrobutamine	2419	(12)	34	28	2345	[12]	5, 10–14
					2360	[12]	15, 23
					2480	[4]	
					2515	[13]	
Pyrrocaine	1968	(3)	3	7			11, 20
Pyrrolidine	695	(1)					2
Pyrroliphene	2396	(1)					10
Pyruvic acid	1249	(1)					10

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
Quinalbarbitone	1791	(24)	29	29			5–7, 9–19, 24, 26, 35
Quinalbarbitone metabolite [5-(1-methylbutyl)barbituric acid]	1660	(1)					35
Quinalbarbitone metabolite [5-(3-hydroxy-1-methylbutyl)-5-(2-propenyl)barbituric acid]	1965	(1)					35
Quinidine	2784	(5)	24	26			5–7, 10, 13
Quinine	2803	(13)	48	47			5–7, 9–11, 13–16, 25, 35
Quinoline					1247	[2]	
					1427	[10]	
Quinone	1250	(1)					7
Racemethorphan	2124	(7)	19	24			11, 12, 20, 21
Racemoramide	2930	(1)					11
Racemorphan	2218	(2)	3	2			11, 21
Rafoxanide	9999	(1)					11
Rescinnamine	2180	(1)					7
Reserpine	9999	(2)					7, 10
Resorantel	9999	(1)					11
Resorcinol	1258	(2)	8	7			10, 35
Riconoleic acid	1991	(1)					10
Rimantadine	1388	(1)					11
Ritodrine	9999	(1)					11
Rolicypram	9999	(1)					11
Ronnel	1893	(4)	13	22			2, 12
Rotenone	3242	(1)					10
Rotoxamine	2049	(1)					10
Saccharin					1764	[10]	
					1875	[10]	
Salicylaldehyde	1072	(1)					10
Salicylamide	1455	(14)	20	45	1560	[24]	5–7, 9, 10, 14, 16, 24, 26, 33, 35
Salicylic acid	1308	(9)	28	22			5–7, 9, 10, 16, 17, 35
Salol					1650	[7]	
					1660	[6]	
					1745	[10]	
Sancticizer 141	2410	(1)					2
Sanguinarine	2880	(1)					5
Santonin	2174	(2)	4	4	1945	[5]	10, 11
Secbutobarbitone	1662	(7)	12	18	1780	[5]	7, 10, 11, 15, 16, 18, 35
Secbutobarbitone metabolite (hydroxysecbutobarbitone)	1928	(1)					35
Secergan	2310	(1)					15
Simazine	1690	(1)					2
Sitosterol	3193	(2)	39	39			10
SKF 525A	2326	(1)					2
Sparteine	1801	(12)	46	29			5, 7, 10–13, 15
Spironolactone	3280	(1)					10
Stearic acid	2174	(3)	4	4			7, 10, 35
Stigmasterol	3234	(1)					2

(Continued on p. 228)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	–	+	*>50*	*[Ref.]*	*Refs.*
trans-Stilbene	1755	(1)					2
Stilboestrol	2298	(2)	3	2			1, 11
STP	1616	(3)	8	4			5, 7, 10
Strychnine	3119	(8)	23	31	3020	[13]	6, 7, 9, 10, 13, 14
					3040	[5]	16, 35
					3060	[31]	
					3280	[25]	
Styramate	1667	(2)	2	1			10, 33
Succinylcholine chloride	9999	(1)					10
Sulphafurazole	1212	(1)					10
Sulphaguanidine	9999	(1)					10
Sulphamoxole	9999	(1)					11
Sulphanilamide	2166	(1)					10
Sulphathiazole	9999	(1)					10
Sulphonal	1478	(1)					11
Sulphosalicylic acid	1265	(1)					10
Sulpiride					2062	[35]	
					2832	[35]	
Symazine	9999	(1)					11
Synhexyl	2616	(1)					10
2,4,5-T Isopropyl ester (2,4,5-trichlorophenoxyacetic acid)	1825	(1)					2
2,4,5-T Methyl ester	1740	(1)					2
Tacrine	2165	(2)	5	5			11, 14
Talbutal	1701	(6)	28	19			10–12, 18, 19, 33
Tartaric acid	1249	(1)					10
Taurolin	9999	(1)					11
o,p'-TDE	2130	(1)					2
p,p'-TDE	2200	(1)					2
Temazepam					2470	[12]	
					2480	[12]	
					2525	[12]	
					2535	[12]	
					2595	[11]	
					2630	[32]	
					2675	[32]	
Terbutryne	1940	(2)	0	0			11
Terodiline	1908	(2)	8	7			11
p-Terphenyl	2208	(1)					2
Terpineol	1127	(1)					2
	1170	(1)					2
	1183	(1)					2
Testosterone	2620	(1)					7
Tetracaine	2215	(9)	35	25			10, 12, 13, 15
Tetrachlorvinphos *Z*-isomer	2430	(1)					2
Tetracycline	1950	(1)					7
Δ^9-Tetrahydrocannabinol	2473	(4)	20	17	2390	[11]	5, 15, 16, 35
Tetrahydrofurfuryl oleate	2290	(1)					2
	2480	(1)					2
	2660	(1)					2
Tetrahydrozoline	1833	(3)	11	19	1750	[25]	9, 11, 15
Tetraphenylethylene	2478	(1)					2
Thebacon	2533	(2)	3	2			11, 35

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Thebaine	2517	(14)	32	18	2395	[12]	5, 7, 9, 10, 13, 14
					2400	[12]	15, 21, 22, 25
					2580	[11]	
Thenalidine	2318	(1)					30
Thenyldiamine	1999	(11)	31	31			1, 8, 10, 12, 15, 28
Theobromine					1840	[35]	
					1847	[10]	
					1910	[14]	
					2250	[25]	
					2470	[11]	
					2845	[7]	
Theophylline	1999	(7)	44	41	2105	[7]	12, 14, 15, 35
					2450	[30]	
Thiabendazole	2040	(5)	39	40			7, 10, 14, 15
Thiacetazone	2038	(2)	3	2			11
Thialbarbitone	2116	(1)					18
Thiambutosine	1715	(1)					11
Thiamylal	1899	(5)	14	18			5, 10, 15, 19
Thianaphthene	1200	(1)					2
Thianthrene	1901	(1)					2
Thiantoin	2145	(1)					5
Thiazesim	2595	(1)					15
Thiethylperazine	3247	(1)					10
Thiobarbituric acid	738	(1)					18
Thiocarlide	2005	(2)	5	5			11
Thiopentone	1859	(19)	24	31	1730	[12]	5, 7, 9, 10, 13, 15
					1740	[12]	16, 17, 18, 19, 24
					1765	[11]	26, 35
					1765	[12]	
Thiopropazate	3465	(2)	15	15			1, 14
Thioproperazine	9999	(1)					7
Thioridazine	3114	(8)	34	31	3176	[9]	5, 10, 13, 14, 23
					3180	[6]	27, 28
Thiosalicylic acid	1494	(1)					10
Thiothixene	3060	(1)					10
Thonzylamine	2203	(7)	48	47	2045	[5]	1, 7, 10, 11, 15, 23
Thozalinone	2040	(1)					15
Thymol	1260	(1)					10
Thymoxamine	1832	(1)					11
Tigloidine	1687	(1)					30
Tiletamine					1695	[15]	
					1720	[30]	
					2195	[11]	
					2200	[11]	
Tilidate	1840	(2)	0	0			16, 35
Tolazamide	1651	(1)					10
Tolazoline	1598	(6)	43	37	1490	[7]	11, 12, 30
					1510	[14]	
Tolbutamide	1683	(2)	13	13			7, 10
o-Toluidine	2356	(1)					10
Tranylcypromine	1223	(12)	23	49	1290	[8]	3, 5, 7–11
					1291	[8]	13, 14, 22, 29, 35

(Continued on p. 230)

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	−	+	*>50*	*[Ref.]*	*Refs.*
Triacetin	1282	(1)					2
Triamcinolone	2970	(1)					10
	3107	(1)					10
Tribenzylamine	2271	(1)					2
Tributyl citrate	2150	(1)					2
Tributyl phosphate	1690	(1)					2
Tributyrin	1552	(1)					2
Tri-(2-chloroethyl) phosphate	1740	(1)					2
Tricresyl phosphate	2695	(1)					2
Triethyl citrate	1655	(1)					2
Tri-(2-ethylhexyl) phosphate	2463	(1)					2
Triethyl phosphate	1109	(1)					2
Triflubazam	2244	(1)					2
Trifluomeprazine	2250	(1)					11
Trifluoperazine	2683	(10)	18	37	2615	[12]	1, 4, 6, 10, 11, 14
					2625	[12]	15, 17, 23, 27
Trifluperidol					2545	[12]	
					2545	[12]	
					2570	[12]	
					2590	[12]	
					2655	[36]	
					2797	[30]	
Triisobutyl phosphate	1483	(1)					2
Triisopropyl phosphate	1182	(1)					2
Trimecaine	1971	(2)	6	6			15, 28
Trimeperidine	1652	(1)					30
	1737	(1)					30
	1808	(1)					30
	1851	(2)	28	27			11, 30
Trimeprazine	2309	(10)	29	36	2230	[12]	1, 4, 6, 7, 10–12
					2245	[12]	23, 27
Trimethobenzamide					3287	[10]	
					9999	[11]	
Trimethoprim	2638	(1)					11
Trimethoxyamphetamine	1748	(1)					10
Trimethylamine	2215	(1)					7
Trimethyl citrate	1442	(1)					2
N,N,α-Trimethyl-β-phenethylamine	1233	(2)	5	5			10, 22
Trimethyl phosphate	995	(1)					2
Trimetozine	2198	(3)	23	17			11, 15
Trimipramine	2201	(11)	36	49			6, 11, 12, 14, 15, 27, 30, 35
Trioctyl phosphate	2445	(1)					2
Trioxsalen	2155	(1)					7
Tripelennamine	1980	(15)	20	30			1, 4–11, 14, 15, 25, 28
Triphenylamine	2055	(1)					2
Triphenyl phosphate	2363	(1)					2
Triprolidine	2253	(7)	8	11			4, 5, 7, 9–11, 14
Tripropyl phosphate	1372	(1)					2
Triptylene	2224	(1)					2
Tris(butoxyethyl) phosphate	2363	(1)					2
Tris(2,3-dichloropropyl) phosphate	2307	(1)					2
Trometamol	1645	(1)					11

TABLE 1 *(continued)*

Compound name	*RI*	*(n)*	*−*	*+*	*>50*	*Ref.*	*Refs.*
Tropacocaine	1952	(4)	17	25			12, 15
Tropicamide	2330	(2)	0	0			7, 15
Tropine	1193	(3)	13	9			7, 10, 22
Troxidone	1100	(1)					5
Tryptamine	1742	(13)	32	48	1800	[13]	2, 4, 5, 7, 10–12, 14, 15, 35
Tuaminoheptane	888	(4)	32	32	785	[9]	10, 11, 22, 25
Tubocurarine	2495	(1)					11
Tybamate					1708	[10]	
					1743	[9]	
					2015	[12]	
					2030	[12]	
Tymazoline	1850	(1)					4
Tyramine	1436	(4)	31	19	1525	[12]	2, 7, 12
					1530	[12]	
Umbelliferone	1828	(1)					10
Urea	9999	(1)					10
Urethane	838	(1)					10
Valnoctamide	1370	(2)	5	5			12
Vinbarbitone	1740	(9)	24	33			9–11, 15, 16, 18, 19, 26, 35
Vinbarbitone metabolite [5-(1-ethyl-*n*-propyl) barbituric acid]	1660	(1)					35
Vinbarbitone metabolite [5-hydroxy-5-(1-ethyl-*n*-propyl)barbituric acid]	1790	(1)					35
Vinbarbitone metabolite [5-(3-hydroxy-1-methylbutyl)-5-vinylbarbituric acid]	1930	(1)					35
Vinylbitone	1720	(1)					16
Warfarin	1432	(3)	27	28	2625	[7]	5, 10, 11
Xenysalate	2443	(2)	3	2			7, 11
Xylometazoline	1940	(4)	21	17	2130	[4]	10, 11, 15
					2520	[25]	
Yohimbine	3269	(3)	13	21	2050	[11]	5, 10, 14
Zoxazolamine	1629	(1)					10
Zylofuramine	1665	(1)					15

values have been reported that differ from the mean by more than 50 retention index units. The determination of the retention index of barbitone is therefore far more reproducible than that for chlorpromazine. The factors affecting the reproducibility of retention indices have previously been discussed[2].

Data concerning reproducibility have been summarised in Table 2 which shows the retention indices and associated standard deviations for twenty compounds for which more than ten values using SE-30 were available. Comparable data for retention indices measured on OV-17 have also been included for comparison but, because

much less OV-17 data were available, a lower limit of five determinations was set. The reproducibility, as measured by the standard deviation, is therefore marginally better using SE-30 than using OV-17. The mean standard deviation of 16.7 for SE-30 compares well with the standard deviations for inter-laboratory measurements of retention indices for basic drugs reported by Moffat[1] which were between 20 and 15 retention index units. The difference of 50 retention index units from the mean, used to identify unusual values, therefore corresponds to 3 standard deviations which would theoretically be expected to encompass 99% of experimentally determined values.

TABLE 2

RETENTION INDICES (*RI*), STANDARD DEVIATIONS (S.D.) AND RETENTION INDEX DIFFERENCES (ΔRI) FOR TWENTY COMPOUNDS FOR WHICH MULTIPLE DETERMINATIONS ON BOTH SE-30 AND OV-17 STATIONARY PHASES WERE AVAILABLE

Compound	*SE-30*		*OV-17*		ΔRI
	RI	*S.D.*	*RI*	*S.D.*	
Amitriptyline	2196	22.13	2518	23.39	322
Amylobarbitone	1718	15.33	1988	10.97	270
Barbitone	1497	14.25	1796	13.01	299
Caffeine	1810	13.22	2246	23.82	436
Chlorpromazine	2486	31.56	2890	18.03	404
Cyclobarbitone	1963	12.30	2351	5.73	388
Dextropropoxyphene	2188	22.36	2455	25.53	267
Glutethimide	1836	17.62	2205	24.10	369
Lignocaine	1870	15.48	2164	26.19	294
Meprobamate	1796	14.51	2182	12.43	386
Methapyrilene	1981	11.70	2296	22.41	315
Methaqualone	2125	15.18	2580	19.85	455
Methylphenobarbitone	1891	16.05	2262	25.73	371
Nicotine	1348	18.65	1553	15.60	205
Pentazocine	2275	22.20	2607	19.35	332
Pentobarbitone	1740	13.46	2017	14.35	277
Pethidine	1751	12.90	1996	9.92	245
Phenacetin	1675	16.25	2040	8.02	365
Phenobarbitone	1957	13.22	2372	16.98	415
Salicylamide	1455	16.46	1802	16.19	347
	Mean S.D.		Mean S.D.		Mean ΔRI
		16.74		17.58	338

Some compounds undergo on-column decomposition and therefore give multiple peaks. An example is chlordiazepoxide, which can give four peaks, and these are reported individually under the parent compound in Tables 1 and 3. Other compounds, such as propantheline bromide, would not be expected to elute as the parent compound, but nevertheless give a peak with a retention index around 2200 or 2350. In these cases the retention indices should be determined in the user's laboratory under specific experimental conditions and special care should be exercised if a mass spectrometer is being used as detector.

Table 3 has been generated from the data in Table 1 to aid the identification of an unknown compound and contains 1820 entries from 1318 compounds in as-

TABLE 3

1820 RETENTION INDICES OF 1318 COMPOUNDS, USING SE-30 OR OV-1 AS THE STATIONARY PHASE, ARRANGED IN ASCENDING ORDER OF RETENTION INDEX

Mean retention indices are given as well as each value which was greater than ±50 retention index units from the mean (indicated by an asterisk).

RI	*Compound name*	*RI*	*Compound name*
695	Pyrrolidine	1109	Triethyl phosphate
738	Thiobarbituric acid	1110	Phenylpropanone
771	Ethylene glycol	1111	β-Phenethylamine
780	Ethanolamine	1118	Heptaminol
785*	Tuaminoheptane	1119	Nicotinyl alcohol
790	Piperidine	1120*	N-Ethylbenzylamine
810	Morpholine	1120*	Paramethadione
813	Piperazine	1123	Amphetamine
838	Urethane	1127	Terpineol
882	Allopurinol	1128	1-Phenylpropylamine
888	Tuaminoheptane	1130	Methyl caprylate
909	Butylamine	1133	Norfenfluramine
917	Cyclohexylamine	1137	Camphor
930	Putrescine	1140	Isopropylhexedrine
981	Phenol	1147	Phentermine
995	Benzylamine	1158	Aniline
995	Trimethyl phosphate	1159	N,N-Dimethylphenethylamine
1000	Acepifylline	1167	Perhydrophentermine
1000	Methylaminomethylheptane	1169	Propylhexedrine
1010*	Dimethylaniline	1170	Terpineol
1017	Fluspirilene	1176	Methylamphetamine
1023	Ethchlorvynol	1176	Phenylpropylmethylamine
1023	*p*-Dichlorobenzene	1180	Benzoic acid
1030*	Heptaminol	1182	Triisopropyl phosphate
1031	α-Phenethylamine	1183	Terpineol
1031	N-Ethylbenzylamine	1185	Chlormethiazole metabolite
1035	Cadaverine	1186	Naphthalene
1046	Benzyl alcohol	1190*	Amphetamine
1046	iso-Amylamine	1192*	Carbromal
1052	Isometheptene	1192	Geraniol
1053	Limonene	1193	Methyl salicylate
1062	Indene	1193	*p*-Dibromobenzene
1072	Salicylaldehyde	1193	Tropine
1078	Methylbenzylamine	1195	α,α-Dimethyl-β-succinamide
1080	N,N-Dimethylaniline	1197	*m*-Dibromobenzene
1081	Diethyl maleate	1200	Thianaphthene
1087	Cyclopentamine	1205	3-Phenylpropylamine
1090*	Amphetamine	1205	Carbromal metabolite
1092	Guaiacol	1206	Ethosuximide
1095	Imidazole	1210	Dimethrin
1100	Linalol	1210	Hexamine
1100	Methyl nicotinate	1212*	Bromvaletone
1100	Monuron	1212	Sulphafurazole
1100*	Nicotinyl alcohol	1213	Dimethyl adipate
1100	Troxidone	1214	Pargyline
1105	Emylcamate	1215	Acetylcarbromal

(Continued on p. 234)

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
1215	Methyl nonanoate	1309	*p*-Aminosalicylic acid
1215	Nicotinyl alcohol	1309	*n*-Butyric acid
1220	Hydroquinone	1309	*cis*-Linoleic acid
1221	*o*-Dibromobenzene	1313	2-Methylnaphthalene
1222	Fenfluramine	1313	Phenylpropanolamine
1223	Tranylcypromine	1318	*n*-Propyl-α-methyl-β-phenethylamine
1227	Ethyl benzoate	1320	Ethosuximide metabolite
1228	N-Ethylamphetamine	1320*	Hydroxyamphetamine
1230	Chlormethiazole	1323	Dichloroaniline
1231*	Apronal	1324	N-Propylamphetamine
1233	N,N,α-Trimethyl-β-phenethylamine	1326	1-Bromodecane
1235	Betahistine	1335*	Methylamphetamine
1236	N,N-Dimethylamphetamine	1335	Nicotinic acid
1239	Mephentermine	1335	Phenelzine
1240	Mebenazine	1340	Carbromal metabolite
1246	Anisaldehyde	1340*	Methylamphetamine
1247	N-Isopropyl-α-methyl-β-phenethylamine	1340*	Methylamphetamine
		1342	Chlorphentermine
1247*	Quinoline	1343	Methyl anthranilate
1249	*n*-Caproic acid	1344	Diethyl adipate
1249	Oxanamide	1345	Cypenamine
1249	Pyruvic acid	1345*	Methylamphetamine
1249	Tartaric acid	1347*	*p*-Hydroxyphenylpyruvic acid
1250	Pyrazinamide	1348	Nicotine
1250	Quinone	1353	Dimetridazole
1252	Promoxolane	1354	Pseudoephedrine
1256	Pentorex	1358	Acetanilide
1257	Amantadine	1361	Methoxyphenamine
1257	N-Isopropylamphetamine	1362*	Norpseudoephedrine
1258	Resorcinol	1363	Ephedrine
1260*	Amphetamine	1363	Ethinamate
1260	Thymol	1365	N-2-Butylamphetamine
1265	*p*-Aminophenol	1365	Chlormethiazole metabolite
1265*	Amphetamine	1365*	*p*-Hydroxyphenylpyruvic acid
1265	Sulphosalicylic acid	1365*	Phentermine
1270	Buphenine	1367*	Phentermine
1272	Citral	1368	Eugenol
1276	Indole	1370	Ethosuximide metabolite
1282	Triacetin	1370*	Mephentermine
1284	Anethole	1370	Valnoctamide
1285*	Diprophylline	1371	N,N-Diethylamphetamine
1285	*p*-Dibutoxyethoxyethyl adipate	1372	Metamfepyramon
1290*	Tranylcypromine	1372	Tripropyl phosphate
1291*	Tranylcypromine	1373	Bemegride
1293	Aletamine	1375*	Mephentermine
1295*	Mephentermine	1375*	Phenylpropanolamine
1302	Norpseudoephedrine	1380	Carbromal metabolite
1303	Octamylamine	1385	Ethosuximide metabolite
1305*	Mephentermine	1385	Methoxyamphetamine
1305	Methyl decanoate	1388	Rimantadine
1305	Piperonal	1389	Biphenyl
1308	Salicylic acid	1390	Ametazole
1309	Acetylsalicylic acid	1390*	Hippuric acid

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
1390	Nicotinic acid amide	1475	Dimethyl terephthalate
1396	Ectylurea	1475*	Leptazol
1396	N-Methyl-N-*n*-propylamphetamine	1478	Sulphonal
1399	*n*-Caproic acid	1480	Beclamide
1400*	Acetylsalicylic acid	1483	Triisobutyl phosphate
1400*	α-Methylphenethylhydrazine	1485*	Diazinon
1400	N-Methylephedrine	1485*	Hordenine
1400	Pheniprazine	1486	Chlorothymol
1402*	Diethylpropion	1486	Diethylpropion
1406	Dimethyl phthalate	1486	N-Methyl-N-*n*-butylamphetamine
1410*	Acetylsalicylic acid	1487	Acetylindole
1415*	Nicotine	1487	Mandelic acid
1415*	Phenylpropanolamine	1487	Protokylol
1418*	Pseudoephedrine	1488	Dimethyl isophthalate
1419	Methyl *p*-hydroxybenzoate	1489	1-Naphthonitrile
1420	Modaline	1490	Butylated hydroxytoluene
1420*	Nicotine	1490	Cantharidin
1422	N-*n*-Butylamphetamine	1490	Carbaryl
1425	2,7-Dimethylquinoline	1490	Diethadione
1426	Isoquinoline	1490*	Hordenine
1427*	Quinoline	1490*	Hordenine
1430*	Nicotine	1490*	Leptazol
1431	Phenmetrazine	1490*	N,N-Diethyl-*m*-toluamide
1432	Warfarin	1490*	Tolazoline
1434	Bromonaphthalene	1494	Thiosalicylic acid
1434	N-Ethyl-*p*-chloroamphetamine	1495*	Diazinon
1436	Tyramine	1495*	Hordenine
1440*	Ephedrine	1495*	Phenmetrazine
1442	Trimethyl citrate	1495*	Phenmetrazine
1443	Kojic acid	1495*	Phenmetrazine
1444	Phendimetrazine	1497	Barbitone
1445*	Ephedrine	1497	Histamine
1446	2,4-Dimethylquinoline	1500*	Nialamide
1448	Naftazone	1505*	Acetylcarbromal
1450	Hydroquinine	1505	Dibutyl maleate
1450	Mevinphos	1507	4,4′-Bipyridyl dihydrate
1455*	Methoxyphenamine	1507	Carminic acid
1455	Salicylamide	1507*	Methocarbamol
1459	Nicotinamide	1510*	Bromvaletone
1460*	Etafedrine	1510*	Cyclopentamine
1462	Butylated hydroxyanisole	1510*	Tolazoline
1465	Pheneturide	1513	Carbromal
1466	Meconic acid	1515*	Cyclopentamine
1467*	Nitrazepam	1515*	Isoniazid
1468	Phenoxypropazine	1515	Pyridostigmine bromide
1470*	Methoxyphenamine	1516	*o*-Chlorobenzylidenemalonitrile
1470*	Methoxyphenamine	1519	Etafedrine
1471*	Benperidol	1519	Homarylamine
1471	2,4-Dinitrophenol	1520*	Diphenylamine
1472	Metharbitone	1520	Phenprobamate
1472	Methylenedioxyamphetamine	1520*	Prolintane
1473	Capric acid	1524	Acetyl-β-phenethylamine
1473	Phenacemide	1524	Cyclododecanone

(Continued on p. 236)

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
1525	Benzenesulphonamide	1598	5-Aminoquinoline
1525*	Isoniazid	1598	Tolazoline
1525*	Isoniazid	1600	Amylocaine
1525	Nikethamide	1600	Lauric acid
1525*	Tyramine	1601	Diphenylamine
1526	N,N-Di-*n*-propylamphetamine	1604	Clorprenaline
1528	*p*-Dimethylaminobenzaldehyde	1605	2,4-D Methyl ester
1528	Hydrallazine	1606	Allobarbitone
1529	Methyprylon	1608	Nicametate
1530*	Tyramine	1608	Nifenazone
1531	α-Naphthylamine	1610	Benzophenone
1542	Etenzamide	1611	4-*tert*.-Octyl-2-methylcyclohexyl acetate
1545	Dipropyl adipate	1613	Pivalylbenzhydrazine
1547	Acetylcysteine	1616	STP
1547	*p*-Aminobenzoic acid	1617	4,6-Dinitro-*o*-cresol
1549	Clofibrate	1620	Propylparaben
1550	Crotamiton	1621	Chlorproguanil
1550*	Diethylpropion	1622	Aprobarbitone
1550	Methimazole	1622	Methsuximide
1551	3,4-Dimethoxyphenethylamine	1628	Demeton S-methyl
1552	Leptazol	1629	Zoxazolamine
1552	Tributyrin	1630	Acetylmethylamphetamine
1555	Benzocaine	1630*	Oxyphenbutazone
1555*	Diethylpropion	1632	Hydroxyamylobarbitone
1555*	Diethylpropion	1633	Diisopropyl phthalate
1555	Ethylallylbarbitone	1634	Dimethyl sebacate
1555*	Phendimetrazine	1634	Phensuximide
1556	Azobenzene	1634	Prolintane
1557	Pyrithyldione	1635	4-Methyl-2,5-dimethoxyamphetamine
1560*	Barbitone	1640	Butoxyethyl nicotinate
1560*	Phendimetrazine	1640	Dipyramidole
1560*	Salicylamide	1640	Piracetam
1561	Enallylpropymal	1645	Trometamol
1564	Diethyl phthalate	1646	3,4-Methylenedioxyphentermine
1565	Hydroxybenzoic acid	1647	3,4-Methylenedioxy-2-methoxy-amphetamine
1567	Bromodiacetylurea		
1567	Probarbital	1650	Guaiphenesin
1567	Propyl *p*-hydroxybenzoate	1650*	Salol
1568	Mephenesin	1651	Tolazamide
1570	3-Ethylamino-3-phenylnorcamphane	1652	Pethidine intermediate A
1578*	Apronal		
1580*	Carbromal	1652	Trimeperidine
1580	Fluorene	1655	Atrazine
1582*	Isoniazid	1655	Orthocaine
1583	N,N-Diethyl-*m*-toluamide	1655	Phenyl salicylate
1585	2-Naphthyl acetate	1655	Triethyl citrate
1585	Butylbenzyl sebacate	1658	*p*-Nitromethylamphetamine
1585	N-Methyl-3,4-methylene-dioxyamphetamine	1660	Diisobutyl adipate
		1660*	Phenylephrine
1590	Hydrastinine	1660*	Salol
1590	Proheptazine	1660	Quinalbarbitone metabolite
1592	Metronidazole	1660	Vinbarbitone metabolite
1593	Iproniazid	1661	Oxyphencyclimine

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
1662	9,10-Dihydroanthracene	1710	β-Benzenehexachloride
1662	Secbutobarbitone	1710	Noramidopyrine
1665	Butobarbitone	1712	Diallyl phthalate
1665	Zylofuramine	1712	Isatin
1667	Styramate	1714	Ergosterol
1668	Butalbital	1715	γ-Benzenehexachloride
1668	Etisazole	1715	Methyl myristate
1670	Isoniazid	1715	Thiambutosine
1675	Methyl hippurate	1718	Amylobarbitone
1675	Phenacetin	1718	Morazone
1675	Phorate	1720*	Chlorothiazide
1676	Dofamium chloride	1720*	Chlorpropamide
1677	Chlorphenesin	1720	Nealbarbitone
1677	Fencamfamin	1720*	Tiletamine
1678	Beclamide	1720	Vinylbitone
1678	Carbimazole	1724	Hydroxyphenamate
1678	Cotinine	1725	Dimethoate
1678	Dyclonine	1726	Methoxamine
1682	Hydroxyephedrine	1728	Chlorzoxazone
1683	Tolbutamide	1730	Acetyltriethyl citrate
1685	CDEC	1730	Isoprenaline
1685*	Etilefrine	1730*	Thiopentone
1687*	Dextropropoxyphene	1734	Diampromide
1687	Paracetamol	1735*	Hippuric acid
1687	Tigloidine	1736	Methallatal
1688	Crotethamide	1737	Methyl phenidate
1688	Mescaline	1737	Trimeperidine
1689	N,N-Di-*n*-butylamphetamine	1738	Benzyl benzoate
1690	α-Benzenehexachloride	1738	Cropropamide
1690	Simazine	1740	α-Methyltryptamine
1690	Tributyl phosphate	1740	2,4,5-T Methyl ester
1693	5-Propyl-5-isobutyl barbituric acid	1740*	Ethamivan
1695	Dibutyl adipate	1740	Pentobarbitone
1695*	Tiletamine	1740*	Thiopentone
1698	5-Allyl-5-butyl barbituric acid	1740	Tri-(2-chloroethyl) phosphate
1700	2,4-D Isopropyl ester	1740	Vinbarbitone
1700	Dimethylmescaline	1742	Butyl aminobenzoate
1700*	Iproniazid	1742	Tryptamine
1700	N-Methylmescaline	1743	Halquinol
1701	Talbutal	1743*	Tybamate
1702	3,4-Methylenedioxy-4-methoxy-amphetamine	1745	Diethyl sebacate
		1745*	Dimethyltryptamine
1702	Carminic acid	1745	Lindane
1703	Phenazine	1745	Norpethidine
1705	3,4-Methylenedioxy-5-methoxy-amphetamine	1745*	Salol
		1746	Dipropyl phthalate
1705	Fluorenone	1748	Profadol
1705*	Leptazol	1748	Trimethoxyamphetamine
1705*	Leptazol	1749	Pentachlorophenol
1708	Dicyclomine	1750	Properidine
1708*	Tybamate	1750*	Tetrahydrozoline

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TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
1751	Pethidine	1796	Meprobamate
1754	Anthracene	1798	Benethamine
1754	Butethamate	1800	α-Benzylphenethylamine
1754	Myristic acid	1800*	Diethyltryptamine
1754	Nitroxynil	1800	Ethotoin
1755	δ-Benzenehexachloride	1800	Methylanhalonidine
1755*	Phenacetin	1800*	Tryptamine
1755*	Phencyclidine	1801	Sparteine
1755	*trans*-Stilbene	1803	Nimorazole
1756	Ethionamide	1804	Pheniramine
1758	Diazinon	1804	Physostigmine
1759	Pethidine intermediate B	1805	2,4-D Isobutyl ester
1760	Alprenolol	1807	Dexpanthelol
1760	Benzphentermine	1808	Cotarnine
1760	Dichlone	1808	Trimeperidine
1760*	Nikethamide	1810	Caffeine
1760*	Paracetamol	1810*	5-Ethyl-5-phenylhydantoin
1760*	Paracetamol	1810*	Methyl phenidate
1764*	Saccharin	1811	Pipobroman
1765*	Fenmetramide	1813	Bromodimethoxyamphetamine
1765*	Thiopentone	1814	Narcobarbital
1765*	Thiopentone	1815*	Mescaline
1766	Methohexitone	1816	Benzamine
1770	Meclofenoxate	1823	Betameprodine
1770*	Methylphenobarbitone	1825	Anhalonidine
1770	N-Methyltryptamine	1825	Bisnortilidine
1770	Neostigmine bromide	1825	Pellotine
1770	Ibomal metabolite	1825	Prilocaine
1775	Ametryne	1825	2,4,5,-T Isopropyl ester
1777	Hexylresorcinol	1828	Umbelliferone
1779	Nicoumalone	1830	Carisoprodol
1780*	Lobeline	1830	Isoaminile
1780	Norpropyphenazone	1832	Thymoxamine
1780*	Paracetamol	1833	Tetrahydrozoline
1780*	Secbutobarbitone	1835	Brallobarbitone metabolite
1782	α-Phenylglutarimide	1835	Nortilidate
1784	N-Benzylamphetamine	1835*	*p*-Hydroxyphenylpyruvic acid
1784	Carbazole	1836	Glutethimide
1784	Clobutinol	1837	5-Allyl-5-(2-hydroxypropyl)barbitone
1785	Allobarbitone metabolite	1840*	Alprenolol
1786	Phenanthrene	1840	Benorylate
1790	Betaprodine	1840	2,4-D Butyl ester
1790	Secobarbitone	1840	Piperoxan
1790	Vinbarbitone metabolite	1840*	Theobromine
1791	Chlorpropamide	1840	Tilidate
1791	Methoin	1842	α-Phenyl-α-ethylglutaconamide
1791	Quinalbarbitone	1843	Ketamine
1792	Alphaprodine	1844	Dimenhydrinate
1795*	5-Ethyl-5-phenylhydantoin	1847*	Theobromine
1795	5-Methyltryptamine	1848	Etryptamine
1795	Brallobarbitone metabolite	1848	Phenazone
1795	Desmetryne	1850	Alphameprodine
1795	Isobutyl aminobenzoate	1850	Anhalonine

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
1850	Lophophorine	1895*	Alphaprodine
1850*	Meprobamate	1898	Dofamium chloride
1850*	Neostigmine bromide	1899	Thiamylal
1850	Tymazoline	1901	Thianthrene
1851	Methyl parathion	1902	Digitalin
1851	Trimeperidine	1902	Digitoxin
1852	Lachesine	1903	Amidopyrine
1853	Diisobutyl phthalate	1903	Cyclandelate
1853	Prometryne	1904	Phencyclidine
1855	Benzphetamine	1906	Doxylamine
1855	Dichlorophenazone	1906	Morinamide
1857	Ethoheptazine	1908	Terodiline
1857	Hexobarbitone	1910	Diethyltryptamine
1858	Brallobarbitone	1910*	Theobromine
1858	Hexethal	1913*	5-Ethyl-5-phenylhydantoin
1859	Diampromide	1913	Dibutyl phthalate
1859	Propyl myristate	1915*	Ethotoin
1859	Thiopentone	1915*	Fenmetramide
1860	Metyrapone	1915	Hydroxypentobarbitone
1860*	Neostigmine bromide	1916*	Dipyrone
1862	Cyclopentobarbitone	1917	Malathion
1866	5-Methyl-5-phenylhydantoin	1920	1-Hydroxyharman
1868*	Bromo STP	1920	Iminodibenzyl
1868	Isobutylcyclohexyl phthalate	1920	Metazocine
1870	Lignocaine	1920*	Norharman
1870	Oxprenolol	1920	Proheptazine
1873	Diphenhydramine	1923	Bibenzonium bromide
1874	4-Hydroxyantipyrene	1924	Di(ethoxyethyl) adipate
1874	*p*-Methoxybenzophenone	1925	Bethanidine
1875*	Ethotoin	1925	Propyphenazone
1875	Glutethimide metabolite	1928	Levamisole
1875	Nicoumalone	1928	Secbutobarbitone metabolite
1875*	Orphenadrine	1929	Allylprodine
1875*	Saccharin	1930*	Diisobutyl phthalate
1880	Dicyclohexyl oxalate	1930*	Ethamivan
1880	Diethyl-*p*-nitrophenyl phosphate	1930	Vinbarbitone metabolite
1880	Heptachlor	1936	2-Mercaptobenzothiazole
1880	Phenylmethylbarbituric acid	1936	Orphenadrine
1883	Clorgyline	1937	Proheptazine
1883	Ibomal	1938	Phenyltoloxamine
1885	Aminoparathion	1939	Azapetine
1885	Dimethylthiambutene	1940	Butalbital metabolite
1885*	Dimethyltryptamine	1940	Butylsextyl phthalate
1885	Methstyridone	1940*	Dextropropoxyphene
1889	Mebutamate	1940	Terbutryne
1889	Methyl palmitate	1940	Xylometazoline
1891	Methylphenobarbitone	1942	Parathion
1893	Octacaine	1943	Aldrin
1893	Ronnel	1943	Ethylmethylthiambutene
1894*	Ethamivan	1945*	Caffeine
1894	Meboral	1945	Lecithin

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TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
1945	Nystatin	1999	Thenyldiamine
1945*	Santonin	1999	Theophylline
1947	Howflex GBP	2000*	Amidopyrine
1949	Isocarboxazid	2000	Captan
1950	Ampyrone	2000	Pyridoxamine
1950	Butylisodecyl phthalate	2002	Chlorpheniramine
1950	Flufenamic acid	2003*	5-Allyl-5-phenyl barbituric acid
1950	Tetracycline	2003	Hymecromone
1952	Harman	2005*	Norharman
1952	Tropacocaine	2005	Thiocarlide
1953	Ethyl parathion	2006	Phenyramidol
1955	1-Hydroxychlordene	2008	Diethylthiambutene
1957	1-Phenyl-3-pyrazolidinone	2008	Etomidate
1957	Phenobarbitone	2010	Anilazine
1960	DCPA	2010	Dyrene
1963	Cyclobarbitone	2010*	Phenobarbitone
1964*	Dextropropoxyphene	2010*	Phenyltoloxamine
1964	Padimate	2011	Cloponone
1965	Hexylcaine	2013	Octaphonium chloride
1965*	Mebutamate	2014	Phenampromide
1965	Quinalbarbitone metabolite	2014	Pilocarpine
1966	Methaphenilene	2015	1-Hydroxyharman
1968*	Doxylamine	2015	Folpet
1968	Pyrrocaine	2015	Heptachlor epoxide
1969*	Pheniramine	2015*	Tybamate
1970*	Aminophylline	2018	Dioxyamidopyrine
1970*	Aminophylline	2018	Procaine
1970	Diphenamid	2020	Chlordane
1971	Caramiphen	2020	Cyclizine
1971	Trimecaine	2020	Cyclopentolate
1972	Diisobutyl terephthalate	2020*	Piminodine
1973	Emepronium bromide	2021	Amisometradine
1973	Palmitic acid	2021	Methadone metabolite
1974	Dofamium chloride	2024	Isopyrin
1975	Ethomoxane	2024	Phenothiazine
1980*	2-Amino-5-chloro-2′-fluorobenzophenone	2025	Butanilicaine
		2025*	Isopropamide
1980	Dimethoxyethyl phthalate	2026	Eucatropine
1980	Methoxsalen	2029	Dimethoxanate
1980	Piperocaine	2030	Bufotenine
1980	Psilocin	2030	Dimethisoquin
1980	Pyridbenzamine	2030	Ketobisnortilidine
1980	Tripelennamine	2030*	Pipobroman
1981	Methapyrilene	2030*	Tybamate
1983	Dipyrone	2034*	Phenobarbitone
1983	Pyrene	2035	Ketobemidone
1988*	Methylphenobarbitone	2035	Physostigmine
1988	Metabutethamine	2037	Pipazethate
1988*	Ibomal	2038	Amprotropine
1990*	Naphazoline	2038	Thiacetazone
1990	Procarbazine	2039	2-Amino-5-chlorobenzophenone
1991	Riconoleic acid	2040	Brallobarbitone metabolite
1993*	Diethyltryptamine	2040	Thiabendazole
1995	Butallylonal	2040	Thozalinone

TABLE 3 *(continued)*

RI	*Compound name*
2042*	α-Methylphenethylhydrazine
2045*	2-Amino-5-chloro-2′-fluorobenzophenone
2045	Chlorbenside
2045	Hydroxypethidine
2045*	Thonzylamine
2048*	Atropine
2049	Rotoxamine
2050	Apoatropine
2050	Hexobarbital metabolite
2050	Norbormide
2050*	Pipobroman
2050	PPO
2050*	Yohimbine
2055	Phenindione
2055*	Phenyltoloxamine
2055	Triphenylamine
2057	Naphazoline
2058	Acetyl-L-tryptophan
2058*	Chloropyrilene
2058	Heptabarbitone
2058*	Isopropamide
2059	Psilocybin
2060*	Iproniazid
2061	Butethamine
2062*	Sulpiride
2065*	Cyclazocine
2065*	Iproniazid
2065	Panidazole
2065*	Phenobarbitone
2066	Dibutyl terephthalate
2070	Berberine
2070*	Cyclazocine
2070	*o,p′*-DDE
2071	Mepivacaine
2072	Chlorphenoxamine
2072	Homatropine
2074*	Dextropropoxyphene
2074	Mercumallylic acid
2075	Dexbrompheniramine
2078	2-Amino-5-chlorodiphenylamine
2079	Diampromide
2080	Carbinoxamine
2080	Chlophedianol
2080	Hexobarbitone metabolite
2080*	Pemoline
2081	Noracymethadol
2082	Benzoctamine
2083*	Aminophylline
2085	5-Ethyl-5-*p*-tolylbarbituric acid
2085	DDA Methyl ester
2085	Endosulphan I
2085*	Lobeline
2086	Methyl oleate
2089	Phenatine
2090*	2-Amino-5-chloro-2′-fluorobenzophenone
2090	β-Hydroxyethyltheophylline
2090	β-Hydroxypropyltheophylline
2091	Normethadone
2096	Brompheniramine
2097	Dicyclomine
2098	Methoprotryne
2099	Diphenylpyraline
2100	Clotrimazole
2100	Methyl linoleate
2100*	Norharman
2103	Di(ethoxyethyl)phthalate
2103	Proxyphylline
2105*	Primaquine
2105*	Procaine
2105*	Theophylline
2106*	5-Allyl-5-phenylbarbituric acid
2106*	Alphenal
2107	2-Methylamino-5-chlorobenzophenone
2108	Dimethocaine
2110	Dieldrin
2112	Dropropizine
2113*	Chlorphenesin
2114	Cycrimine
2115	Methyl stearate
2116	Disextyl maleate
2116	Thialbarbitone
2120*	2-Amino-5,2′-dichlorobenzophenone
2120	Glycopyrrolate
2122	Nomifensine
2124	Racemethorphan
2125*	2-Amino-5-chlorobenzophenone
2125	Methaqualone
2127	Diamyl phthalate
2128	Isomethadone
2129	Chlorothenylpiramine
2130	Butylbenzyl sebacate
2130*	Levomethorphan
2130	*o,p′*-TDE
2130	*p,p′*-DDE
2130*	Xylometazoline
2133	Chloropyrilene
2135	Brallobarbitone metabolite
2135*	Carisoprodol
2135	Dibenzyl sebacate
2135*	Phenelzine

(Continued on p. 242)

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
2137	Dibutyl sebacate	2180*	Propantheline bromide
2138	Pyrimethamine	2180	Pseudococaine
2140	Dextromethorphan	2180	Rescinnamine
2140	Dichlorophen	2181	Butriptyline
2141	Disulfiram	2183	Endrin
2142	Alverine	2184	Acetyl-D-tryptophan
2145*	Atropine	2184	Ergocryptine
2145	Chlorthalidone	2185	Embramine
2145	Pipradrol	2185	Levopropoxyphene
2145	Thiantoin	2186	Adiphenine
2146	3-Methoxymorphinan	2187	Alphacetylmethadol
2147	Lecithin	2187	Cocaine
2148	Methadone	2187	Methazolamide
2150	3-Isobutyl-1-methylxanthine	2188	2,5-Diamino-2′-fluorobenzophenone
2150*	Chlormezanone	2188	Dextropropoxyphene
2150	Tributyl citrate	2190*	Cannabidiol
2153	Chlorothen	2190*	Chlordiazepoxide
2153	Perhexiline	2190	Cyclobarbitone metabolite
2155	Bromdiphenhydramine	2190*	Methaqualone
2155*	Levomethorphan	2190*	Methaqualone
2155	Mephenoxalone	2190	Physostigmine
2155	Trioxsalen	2192	Hyoscyamine
2156	Procyclidine	2195*	2-Amino-5,2′-dichlorobenzophenone
2157	Butyl stearate	2195*	Bromo STP
2157	Propranolol	2195*	Cyclazocine
2158*	Phenylephrine	2195	Pentifylline
2159	Isobutylcyclohexyl phthalate	2195*	Tiletamine
2160*	Dimethoxanate	2196	Amitriptyline
2163	Metaxalone	2198	2,5-Diaminobenzophenone
2165*	Chlormezanone	2198	Trimetozine
2165*	Glutethimide	2199	Atropine
2165*	Imipramine	2200	*p,p′*-TDE
2165*	Primidone	2200*	Pipobroman
2165	Tacrine	2200*	Tiletamine
2166	Sulphanilamide	2201	Mefenamic acid
2167	Phenindamine	2201	Trimipramine
2168	Oxymetazoline	2203	Thonzylamine
2170	Cyclobarbitone metabolite	2205*	Biperiden
2170*	Desipramine	2205	Phenylalanine
2170*	Dieldrin	2205*	Picrotoxin
2170	Methaqualone metabolite	2205*	Pipethanate
2170*	Primidone	2205*	Promethazine
2174	Santonin	2205*	Propantheline bromide
2174	Stearic acid	2207	Perphenazine
2175*	Dieldrin	2208	*p*-Terphenyl
2175	Dopamine	2210	2-Benzhydroxymethyl-2-imidazoline
2175	Endosulphan II	2210	Nortriptyline
2175	Ethyl oleate	2211	Bamipine
2175	Ethylan	2211	Flupromazine
2175	Perthane	2213	Phenetamine
2178	Linolenic acid	2215*	Dieldrin
2179	*cis*-Linoleic acid	2215*	Propantheline bromide
2180	Alphamethadol	2215	Trimethylamine

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
2217	Doxepin	2245*	Cocaine
2218	Dicophane (*o,p'*-isomer)	2245*	Hyoscine
2218	Racemorphan	2245	Phenazopyridine
2219	Amethocaine	2245*	Trimeperazine
2219	Benzhexol	2247	Primidone
2220	Ethion	2248	Benactyzine
2220	Mepyramine	2248	Clonidine
2220*	Pilocarpine	2248	Procainamide
2220*	Procyclidine	2250*	Adiphenine
2223	Imipramine	2250*	Atropine
2224	Citroflex A4	2250	5-Chloro-2-methylaminodiphenylamine
2224	Triptylene	2250*	Meprobamate
2225	Chlorcyclizine	2250	Prolan
2225*	Cyclomethycaine	2250*	Theobromine
2226	Amolanone	2250	Trifluomeprazine
2226	Medazepam	2253	Acetyltributyl citrate
2226	Metabutoxycaine	2253	Triprolidine
2227	Aminoglutethimide	2255	Bromazepam decomposition product
2227*	Cyclomethycaine		
2227	Deanol acetamidobenzoate	2255	Carbophenothion
2229	Chloroprocaine	2255*	Levomethorphan
2230*	Levomethorphan	2255	Mecloqualone
2230*	Phenylbutazone	2258	Dimethindene
2230*	Trimeprazine	2259	Promethazine
2231	Noracymethadol	2260*	Oxazepam
2232	Carbetapentane	2260	Pindolol
2232	Diampromide	2261	Protriptyline
2232	Dihydrodesoxymorphine	2265	2-Hydroxyiminodibenzyl
2233	Phenoxybenzamine	2265	Cyheptamide
2234	Halopyramine	2266	Biperiden
2234	Levorphanol	2267	Isothipendyl
2235	Ambucetamide	2267	Noxiptyline
2235	Butylsextyl phthalate	2268	Melitracene
2235*	Methaqualone	2270	Di(ethoxyethyl)sebacate
2235*	Phenylbutazone	2270*	Ethopropazine
2237	3-Hydroxymorphinan	2270*	Oxazepam
2238	Chlormezanone	2270*	Phenytoin
2240	Aminacrine	2270*	Phenytoin
2240*	2-Amino-5,2'-dichlorobenzophenone	2271	Diclofenac
2240	Chlordecone	2271	Tribenzylamine
2240*	Cocaine	2273	Anisindione
2240*	Hyoscine	2273	Bupivacaine
2240	Leucinocaine	2274	Clopenthixol
2240	Methaqualone metabolite	2275*	Benzhexol
2240	N1-Desalkyl-3-hydroxyflurazepam	2275*	Desmethylmedazepam
2240*	Pemoline	2275	Diphenazoline
2242	Desipramine	2275	Heptabarbital metabolite
2243	Azacyclonol	2275	Pentazocine
2243	Bromazepam metabolite	2280*	Aconitine
2244	Norlevorphanol	2280*	Amethocaine
2244	Triflubazam	2280*	Benzhexol

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TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
2280*	Picrotoxin	2325*	Hydrocodone
2281	Pramoxine	2325*	Lorazepam
2282	Dicyclohexyl adipate	2326	SKF 525A
2285*	Amethocaine	2327	Butylbenzyl phthalate
2285	Galantamine	2327	Mepenzolate
2285*	Imipramine	2328	Antazoline
2285*	Medazepam	2328*	Promethazine
2288	9,10-Anthracene-dicarbonitrile	2330*	Bromhexine
2290	Benzylbutyl phthalate	2330	2′-Chloro-2,5-diaminobenzophenone
2290	Carbamazepine	2330	Cocaethylene
2290	2-Chloroprocaine	2330*	Lorazepam
2290*	Codeine	2330	Phenytoin
2290*	Dihydrocodeine	2330	Tropicamide
2290	1-Hydroxyharman	2335	Chlormezanone
2290	Tetrahydrofurfuryl oleate	2335*	Clomipramine
2291	Harmine	2335*	Diazepam
2292	Diethylaminoethyl diphenylpropionate	2335*	Ethylmorphine
2295*	Bromhexine	2335*	Ethylmorphine
2295*	Bromhexine	2335	Iprindole
2295*	Codeine	2335*	Morphine
2295	Desmorphine	2335	Propoxycaine
2295*	Dihydrocodeine	2336	Oxazepam
2295*	Imipramine	2336	Probenecid
2298	Stilboestrol	2339	Prothipendyl
2299	Dicophane (*p,p′*-isomer)	2340	Acetylprocaine
2300*	2,5-Diaminobenzophenone	2340*	Bromhexine
2300*	Chlordiazepoxide	2340	Dexoxadrol
2300	Dimophebumine	2340*	Doxepin
2300	Isoxsuprine	2340*	Hydrocodone
2301	Bufylline	2340*	Hydrocodone
2303	Hyoscine	2340*	Morphine
2305	Methyldesorphine	2340*	Protriptyline
2307	Tris(2,3-dichloropropyl) phosphate	2342*	Cyclazocine
2309	Trimeprazine	2342*	Dimethindene
2310	Bulan	2345	2,5-Diamino-2′-chlorobenzophenone
2310	Chloramphenicol	2345*	Clomipramine
2310	Domiphen	2345*	Diazepam
2310*	Piminodine	2345*	Methoxychlor
2310	Secergan	2345*	Phenazopyridine
2314	Benztropine	2345*	Pyrrobutamine
2314	Buphenine	2346*	Phenazopyridine
2314	Primaquine	2347*	Chloroprocaine
2315	Dihexyverine	2349*	Propantheline bromide
2315	Dihydroergotamine	2350*	Medazepam
2315	Leucinocaine	2352	Gentisic acid
2316	Promazine	2355	Mazindol
2318	Piperidolate	2355*	Methoxychlor
2318	Thenalidine	2355	Oxypertine
2320	Heptabarbital metabolite	2356	Maprotiline
2321	Phenglutarimide	2356	*o*-Toluidine
2323	Dioxadrol	2356*	Picrotoxin
2323	Proxymetacaine	2357	Ethopropazine
2325	Etoxeridine	2359	Levallorphan

TABLE 3 *(continued)*

RI	*Compound name*
2360*	Bupivacaine
2360	Methaqualone metabolite
2360*	Pyrrobutamine
2362	Butyl stearate
2363	2-Amino-2′-fluoro-5-nitrobenzophenone
2363	Dihydrocodeine
2363	Triphenyl phosphate
2363	Tris(butoxyethyl) phosphâte
2365*	Hydrocodone
2365*	Hyoscine
2365	Phenylbutazone
2366	Cyproheptadine
2366	Diethylstilboestrol
2366	Ergotamine
2368	Benzydamine
2370	Diperodon
2370	N-Allyl-3-hydroxymorphinan
2370	Noxythiolin
2375	Dextropropoxyphene metabolite
2375	Pizotifen
2376	Codeine
2377*	Azacyclonol
2377	Diethazine
2380*	Bromhexine
2380*	Colchicine
2380	Dothiepin
2380*	Hydrocodone
2380*	Hydrocodone
2380	Methyldihydromorphine
2380*	Metopon
2380*	Morphine
2380	Nifuroxime
2380	Polythiazide
2381	Di-(2-ethylhexyl) adipate
2381*	Pholcodine
2383	Cannabidiol
2383	Dioctyl adipate
2383*	Isopropamide
2384*	Buphenine
2384	Diphenidol
2385*	Bromhexine
2385	2-Cyclopropylmethylamino-5-chloro-benzophenone
2385*	Dihydroergotamine
2388	2-Amino-5-nitrobenzophenone
2388	Norcodeine
2390	2-Amino-5-chloro-3-hydroxy benzophenone
2390*	Hydrocodone
2390*	Δ^9-Tetrahydrocannabinol
2390*	Morphine
2395	Neopine
2395	Norpropoxyphene
2395*	Thebaine
2396	Pyrroliphene
2397	Dextrorphan
2397	Diphenyl adipate
2397	Diphenyl mercury
2398*	Isoxsuprine
2400*	Chlorpromazine
2400*	Chlorprothixene
2400	Methaqualone metabolite
2400*	Morphine
2400*	Phenytoin
2400*	Thebaine
2402	Hexoestrol
2402	Lorazepam
2402*	6-Monoacetylmorphine
2406	Clomipramine
2406	Levophenacylmorphan
2407	5-Chloro-2-cyclopropylmethylamino-benzophenone
2410*	Chlorpromazine
2410*	Chlorprothixene
2410*	Desmethylmedazepam
2410	Flunitrazepam decomposition product
2410	Methaqualone metabolite
2410	Sancticizer 141
2411	Ethylmorphine
2415	Azatadine
2415*	Chlorpromazine
2415	Clemastine
2415	4-Hydroxyphenobarbitone
2415*	Oxazepam
2417*	Dihydrocodeine
2417	Methoxychlor
2419	Pyrrobutamine
2420	Diloxanide
2420	Meclofenamic acid
2425	Diazepam
2425*	Oxazepam
2425*	Oxycodone
2430	Azinphos-methyl
2430	2′-Fluoro-2-methylamino-5-nitro-benzophenone
2430*	Isopropamide
2430*	Oxazepam
2430	Tetrachlorvinphos (*Z*-isomer)
2433	Propanidid
2434	Diampromide
2434	Proquamezine

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TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
2438	Normorphine	2480*	2-Amino-5,2′-dichlorobenzophenone
2440	Hydrocodone	2480	Desmethylchlorpromazine
2440*	Methotrimeprazine	2480	Didesmethylchlorpromazine
2442	Glyceryl dibenzoate	2480*	Mefenamic acid
2443	Dibenzepin	2480*	Pyrrobutamine
2443	Diphenoxalate	2480*	Temazepam
2443	Xenysalate	2480	Tetrahydrofurfuryl oleate
2444	Diisooctyl adipate	2484	Dinonyl adipate
2445	Ethynodiol diacetate	2485*	Methocarbamol
2445*	Pecazine	2485	Oxycodone
2445*	Pecazine	2485*	Phenyramidol
2445*	Methotrimeprazine	2486	Chlorpromazine
2445	Trioctyl phosphate	2486*	Phenytoin
2446	Cyclohexylisooctyl phthalate	2487	Chlorprothixene
2450*	Oxycodone	2488	Androsterone
2450*	Theophylline	2488	1-Benzyl-3-ethyl-6,7-dimethoxy-isoquinoline
2451	Dihydromorphine		
2453	Chlordiazepoxide	2488	Norpipanone
2453	Isobutylcyclohexyl phthalate	2490	Butalamine
2454	Morphine	2490*	Colchicine
2455	Acetyldihydrocodeine	2490*	Diazepam
2455	Ethylisobutrazine	2490	Methaqualone metabolite
2457	Butacaine	2490	3-Monoacetylmorphine
2457	Clorazepate	2491	Dioxaphetyl butyrate
2459	Metopon	2495	Cinnamylcocaine
2460*	Phenytoin	2495	Ergocristine
2461	Dicyclohexyl phthalate	2495	Neocinchophen
2461	Methixene	2495	Tubocurarine
2463	Tri-(2-ethylhexyl) phosphate	2496	Desmethyldiazepam
2464*	Picrotoxin	2500	Bromazepam metabolite
2464	Piperilate	2500	Diheptyl phthalate
2465	Mebhydrolin	2500*	Hydrastine
2465	Molindone	2500*	Hydrocodone
2465	Protoveratrine A	2500	N-Methyl-2,3-dihydroxymorphinan
2465	Protoveratrine B	2505*	Diamorphine
2467	Hydromorphone	2505*	Diazepam
2467	Methdilazine	2505*	Dibenzepin
2470*	Diprophylline	2505	Emetine
2470*	Hydroxyzine	2505*	Ethylmorphine
2470	Mirex	2507	Di-(2-ethylhexyl)phthalate
2470*	Temazepam	2510	Acetylcodeine
2470*	Theobromine	2510	Chlorprothiazide
2471	N1-Desalkylflurazepam	2510*	Diamorphine
2471	Oxybuprocaine	2510*	Diamorphine
2472	5-Chloro-2-fluoro-2-hydroxyethylamino-benzophenone	2510*	Diazepam
		2510*	Morphine
2472*	Norpropoxyphene amide	2511	Diisodecyl phthalate
2473	Δ^9-Tetrahydrocannabinol	2514	Methotrimeprazine
2474	Dipipanone	2515*	Cyclomethycaine
2475*	2-Amino-5-nitrobenzophenone	2515	Dioctyl phthalate
2475*	Pipethanate	2515*	Lorazepam
2478*	Lorazepam	2515*	Meclozine
2478	Tetraphenylethylene	2515*	Pyrrobutamine

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
2516	2-Amino-2′-chloro-5-nitrobenzophenone	2578	Ethoxzolamide
2517	Thebaine	2580*	Hydromorphone
2518	Cyclohexyltridecyl phthalate	2580*	Norpropoxyphene amide
2520	Butylbenzyl sebacate	2580*	Thebaine
2520	Cannabinol	2583	Cinchonine
2520	Methaqualone metabolite	2584	5-(*p*-Methylphenyl)-5-phenyl-hydantoin
2520*	Xylometazoline		
2522	Phenadoxone	2585*	Clemizole
2524	Oxycodone	2585*	Hydromorphone
2524	Pecazine	2588*	Methotrimeprazine
2525	Diisooctyl phthalate	2590	Chloroquine
2525	Methaqualone metabolite	2590*	Clemizole
2525	Oxethazaine	2590	Nifuratel
2525*	Temazepam	2590*	Trifluperidol
2529	Demoxepam	2595*	Temazepam
2530	Apomorphine	2595	Thiazesim
2530	Chlordiazepoxide	2598	Cinchonidine
2530	Loxapine	2610	Fosazepam
2532	Cyclohexylisooctyl phthalate	2612	Oestrone
2532	Oxymorphone	2612	Mestranol
2533	Thebacon	2613*	Buphenine
2535*	Flunitrazepam	2614	Diamorphine
2535	Morpheridine	2615	Deptropine
2535*	Temazepam	2615*	Trifluoperazine
2536	Pyrathiazine	2616	Synhexyl
2537	6-Monoacetylmorphine	2617	Chlorproethazine
2539	Deanol	2617	Phenacaine
2540	Octyldecyl adipate	2620	Testosterone
2542*	Chlorprothixene	2625*	Gallamine
2542	Methoxypromazine	2625	Norethisterone
2545*	Picrotoxin	2625*	Trifluoperazine
2545*	Trifluperidol	2625*	Warfarin
2545*	Trifluperidol	2630	Metoclopramide
2550	Diphenyl phthalate	2630*	Temazepam
2550	Oxyphencyclimine	2635	Metofoline
2551	Dextropropoxyphene metabolite	2637	Furethidine
2551	Norethynodrel	2638	Trimethoprim
2553*	Chlorpromazine	2640	Naloxone
2555	3-Hydroxybromazepam	2641	Prazepam
2555	Dimefline	2643	Methyltestosterone
2555*	Flurazepam	2643*	Nalorphine
2555	Pentaquin	2644*	Cinchonine
2557	Prenylamine	2645*	Clobazam
2558	5-Chloro-2-diethylaminoethylamino-2′-fluorobenzophenone	2645	Flunitrazepam
		2649	Dinonyl phthalate
2560	Methaqualone metabolite	2650	Fentanyl
2570	Benzoylecgonine	2655*	Clorazepate
2570	Methylnitrazepam	2655*	Trifluperidol
2570	Nordiazepam	2659	Oestradiol
2570*	Trifluperidol	2660*	Chlordiazepoxide
2577	Nalorphine	2660*	Chloroquine

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TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
2660*	Clobazam	2760*	Hydroxyzine
2660*	Dihydrocodeine	2760*	Hydroxyzine
2660	Laudanosine	2760*	Perazine
2660	Tetrahydrofurfuryl oleate	2762*	Bromazepam
2663	Bromazepam	2763*	Hydroxyamphetamine
2670*	Aconitine	2770*	Papaverine
2670	Phenbutrazate	2770	Pseudomorphine
2672	Methandienone	2774	Captodiame
2674*	Nitrazepam	2777*	Clobazam
2675	Clemizole	2779	Ethynodiol diacetate
2675*	Temazepam	2779*	Fentanyl
2680	Benzthiazide	2782	Dioctyl sebacate
2680*	Nalorphine	2784	Quinidine
2683	Trifluoperazine	2785	Flurazepam
2684	Phenazocine	2792	Di-(2-ethylhexyl) sebacate
2685*	Chlorpromazine	2793	Progesterone
2685	Indomethacin	2797*	Trifluperidol
2685	Methiomeprazine	2799	Chlordiazepoxide
2686	Broxaldine	2800	Ethoxyquin
2688	N1-Hydroxyethylflurazepam	2800	Propionylpromazine
2690	Dibenzyl phthalate	2803	Quinine
2690*	Nitrazepam	2807	Hexachlorophane
2692*	Diamorphine	2810*	Haloperidol
2694	Acepromazine	2810	Hydroquinidine
2695	Benzethidine	2815*	Hordenine
2695	Laudanine	2815*	Norclobazam
2695*	Norclobazam	2820	Bisacodyl
2695	Tricresyl phosphate	2820	Chromonar
2700	Di(butoxyethyl) sebacate	2825	7-Aminonorflunitrazepam
2700	Griseofulvin	2825	Papaverine
2701	Cinchocaine	2828	7-Aminonitrazepam
2703	5-Amino-2′-fluoro-2-methylamino-benzophenone	2830	Fenethylline
		2830*	Nitrazepam
2705	Ajmaline	2832*	Sulpiride
2705	Azaperone	2835*	Haloperidol
2710*	Prazepam	2835	Oxymetholone
2715*	Prazepam	2845*	Theobromine
2719	Ethinyloestradiol	2849	Hydroxyzine
2720*	Fentanyl	2850*	Acepromazine
2723	7-Aminoflunitrazepam	2850	Anileridine
2725	Oxomemazine	2850	Clonazepam metabolite
2730	Di(2-ethylhexyl) isophthalate	2850	Di(butoxyethyl) phthalate
2730	Phenazone metabolite	2850	Gelsemine
2738	Propiomazine	2852	5-α-Cholestane
2740	Norflunitrazepam	2858*	Flurazepam
2745	BBOT	2860	3-Hydroxyprazepam
2745	Diisodecyl adipate	2872	Hydroxychloroquine
2745	Octyldecyl adipate	2872	Ibogaine
2750	Calusterone	2872	Phenoperidine
2750*	Hydroxyzine	2880	Dapsone
2750	Nitrazepam	2880	Sanguinarine
2760*	Clorazepate	2885	Clonazepam
2760*	Hydroxyzine	2885*	Desmethylchlordiazepoxide

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
2885*	Piminodine	3086	Cholesterol
2890	7-Amino-3-hydroxyclonazepam	3089	Methysergide
2893*	Chlordiazepoxide	3100	Desmethylchlordiazepoxide
2895	Dioxyline	3103	Isooctylhydrocupreine
2900	7-Aminoclonazepam	3105	Clonazepam metabolite
2900*	Papaverine	3107	Triamcinolone
2905	Di(*n*-decyl) adipate	3114	Thioridazine
2905*	Haloperidol	3115	7-Acetamidoflunitrazepam
2906	Doxapram	3119	Strychnine
2913*	Nitrazepam	3120	Noscapine
2915*	Clozapine	3120*	Prochlorperazine
2925*	Prajmalium bitartrate	3149*	Haloperidol
2930	Clomiphene	3170*	Pholcodine
2930*	Desmethylchlordiazepoxide	3175	Etodroxizine
2930*	Perazine	3176*	Thioridazine
2930	Racemoramide	3180*	Thioridazine
2934	Diphenadione	3193	Sitosterol
2940	Dextromoramide	3202	Levophenacylmorphan
2940	Octyldecyl adipate	3205	7-Acetamidonitrazepam
2949*	Piminodine	3230*	Pericyazine
2954	Prochlorperazine	3234	Stigmasterol
2965*	Clonazepam	3240*	Noscapine
2965*	Haloperidol	3242	Rotenone
2970	Dexamethasone	3247	Thiethylperazine
2970	Oestriol	3260	Pipamazine
2970	Triamcinolone	3263	7-Acetamidoclonazepam
2972*	Cyclomethycaine	3269	Yohimbine
2975*	Benperidol	3280	Brucine
2980*	Haloperidol	3280	Spironolactone
2980*	Hydroxyzine	3280*	Strychnine
2980	Levomoramide	3286	Buclizine
2980	Miconazole	3287	Phytomenadione
2988	Hydrastine	3287*	Trimethobenzamide
3005*	Pholcodine	3327	Nicodicodine
3008*	Cholesterol	3340*	Colchicine
3011*	Clozapine	3340*	Pericyazine
3014*	Doxapram	3342	Butyl PBD
3015	Benzylmorphine	3365*	Buclizine
3020*	Cholesterol	3380	Penfluridol
3020*	Haloperidol	3408	Nicocodine
3020*	Strychnine	3430*	Droperidol
3030*	Pholcodine	3445	Lysergide
3033	Meclozine	3465	Thiopropazate
3040*	Strychnine	3466*	Colchicine
3059	Famprofazone	3525	POPOP
3060*	Strychnine	3560*	Brucine
3060	Thiothixene	3590	Carphenazine
3065	Cinnarizine	3618	Dimethyl POPOP
3065	Fluphenazine	3710	BBO
3070	Pipamperone	3750	BBOT
3078	Dimethothiazine	9999	Acetophenazine

(Continued on p. 250)

TABLE 3 *(continued)*

RI	*Compound name*	*RI*	*Compound name*
9999*	Aconitine	9999	Methallibure
9999	Amidephrine	9999	Methyclothiazide
9999	Aminonitrothiazole	9999	Methylergometrine
9999	Aminotriazole	9999	Morantel
9999	Amodiaquine	9999*	Nialamide
9999	Amotriphene	9999	Nicergoline
9999	Benzonatate	9999	Nifursol
9999	Brocresine	9999	Obidoxime bromide
9999	Bunamidine	9999	Orciprenaline
9999	Chloral hydrate	9999	Oxyclozanide
9999*	Chlorothiazide	9999*	Oxyphenbutazone
9999	Cinchophen	9999*	Paramethadione
9999	Clioxanide	9999	Parbendazole
9999	Clofazimine	9999	Pentapiperide
9999	Coniine	9999	Phentolamine
9999	Debrisoquine	9999*	Phenylephrine
9999	Decoquinate	9999	Phthivazid
9999	Diazoxide	9999	Picloxydine
9999*	Droperidol	9999	Pimozide
9999	Ecgonine	9999	Piperacetazine
9999	Ethacrynic acid	9999	Practolol
9999	Ethoxazene	9999*	Prajmalium bitartrate
9999*	Etilefrine	9999	Propyliodone
9999	Flavoxate	9999	Pyrantel
9999	Flupenthixol	9999	Rafoxanide
9999*	Galamine	9999	Reserpine
9999	Gitalin	9999	Resorantel
9999	Guanethidine	9999	Ritodrine
9999	Hexobendine	9999	Rolicypram
9999	Hydrochlorothiazide	9999	Succinylcholine chloride
9999*	Ibogaine	9999	Sulphaguanidine
9999	Imidocarb	9999	Sulphamoxole
9999	Isometamidium	9999	Sulphathiazole
9999	Lidoflazine	9999	Symazine
9999	Mafenide	9999	Taurolin
9999	Mebeverine	9999	Thioproperazine
9999	Mesoridazine	9999*	Trimethobenzamide
9999	Metaraminol	9999	Urea
9999	Metformin		

cending order of retention index. The entries include not only the mean retention index, but also each of the values which were greater than ± 50 retention index units from the mean, these being indicated by an asterisk. Table 3 can therefore be used to obtain a tentative identification of an unknown compound before referring to Table 1 for more complete data.

The data in Table 3, excluding the 9999 entries, have been used to generate the histogram in Fig. 1. Thus, Fig. 1 may help in determining the usefulness of the retention index measurement for an unknown compound for identification purposes, *e.g.* a value of around 2200 is much less useful than one of around 3500.

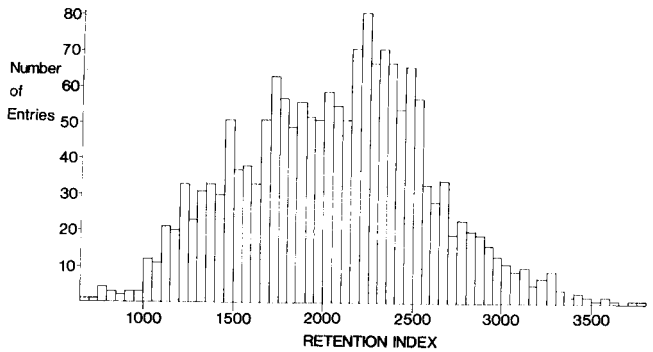

Fig. 1. Histogram of 1742 retention index values associated with 1318 compounds.

4. CONCLUSIONS

It is hoped that this compilation will enable those involved with the analysis of drugs to identify more easily unknown compounds when using SE-30 or OV-1 stationary phases. Table 1 may be used to obtain the retention index of a compound together with an estimate of its reproducibility and Table 3 can be used with Fig. 1 to help identify an unknown compound. The retention indices quoted in this work may be easily converted to retention times or relative retention times, if they are considered more appropriate to particular needs, by the use of appropriate calibration graphs. Analysts' collections of retention parameters may therefore be supplemented and checked for unusual results regardless of the retention parameter used.

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6. SUMMARY

Retention indices associated with 1318 substances likely to be encountered in toxicological analyses are presented. They are listed in ascending order of retention index for identification purposes and also in alphabetical order of compound name. The 4586 values used in this collection have been extracted from 36 sources, many of which have not been previously published. In many cases, where the quoted retention index is the mean of several determinations, the reproducibility and reliability of this value may be assessed.

A histogram of the 1742 values listed is provided to help in determining the usefulness of a retention index for identification purposes.

The reproducibility of inter-laboratory retention index measurements for twenty compounds on both SE-30 and OV-17 are presented and show the former, on average, to give more reproducible results.

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ANALYSIS OF BARBITURATES BY GAS CHROMATOGRAPHY

DILRANJAN N. PILLAI
Department of Clinical Chemistry, Prince Henry Hospital, Sydney, N.S.W. (Australia)
and
SERGIO DILLI*
Department of Analytical Chemistry, School of Chemistry, University of N.S.W., P.O. Box 1, Kensington, N.S.W. (Australia)
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1. INTRODUCTION

Barbiturates are derivatives of malonyl urea, formed by replacement of both hydrogen atoms on the carbon at position 5 by alkyl, aryl or alicyclic groups. The synthesis of barbituric acid (or malonyl urea) [2,4,6-(1H,3H,5H)pyrimidinetrione] was reported by Von Baeyer[1] as early as 1864, but it was only the subsequent discovery of the hypnotic properties of barbital, by Fischer and Von Mering[2] in 1903, which led to the extensive development of the barbituric acid class of drugs. They are most frequently used as sedative hypnotics and anticonvulsants but can also be employed intravenously to effect surgical anaesthesia. Their action on the central nervous system (CNS), and the extent of CNS depression, is dependent on the particular barbiturate. Although variations in the pharmacological properties of the various barbiturates depend on the nature of the 5-substituted entities, the chemical groups of major importance are the imino hydrogens. A definitive publication by Doran[3] in 1959, appears to be the only monograph providing useful information including details of syntheses, chemical and physical properties, reactions, as well as pharmacology.

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Despite the numerous publications that describe the gas chromatography (GC) of barbiturates, there are few major reviews devoted solely to the subject. The literature to 1966 has been reviewed by Brochmann-Hanssen[4], otherwise the subject appears to have been treated only in reviews on general methods[5–7] or in applications of GC in toxicology[8]. The use of paper chromatography, thin-layer chromatography (TLC) and GC in barbiturate analysis have been reviewed by Melzacka[5] in 1971, and an assessment of methods available in 1972 was made by Kananen *et al.*[6]. Selected methods for the screening and identification of barbiturates from biological fluids were again reviewed by Jain and Cravey in 1974[7].

Apart from GC, numerous other methods for the determination of barbiturates include ultraviolet (UV)[9], infrared (IR)[10], nuclear magnetic resonance (NMR)[11,12] and mass spectroscopy (MS)[13,14]; TLC[15,16] and high-pressure liquid chromatography[17], as well as enzyme-[18] and radioimmunoassay[19,20] methods. This variety implies as many different types of problems and shows an historical development also.

In this review, an attempt has been made to comprehensively survey the advances in the analytical chemistry of the barbiturates studied by the GC technique.

2. ANALYSIS OF FREE BARBITURIC ACIDS

GC methods for the analysis of barbiturates were first reported in 1960 by Janak[21]. His method involved heating the sample to 800°C, followed by chromatographic separation of the pyrolytic products. The characteristic pyrolysis pattern facilitated quantification as well as identification. In 1962, a similar procedure[22] gave unique patterns for 22 barbiturates in which the most significant pyrolytic products were identified as nitriles. While these methods appeared to be satisfactory for the analysis of single barbiturates, pyrolysis of mixtures were not considered and would probably have given complicated patterns incapable of resolution. The lack of further reports on pyrolysis methods for barbiturates is indicative of their limited usefulness.

Significant early work on the GC of barbiturates in biological fluids, was done by Baerheim Svendsen and Brochmann-Hanssen[23], Parker and co-workers[24,25] and Anders[26], and the use of two columns was commonly recommended[23,27,28] for barbiturate mixtures which could not be separated on a single column. Of particular relevance are the problems with tailing and adsorption noted in much of the early work. To overcome these, Cieplinski[29] incorporated high-molecular-weight organic acids into the stationary phase to neutralize active sites in the column and reduce peak tailing. McMartin and Street[30,31] obtained similar results with tristearin.

After Bohemen *et al.*[32] showed that absorptive inertness was conferred on diatomaceous-earth supports by silylation, this technique found wide application in the GC of barbiturates. Another approach introduced to minimise adsorptive losses was the saturation of the active sites of the column by the injection of large amounts of barbituric acids[26,33–36] onto the column. However, deactivation here was probably only temporary due to slow elution of the barbiturate from the column, causing re-exposure of the active sites. Predictably, variations in the retention times of barbituric acids were observed in cases where adsorption was suspected[33,34,37].

Studies in 1963 showed that addition of formic acid vapour to the carrier gas, improved the chromatographic properties of several fatty acids[38]. This technique when

first applied to the barbiturates in 1970[39], improved the resolution of 6 barbiturates on an Apiezon L column, yielding good peak symmetry from nanogram quantities. Ioannides *et al.*[40] explained the improvement by postulating that adsorption could result from hydrogen bonding of the imino-protons of a barbiturate with Si–OH and Si–O–Si groups in the diatomite support. The former groups, of course, were deactivated by silanization but the latter acted as proton acceptors to form hydrogen bonds with barbiturates, as had been demonstrated for barbiturates with adenine derivatives[41]. Because of its strong tendency to form hydrogen bonds, it was postulated that formic acid occupied all Si–O–Si sites, thereby preventing adsorption of the barbiturates.

In an application of this technique to the analysis of pharmaceuticals, Greenwood *et al.*[42] demonstrated the on-column liberation of the free acids following direct injection of barbiturates as their sodium salts. Barbiturates extracted from blood[43,44] were well resolved on SE-30 columns with such a system, despite the fact that decreased column life and increased noise were noted[45]. Although several methods for the saturation of carrier gas with formic acid are known, Woo and Lindsay[46] have recently described a simple device, claimed to be safe and effective, for barbiturate and fatty acid analysis.

The use of a wide variety of stationary phases are a feature of the literature on the GC of barbiturates. For example, in an attempt to identify a *single* column capable of specific and reliable identification, a critical examination of 12 different columns was made by Berry[47] who found a moderately polar 4% CDMS column most satisfactory, with 3% OV-225 as the second choice. Mixed liquid phases in columns, have been investigated too in attempts to optimize the separation of barbiturate mixtures. Phases investigated were SE-30–Carbowax[48,49], Apiezon L–NPGA[50], SE-30–XE-60[51] and Apiezon L–SE-30–Tristearin[43]. Of these, the last appeared to provide the best resolution.

Solid injection techniques which produce solvent-free chromatograms have useful application in the GC of barbiturates[52–55]. Optimal conditions for their determination were investigated by Rasmussen *et al.*[52] who found no difference in analytical precision with either liquid or solid injection at flash-heater temperatures over 230°C and injection times of 30 sec. Micropacked[45] and support-coated, open-tubular columns[56] in conjunction with solid injection, have also been used. For the latter procedure, columns with high plate numbers, sensitivity of the order of 10^{-10} g, and high precision were claimed.

Many methods have been proposed for the determination of barbiturates in the presence of other drugs[57–64] and specific procedures for the estimation of barbiturates in pharmaceutical preparations also exist[65–67]. Progressively, with increases in the sensitivity of GC methods, pharmacokinetic studies became possible and levels of amobarbital[68–74], pentobarbital[73,75,76] and phenobarbital[77] were determined in both humans and animals. In most cases, pharmacokinetic parameters were evaluated from concentration–time plots. In another case, the primary metabolite of amobarbital, 3′-hydroxyamobarbital was estimated in blood[54,69,78] and urine[68,69,74,78]. Here, use of the polar phase FFAP permitted the determination of 2 μg of this metabolite in either plasma or urine[78]. More recently, the use of an even more polar phase WG11 for estimation of 3′-hydroxyamobarbital, was reported by Kinsella *et al.*[74]. However, Garrett *et al.*[70] in a detailed study of the pharmacokinetics of amobarbital,

pre-saturated the cyanoalkylsilicone stationary phase (GE-XE-60) with repetitive injections of the metabolite to obtain high precision analysis at the 0.1 μg/ml level, in plasma. In contrast to the use of these polar phases, the principal metabolite of hexobarbital, [3,5-dimethyl-5-(3-oxocyclohexenyl)barbituric acid] has been determined in 0.5 ml of blood with 10% UCC W-982 in a stainless-steel column[79].

Following the advent of the nitrogen-specific detector[80], which exhibits a reduced response to many co-extractable interferents in serum, determinations have been reported of nanogram amounts of thiopental[81–84], pentobarbital[83] and hexobarbital[85] in blood, and trace amounts of phenobarbital in plasma[86] and brain tissue[87]. However, adsorption of barbituric acids by the column appear to limit a further lowering of detection limits. Thus, Dvorchik[88] found that at least 10 ng of barbituric acid had to be injected onto the column before adsorption effects were negligible.

Perhaps the most novel application[89,90] to barbiturate analysis is that of the electrolytic conductivity detector. Up to 0.1 μg/ml of barbiturate in serum or urine was determined without sample clean-up, although column resolution deteriorated gradually with injection of direct extracts.

3. PRIOR DERIVATIZATION OF BARBITURIC ACIDS

Despite increases in sensitivity and selectivity made possible by improvements in column technology and detector specificity, GC methods involving underivatized barbiturates are clearly limited by column adsorption. Since 1975, the authors of over 75% of important publications on the GC of barbiturates, have utilised derivatization procedures prior to analysis.

During the GC separation of many drugs, compounds capable of hydrogen bonding appear to adsorb strongly and, for the barbituric acids adsorption of submicrogram quantities on chromatographic columns is common. The consequences of adsorption are the loss of material, column contamination and unsatisfactory peak profiles, with tailing increasing in severity as sample size or concentration is reduced.

Reduction in polarity of the free acid has been the primary objective in the derivatization of barbituric acids and is virtually limited to alkylation. These derivatives are far less polar than the free acids due to conversion from secondary to tertiary amides so that stationary phases suitable for derivatized barbiturates are also generally less polar than those employed for the free acids. SE-30 and OV-17 now appear to be the phases of choice.

3.1 Methyl derivatives

The GC of barbiturate derivatives was first reported by Cook *et al.*[91] in 1961. Here, overnight methylation with diazomethane was followed by chromatographic separation of 11 barbiturates as their 1,3-dimethyl derivatives. Unfortunately, inadequate resolution of mixtures of barbiturate derivatives still necessitated the use of two columns. Later, in 1966, Stuckey's method[92] for alkylation with dimethyl sulphate was adapted by Martin and Driscoll[93] for the microscale methylation of several barbiturates. In this method, the free acid extracted from 2 ml of serum was heated briefly with the alkylating agent, then after acidification and reextraction, the extract was chromatographed. Another method requiring only 15 min was reported by Stewart *et al.*[94] who subjected the barbituric acids in serum or biological tissue to

direct methylation. Good recoveries of phenobarbital (78–98 %)[95] and other barbiturates (96–104 %)[96] were also reported for methylations with dimethyl sulphate. Subsequently, successful use of this reagent for derivatization purposes has been described by other workers[97–99].

Since the amide functionality of the barbiturate pyrimidinetrione ring can tautomerise to the lactim form, methylation can result in the formation of either N-methylated and/or O-methylated derivatives. N,O′-methylated derivatives are also feasible. Although NMR studies by Neville[100] indicated that N-methylation was exclusive with dimethyl sulphate, and predominant with diazomethane, evidence exists for formation of small amounts of N,O′-dimethyl[101] and N,O′-diethyl derivatives[102,103] when barbiturates are alkylated by the respective dialkyl sulphate.

The Claisen synthesis of allyl-phenyl ethers was adapted by Dünges and Bergheim-Irps[104] in 1973 for the methylation of barbiturates. This was achieved by refluxing an acetone solution of the barbituric acid with the alkylating agent (methyl iodide) and a condensing agent (potassium carbonate) and resulted in a yield of 98 % ± 6 % (standard deviation, SD). The procedure was later extended by Dünges, to alkylations involving ethyl, allyl, methoxymethyl and benzyl derivatives[105,106]. Features of this technique were the direct injection of the reaction mixture into the GC and a micro-refluxer for handling microlitre[106,107] or millilitre[101] amounts of reactants. During the methylation of barbiturates with alkaline methyl iodide, Wu and Pearson[108] found, in a variation of this procedure, that improved reaction rates were obtained with a mixed solvent system of acetone–methanol than with acetone alone. The improvement was attributed to the enhanced polarity of the mixed solvents. Recently, Dünges *et al.*[109] reported the determination of several barbiturates as the allyl, alkyl or benzyl derivatives with glass capillary columns, obtaining good resolution after extraction from blood. Also in 1979, Sun and Hoffman[110] utilised the method of Dünges and Bergheim-Irps[104] to estimate several barbiturates in serum, using nitrogen-specific detection to successfully improve selection and sensitivity.

Methylation of hydroxylated barbiturate metabolites results in alkylation of the imino protons but not necessarily of the hydroxyl group attached to substituents at C-5. To avoid confusion over the identity of the products when barbiturate metabolites were alkylated, Horning *et al.*[111] silylated the hydroxyl group *after* the methylation procedure. The derivatized products were presumably, identical to those identified in later publications from the same laboratory[112,113]. Here, the methylation-silylation procedures resulted in conversion of the imino protons of the barbituric acids and their metabolites, to N-methylated groups. Additionally, any aromatic hydroxyl groups formed a mixture of methyl and trimethylsilyl ethers whereas non-aromatic hydroxyl groups were converted to trimethylsilyl ethers. Identification of these products was made by GC–MS and the technique utilised for the detection of the epoxide metabolites of some barbiturates in rat urine.

Methylation of barbituric acids by extractive alkylation was first reported by Ehrsson[114]. In this reaction, pentobarbital and phenobarbital were extracted as ion pairs from an aqueous phase into an organic phase having a weak solvating capability, resulting in enhanced susceptibility of the barbiturates to the nucleophilic displacement reaction with methyl iodide.

3.2 Other alkyl derivatives

Since the technique of methylation was incapable of distinguishing between mephobarbital and phenobarbital because the methylated products were identical, it is not surprising that to overcome this limitation, and also improve the resolution of other barbiturates, derivatization to form higher alkyl homologues was examined. Several different reactions were employed to achieve derivatization.

Extractive alkylation with ethyl iodide and tetrabutylammonium hydrogen sulphate was employed for the formation of ethylated barbiturate derivatives[115]. Here, satisfactory separation of 15 ethylated barbiturates on an SE-30 support-coated, open-tubular column was reported, although mephobarbital was not included. The Claisen type reaction for preparation of propyl derivatives was described by IJdenberg[116] who reported successful resolution of mephobarbital and phenobarbital, as well as several other anticonvulsants, on a 3.8% SE-30 column which was temperature-programmed. Propylation was effected by heating for 1 h in a sealed tube containing nitrogen.

Butylation of several barbiturates was reported by Greeley[117] in 1974. Derivatization depended on formation of a soluble tetramethylammonium salt of the barbiturate in a highly polar solvent system, followed by a fast S_N2 reaction of the anion of the salt with iodobutane. Separation of 14 barbiturates was obtained, although overlap with some uncommon barbiturates occurred. More recently in 1979, the butylation of several barbiturates, amongst other drugs, was described by Roseboom and Hulshoff[118]. After extraction from acidified plasma and back extraction into tetramethylammonium hydroxide, the drugs were reacted with N,N′-dimethylacetamide and *n*-butyliodide prior to GC. Mephobarbital, phenobarbital and heptabarbital were satisfactorily resolved from each other on a 3% OV-17 column.

Menez *et al.*[119] made a systematic study of the GC behaviour of several barbiturates after N-alkylation with straight-chain alkyl groups from C_1 to C_6, using the technique of Greeley[117]. GC on OV-101, Dexsil 300 GC, SP-2250 and OV-7 columns showed that the smallest change in retention time was observed between methyl and ethyl derivatives, so that separation of methylated and ethylated barbiturates was not always achieved. Propylated derivatives were considered to exhibit the most desirable chromatographic properties and optimum separation was obtained by temperature programming the Dexsil 300 GC column at 4°C/min, after an initial pause at 140°C for 15 min.

The use of dimethylformamide dimethylacetal for the derivatization of barbiturates has also been investigated[120]. Decomposition of this reagent during the reaction with barbiturates, results in the formation of both CH_3^+ and OCH_3^- species and, thus, either N-methylation or acetal-formation is possible, depending on whether carbonyl polarization is preferred to proton abstraction. In fact, acetal-formation was predominant, and quantitative recoveries of several barbiturates was reported.

An attempt to permethylate barbiturates with methyl iodide and the methylsulphinylmethide carbanion, resulted in the formation of mixtures of three permethylated products for each barbiturate[121]. Efforts to obtain only one derivative for each barbiturate were unsuccessful with the exception of secobarbital. It was concluded that the derivatization, later shown to be useful for estimation of polar glucuronide metabolites[122], was of limited value for analysis of free barbituric acids.

3.3 Electron-capture detection of barbiturates

Although the response of the electron-capture detector (ECD) to free barbituric acids was examined as early as 1965[33], derivatization with a suitable electrophore was only reported recently. Pentafluorobenzyl bromide (PFBB) was employed by Walle[123] in 1975 to alkylate several barbituric acids in which triethylamine was used as the base catalyst in preference to potassium carbonate, since the latter caused hydrolysis of the barbituric acids. Response at picogram levels was obtained and, despite a large increase in molecular weight upon derivatizaton, only a 3 to 4-fold increase in retention times (ranging from 5 min for barbital to 21 min for phenobarbital) was observed on 3% OV-17 at 210°C.

Pentafluorobenzylation of a barbiturate extracted from a biological matrix was first accomplished by Gyllenhaal *et al.*[124]. Here, extractive alkylation with tetrabutylammonium ion and PFBB enabled the determination of 60 ng of phenobarbital in 100 μl of saliva to be made, with a precision of 1.9% (S.D.), after a recovery of 93%. However, the procedure required a pre-column venting system for the removal of excess PFBB from the column to avoid the pronounced detector response which would otherwise make quantification impossible. This method may also be unsuitable for barbiturates with retention times smaller than that of phenobarbital, due to two large unidentified peaks seen in the chromatogram of the extracted saliva sample. This limitation would exclude most barbiturates.

Pentafluorobenzylation of pentobarbital prior to EC detection has been reported by Sun and Chun[125]. The barbiturate extracted from serum was reacted with PFBB and sodium carbonate without apparent interference from excess reagent or from interfering peaks. However, the extraction procedure was time consuming (1 h) and prolonged heating of the reaction mixture (4 h) was required. In addition, further washing and concentration steps were necessary prior to GC. Values for the recovery in the derivatization were not given.

Dilli and Pillai[126] recently described the chloroethylation of several barbiturates, prior to electron-capture detection. After quantitative extraction from saliva, the barbiturate was reacted with triethylamine and bis(chloroethyl) sulphate. Chromatography was effected after washing and concentration steps, the entire procedure taking 2 h for duplicate samples of saliva. Amobarbital, pentobarbital and phenobarbital were determined at levels of 0.10–1.0 μg/ml in saliva. A pharmacokinetic study also enabled the estimation of the *in vivo* biological half lives of amobarbital and pentobarbital to be made.

4. ON-COLUMN DERIVATIZATION OF BARBITURIC ACIDS

The on-column derivatization technique involving *in situ* formation of derivatives in the injection port of the gas chromatograph, was established principally by Robb and Westbrook[127]. The technique is considered by many to be the method of choice for routine analysis of barbiturates and related drugs, due to its rapidity and simplicity.

4.1 Trimethylsilyl derivatives

The estimation of 3′-hydroxyamobarbital by on-column silylation with TMCS and HMDS, was reported by Kamm and Van Loon[128] as early as 1966. Extracted

from urine, the metabolite was converted to a "silyl ether" whose structure was not further specified. In 1969, several barbiturates were derivatized by Street[129] with BSA. Again, the resultant structures were unspecified, although, it was postulated that the barbiturates were monosilylated, at either of the nitrogen atoms. In 1971, 3′-hydroxyamobarbital, extracted from rat-liver homogenate, was silylated with BSTFA and TMCS[130]. Here, GC–MS studies showed a peak at *m/e* 458, indicating formation of the tris(trimethylsilyl)-derivative. It was observed[78,132], however, that the relative instability of N-trimethylsilylated barbiturates caused unspecified interference during GC. Variations in the recoveries of silylated barbiturates led Street[131] to recommend trimethylsilylation for qualitative purposes only.

4.2 Methyl derivatives

The on-column methylation of barbituric acids with tetramethylammonium hydroxide (TMAH), was first attempted by Stevenson[133] who injected solutions of the barbituric acids in methanolic TMAH onto a temperature-programmed 5% SE-30 column. Most of the 18 barbiturates investigated, were adequately resolved, however, the presence of an "early peak", with retention time smaller than that of the N,N′-dimethyl derivative, was observed for barbiturates with a phenyl substituent at C-5. Similar results were also observed by Parker *et al.*[134]. With other barbiturates, the appearance of multiple peaks has also been noted[132,135] during on-column alkylation with TMAH. Pippenger and Kutt[136] observed barbiturate decomposition by the alkaline TMAH reagent, even at room temperature. Despite these observations, TMAH has been widely used for derivatization of phenobarbital[137–141] and secondary peak formation has either been absent or, if present, been ignored.

In earlier efforts to find an alternative alkylating agent, Brochmann-Hanssen and Oke[132] noted that a quaternary ammonium base producing a better leaving group than trimethylamine was desirable so that shorter reaction times, and milder reaction conditions conducive to thermal stability, could be used. Such a base, trimethylphenylammonium hydroxide (TMPAH), was claimed to be superior to TMAH.

Quantitative studies on the methylation of barbiturates with TMPAH were first conducted in 1970 to determine[131,142,143] therapeutic amounts of phenobarbital in plasma. On-column methylation with TMPAH was extended to other barbiturates[6,144] in 1972. For these studies, TMPAH was prepared by reaction of trimethylphenylammonium iodide[132,142–144] with silver oxide[6]. Fortunately, during 1973 TMPAH became available commercially, and its time-consuming synthesis was then unnecessary. Now it is probably the most widely used alkylating agent for the determination of barbiturates and anticonvulsant drugs[108,145–175] although difficulties have also been encountered with this reagent. An early study of the degradation of barbiturates showed their decomposition by both TMPAH and TMAH, however, degradation of phenobarbital with TMPAH was not as rapid as with TMAH[136]. It may be noted that of the common barbiturates, it is the least stable to aqueous alkali at room temperature[176]. Subsequent studies of barbiturate degradation with TMPAH have been confined to phenobarbital because of its extensive use as an anticonvulsant.

On-column methylation of phenobarbital with TMPAH results primarily in the formation of the N,N′-dimethylated compound[132], however, an additional peak with a much shorter retention time has also been reported for alkylations with

TMPAH[6,146,149,177] and TMAH[133,140]. The compound responsible was termed "early phenobarbital"[6] and, on the basis of retention times[177], was thought to be 2-ethyl-2-phenylmalondiamide[136], until GC–MS studies by Wu[178] established that the compound in question was actually N-methyl-2-phenylbutyramide (MPB). This was confirmed by Osiewicz *et al.*[151] following synthesis and chemical ionization MS studies of MPB.

There has been considerable interest in the mechanism of the high-temperature reaction of phenobarbital with TMPAH, in the injection port. This originates from the suggestion of Kelly *et al.*[165] that the monomethylated derivative, formed by reaction of phenobarbital with TMPAH, was the principal precursor of MPB. Their proposal was supported by the observation that N-methylphenobarbital was dramatically more prone to ring cleavage than was phenobarbital, when subjected to alkaline hydrolysis[179]. In addition, several steps in the suggested pathway were similar to known decomposition reactions of barbiturates or structurally related compounds. At about the same time, Callery and Leslie[180,181] concluded that MPB was produced during the extensive degradation of N,N′-dimethyl phenobarbital by TMPAH in the injection port. These studies indicated that MPB formation occurred from decomposition of either the monomethyl or dimethyl derivatives of phenobarbital. More recently, Kurata *et al.*[174] reported that MPB formation occurred from the injection-port hydrolysis of phenobarbital itself and was caused by water in the sample or reagents. Thus, with this uncertainty it appears that final clarification of the mechanism of MPB formation must still await further studies.

Several approaches to the problems caused by alkaline degradation of barbiturates by TMPAH include the estimation of phenobarbital by measurement of the degradation products of the on-column reaction. Thus, Perchalski *et al.*[146] determined phenobarbital using the combined peak areas of N,N′-dimethylphenobarbital and two decomposition products. Again, Osiewicz *et al.*[151], using a high concentration of TMPAH, showed that the amount of MPB formed was a reproducible, linear function of the amount of phenobarbital injected onto the column. This method was, however, not entirely satisfactory due to the close proximity of the MPB and solvent peaks and the requirement that the extract be slowly and reproducibly injected to obtain reliable results. Surprisingly, the claim that the decomposition product was a reproducible measure of the phenobarbital present, could not be substantiated by Serfontein and De Villiers[168]. Despite this, Kurata *et al.*[174] recently proposed that the sum of the methylated phenobarbital and MPB was an accurate measure of the amount of phenobarbital present.

In another approach aimed at reducing the decomposition product MPB, reduction or elimination of the interfering peak was reported when a solution of TMPAH was neutralised with buffer, prior to on-column alkylation[132]. Here, back extraction of the drug with an aqueous solution of the reagent, was followed by adjustment to pH 8–10, prior to GC. The idea of reducing the alkalinity was further developed by Mraz and Sedivec[182,183] who used a neutral quaternary ammonium salt (trimethylphenylammonium acetate) as the on-column alkylating agent, and obtained only peaks of N,N′-dimethyl derivatives. Similar results were produced with tetramethylammonium acetate. Important here was the fact that the reaction was unaffected by variations in injection port temperatures (200–300°C), or by excess alkylating agent (5–500-fold excess), but no estimate of percentage conversion was indicated. A fur-

ther refinement of this procedure is illustrated by the work of Vincent *et al.*[175] who recently described on-column methylation of 11 barbiturates with TMPAH. Here, degradation was avoided by using a dilute solution of TMPAH (0.2 *M*) and allowing minimal contact of barbiturate with TMPAH before GC. Thus, to achieve this, TMPAH and internal standards were drawn into the syringe *before* the barbiturate (in carbon disulphide) and the contents immediately injected onto the column.

The role of the solvent in the injection-port degradation of phenobarbital in TMPAH was investigated by Kelly *et al.*[165] who found that MPB interference was inhibited by viscous polyhydric alcohols whereas certain aprotic solvents appeared to promote the decomposition reaction. In the former, an inhibitory effect exerted by the solvent on the activity of the hydroxide ion appears responsible. The formation of anisole as a by-product in on-column methylations involving TMPAH is also known[184], due possibly as the result of nucleophilic attack by the solvent on the strongly alkaline TMPAH reagent in the injection port.

In yet another approach, several authors[164,185,186] have initiated the methylation reaction by pre-heating the reaction mixture at 85–100°C for 5–10 min prior to GC. Reproducible results, with no interfering peaks, were claimed with these methods which are not strictly on-column methods. However, prolonged contact (>10 min) between phenobarbital and TMPAH can result in decomposition of phenobarbital[156].

Finally, reference is made to a report by Wong *et al.*[158] of the presence of an endogenous methylating agent in serum. It was observed that, whereas urine from phenobarbital-treated patients usually contained only phenobarbital, corresponding serum samples extracted at pH 7 with dichloromethane invariably contained small amounts of N-methylphenobarbital. After ruling out the possibility of *in vivo* methylation, lecithin was implicated in the thermally-induced methylation of phenobarbital in the injection port. A deuterated analogue of TMPAH was also recommended for quantification of phenobarbital and mephobarbital in the serum of patients prescribed both drugs, since patients[187] receiving mephobarbital have higher plasma levels of phenobarbital than the parent drug.

4.3 Other alkyl derivatives

MacGee[135] first reported the on-column ethylation of barbiturates with tetraethylammonium hydroxide. No interfering peaks were observed, and separation of mephobarbital from phenobarbital was obtained on 0.05% OV-101. A slow injection technique (10 sec) appeared to markedly reduce tailing of the solvent peak, however, the high injection-port temperature of 360°C may have been responsible for column bleeding and contributed to loss of resolution observed after prolonged use. Using this procedure poor separation of mephobarbital and phenobarbital was obtained on conventional 3% SE-30 or 2.5% OV-17 columns[188].

Ethylation with tetraethylphenylammonium hydroxide has been reported[189] and although phenobarbital was successfully determined, this reagent was unsuitable for quantification of mephobarbital because of the high level of transethylation of the latter (*ca.* 20%) to form N,N′-diethylphenobarbital. Again, separation of the ethyl derivative of phenobarbital and mephobarbital on 3% OV-1 was very poor, but better reproducibility was achieved with a rapid injection technique, in contrast to the slow injection method of MacGee[135].

On-column butylation with tetrabutylammonium hydroxide[190] resulted in a difference of 2 min in the retention times of phenobarbital and mephobarbital on 3% OV-17. Although secondary peaks were absent, solvent peak tailing was far more pronounced when compared to an on-column methylation procedure with TMPAH. Degradation during on-column butylation was observed by Hooper *et al.*[191] with mephobarbital and phenobarbital, each giving two peaks when injected with tetrabutylammonium hydroxide. These barbiturates were quantified only after selection of chromatographic conditions led to elution of the interfering peak at short retention time, with the solvent.

A comparison was made recently between a pre-column and an on-column butylation procedure for barbiturates extracted from plasma with toluene–methanol[192]. The latter method involved treatment of the toluene layer with tetrabutylammonium hydroxide in methanol–water solution, while the former technique involved back-extraction of the toluene layer with TMAH, followed by treatment with dimethylacetamide and iodobutane. The back extraction was found to improve the extraction efficiency of several barbiturates and also led to cleaner chromatograms, whereas the on-column procedure though quicker, resulted in some decomposition of barbiturate in the injection port.

On-column derivatizations have been extended to the higher alkyl homologues of TMAH[153]. Thus, with phenobarbital, minor secondary peaks were noted with tetrapropyl, tetrabutyl and tetrapentylammonium hydroxides. Although the use of trialkylphenylammonium hydroxides with better leaving groups was considered, steric hindrance prevented synthesis of such bases with alkyl moieties longer than the ethyl group. Reports of on-column alkylations with tetrahexyl-[193,194] and tetraheptylammonium[194] hydroxides allowed identification and quantification of 12 out of 17 barbiturates on an OV-17 column. Alkaline degradation was not apparent, and, although phenobarbital could not be resolved from cyclobarbital, very good resolution of phenobarbital and mephobarbital was produced with either alkylation procedure.

It may be concluded that on-column derivatization of barbiturates is greatly advantageous in many situations due to its rapidity and simplicity, however, results of quantitative estimation of barbiturates, especially phenobarbital, should be treated with caution. Since most on-column techniques are prone to interferences from minor-peak formation to an extent which is unpredictable and probably promoted by the alkaline derivatizing agents, the use of neutral on-column alkylating reagents appears desirable. Another factor influencing the choice of the reagent is the pronounced tailing of the solvent peak, presumably related to it, which may interfere with the peaks of barbiturates having relatively short retention times.

5. GC–MS STUDIES

Reports on the analytical application of GC–MS to the detection of barbiturates first appeared in 1970, when Bonnichsen *et al.*[195] identified several barbituric acids in biological samples. In the same year, Gilbert *et al.*[196] utilised the technique, in metabolic studies of barbiturates. As in conventional GC, widespread recognition of the value of derivatization prior to analysis with GC–MS has not only overcome problems such as tailing and adsorption but, in addition, ion-source contamination is avoided when compounds are converted to more volatile derivatives. Consequently,

TABLE 1

GC–MS STUDIES OF BARBITURATE METABOLITES IN URINE

ns = Not specified

Barbiturate	*Metabolite*	*Dose excreted (%)*	*Notes*	*Reference and (year)*
Phenobarbital	(a) 5-Ethyl-5-(4-hydroxyphenyl) barbituric acid	ns	(a) is the major metabolite	200 (1971)
	(b) 5-(3,4-Dihydroxyphenyl)-5-ethyl barbituric acid	ns	(b) and (c) are also metabolites of	201 (1972)
	(c) 5-(3,4-Dihydroxy-1,5-cyclohexadien-1-yl)-5-ethyl barbituric acid	ns	mephobarbital	201 (1972)
	(d) 5-(1-Hydroxyethyl)-5-phenyl barbituric acid	ns		201 (1972)
Pentobarbital	5-Ethyl-5-(3-hydroxy-1-methylbutyl) barbituric acid	ns	Metabolite formed by in-vivo incubation	130 (1971)
(Several)	Intact glucuronide conjugates of mephobarbital, phenobarbital and hexobarbital were detected	ns	Glucuronides of secobarbital and butalbital were not found	122 (1973)
Secobarbital	(a) 5-(3-Hydroxy-1-methylbutyl)-5-(2-propenyl) barbituric acid	ns		207 (1973)
	(b) 5-(2,3-Dihydroxypropyl)-5-(1-methylbutyl) barbituric acid	ns		
Nealbarbital	5-(2,3-Dihydroxypropyl)-5-(2,2-dimethylpropyl) barbituric acid	30–40	Metabolite isolated for the first time	211 (1973)
Heptabarbital	(a) 5-Ethyl-5-(3-hydroxycyclohepten-1-yl) barbituric acid	18–21	Metabolites (a) and (c) were new; the free acid was absent in urine; (c) was isomeric with (a)	212 (1973) and 213 (1974)
	(b) 5-Ethyl-5-(3-oxocyclohepten-1-yl) barbituric acid	4–8		
	(c) An unidentified hydroxy derivative	10–17		
Butobarbital	(a) 5-Ethyl-5-(3-hydroxybutyl) barbituric acid	22–27	(a), (b) and (c) were isolated for the first time in humans	214 (1974)
	(b) 5-Ethyl-5-(3-oxobutyl) barbituric acid	14–18		
	(c) 5-(3-Carboxypropyl)-5-ethyl barbituric acid	4–8		
	(d) Unchanged butabarbital	7–9		
Mephobarbital	(a) 5-Ethyl-5-phenyl barbituric acid	ns	(2,4,5-^{13}C) phenobarbital was used	215 and 216
	(b) Unchanged mephobarbital	ns	as a homologue, to quantify empho-barbital, and as a stable isotope analogue to quantify phenobarbital	(1974)
Amobarbital	(a) 5-(3-Hydroxy-3-methylbutyl)-5-ethyl barbituric acid	ns	(b) was the first example of an N-hydroxylated metabolite of any barbiturate	209 (1975)
	(b) N-Hydroxy-5-ethyl-5-(3-methylbutyl) barbituric acid	ns		
	(a) and (b) as above	ns	An on-column methylation method for	217 (1977)

analytical studies of barbiturates with GC–MS, have usually involved the derivatized species.

Skinner *et al.*[197] described GC–MS studies of barbiturates derivatized by on-column alkylation with TMPAH. However, TMPAH did not react reproducibly with the 3′-hydroxylated metabolites of several barbiturates[198]. Diazomethane now appears to be the derivatizing agent of choice as it gives a rapid and quantitative reaction, and leaves no solid residue after derivatization, effected simply by mixing at room temperature for 15 min. Yet, with diazomethane, methylation results in the formation of the N,N′-dimethylated derivatives, together with the N,O′- and O,O′-dimethylated isomers that can account for 10–15% of the total yield[199]. In the particular case of urine where hydroxylated metabolites are present, derivatization by methylation is often followed by silylation. Predictably, this procedure results in the formation of several different derivatives from a single barbiturate, as shown during the analysis of urinary metabolites of phenobarbital[200,201].

In efforts to detect microgram amounts of various drugs in human biological specimens, computer-assisted GC–MS identification procedures have been invaluable. The first of such programmes, described by Finkle and Taylor[202] in 1972, involved the compilation of a MS data system for 11 barbiturates and over a hundred other drugs extracted and presented to the GC–MS instrument in a form comparable with that encountered in toxicological practice. Also in 1972, Bonnichsen *et al.*[203] described the use of a computer to evaluate and process the MS data for several barbiturates, recorded on a digital tape, off-line system. The barbiturates were isolated from the blood or liver of suicide cases, prior to analysis by GC–MS.

Since these developments, several additional computer-assisted GC–MS systems suitable for a variety of needs, have been described[204–206]. In a recent report[206], relative intensities of fragment ions from barbiturates methylated with diazomethane, showed some differences to those of authentic N,N′-dimethyl barbiturate derivatives. These differences, possibly due to formation of small amounts of the N,O′- and O,O′-dimethyl derivatives, were obviated by storage and processing of both spectra in the data system.

Use of stable isotopes for the quantification of barbiturates with GC–MS was introduced in 1973[207]. Internal standards labelled with stable isotopes were added to the biological fluid containing the barbiturate. Extraction and derivatization was followed by selective monitoring of ions corresponding to base peaks of sample and internal standard, followed by computer measurement of peak-height ratios. In this way, [2,4,5-^{13}C]pentobarbital, was used to quantify amobarbital, secobarbital and phenobarbital in plasma[207]. Increasing availability of stable, isotope-labelled barbituric acids has led to the determination of many other barbiturates[208–210].

GC–MS procedures have facilitated the identification of urinary metabolites of several barbiturates for the first time. Thereafter, structural identity of the metabolite has usually been confirmed by synthesis and subsequent characterization. Table I lists some important contributions to studies in barbiturate metabolism by GC–MS methods.

GC–MS procedures employing chemical ionization mass spectroscopy (CI-MS), were demonstrated by Horning *et al.*[200] as early as 1971. The CI-MS mode was preferred to the conventional electron impact (EI-MS) mode because of reduced fragmentation and the marked reduction in the probability of fragment ions from

other compounds contributing to the intensity of the ion being monitored. Again, CI-MS spectra of diazomethane-methylated barbiturates are less liable to misinterpretation than corresponding EI-MS spectra, since all isomeric N,N′-, N,O′- and O,O′-dimethylated barbiturates would be expected to give a single peak for the corresponding M + 1 ion. In fact, both N,N′- and N,O′-isomers of phenobarbital gave virtually identical CI-MS spectra[200]. More recently, the use of low-resolution field desorption and field ionization mass spectroscopy in GC–MS methods has been documented[219]. Relatively small samples gave good field desorption spectra, and 1–10 μg of underivatized barbiturate and less of the methylated compounds were required for satisfactory field ionization spectra.

GC–MS methods have been particularly valuable in pharmacokinetic studies due to the specificity of detection, with metabolites being readily distinguished from the parent barbiturate. Furthermore, because of its inherent sensitivity, only small samples are necessary so that repetitive sampling from humans has been facile. An example of this work is the investigation of the kinetics of hydroxylation of amobarbital in liver tissue, where amobarbital was measured in an incubation derived from less than 3 mg of liver tissue obtained by needle biopsy[220]. A total sample weight of 29 mg of liver tissue was sufficient for the determination of the kinetic parameters K_M (Michaelis constant) and $V_{max.}$ (maximal velocity) for the hydroxylation reaction.

In another study of barbiturate levels in the breast milk of nursing mothers[221] it was shown that, while the short acting barbiturates were present in low concentrations, the long acting barbiturate, phenobarbital, reached high levels. Despite interferences from large quantities of free fatty acids present in breast milk, a limit of detection of 0.4–0.5 ng was obtained with the GC–MS system used.

GC–MS methods have enabled the determination of *in vivo* plasma half-lives of amobarbital and 3′-hydroxyamobarbital after ingestion of therapeutic doses[198,222]. Mephobarbital half-lives were estimated similarly, by computer assisted GC–MS[223,224]. Several kinetic parameters for *in vitro* metabolism of secobarbital in rat-liver homogenate, were evaluated in the same study. Again, GC–MS methods have enabled the study of amobarbital, both as a probe drug for hepatic oxidation[210] as well as for an investigation of the influence of genetic factors on drug elimination[225].

A valuable application of the GC–MS–computer method, was demonstrated by Horning *et al.*[226], who utilised it as a reference procedure for some other methods used in a clinical chemistry laboratory. Concentrations of phenobarbital in saliva and plasma measured by enzyme immunoassay, were 10–15% higher than those obtained with a GC–MS system, suggesting that metabolites as well as parent drug were being measured by the immunoassay procedure used.

6. INTERFERENCES IN GC ANALYSIS OF BARBITURATES

Problems encountered during the analysis of barbiturates have arisen primarily from endogenous artifacts or as a result of manipulative procedures, prior to the actual GC. An example of the latter is the adsorption of barbiturates on glassware which may explain the anomalous losses of these polar molecules during analytical procedures. Pronounced losses at the 0.75 μg/ml level, with complete loss at 0.50 μg/ml are known[239]. Such losses can be prevented by silylation of glassware with silylating reagents applied in solution[89] or the vapour phase[239].

Multiple solvent extractions after acidification of the sample form the basis of most methods for the extraction of barbiturates and their metabolites from biological fluids.

6.1 Endogenous substances

There has not been an extensive investigation of endogenous sources of interference in the analysis of barbiturates by GC, although Niyogi and Rieders[228] have described a number of endogenous compounds that could be mistaken for barbiturates after direct extraction from blood with chloroform. Indicative of the need for a greater understanding of interference by artifacts are prominent, unidentified peaks in chromatograms obtained during the analysis of barbiturate extracts from blood[45,56,78,96,159,162,169,188]. Reported for the first time by Cook[229] in 1963, fatty acids present in blood constitute a primary and predictable source of interference due to their co-extraction in significant amounts by most organic solvents. Extractions with non-polar solvents such as isooctane[111] and cyclohexane[148] have been reported. Although fatty acids were largely removed by these procedures, some concomitant loss of barbiturate was also observed[111].

Selective alkylation of barbiturates in the presence of fatty acids has been reported by Kumps and Mardens[188] who observed the fatty acid alkyl ester peaks in the chromatograms of phenobarbital extracted from blood and subjected to on-column alkylation with methanolic TMAH or aqueous tetraethylammonium hydroxide (TEAH). When methanolic TEAH was used, the reaction was not observed and, furthermore, no reason for this behaviour was given. In another instance[118] of the analysis of barbiturates and other acidic drugs, use of TMAH in a back-extraction of the organic phase obtained after extraction of an acidified plasma was found to reduce substantially the interference by fatty acids. Here, a recovery study with palmitic acid showed that only 0.1 % was extracted from toluene with TMAH.

A different approach to overcome the problem of fatty acids was introduced by Mraz and Sedivec[183] who exploited the relative insolubility of the barium salts of fatty acids in diethyl ether in an effort to separate them from barbituric acids in serum. A back-extraction of the organic phase with barium hydroxide also had the advantage of minimising the alkaline degradation of barbiturates, an aspect which appears to have been largely overlooked in most analytical procedures utilising a back-extraction step with strong bases such as sodium hydroxide. Another direct procedure has been reported[230] recently for removing large amounts of free fatty acids co-extracted with barbiturates from autopsy liver and blood samples. Its success depends upon the selective alkylation of the carboxylic acids, under anhydrous conditions, with methanol–HCl. Barbituric acids were then removed and converted to dimethyl derivatives for GC.

As stated earlier, lecithin is responsible for on-column methylation of barbiturates but has also been implicated[158] in the methylation of several fatty acids. This second reaction has been confirmed by GC–MS studies of serum extracts which showed that methyl esters of palmitic, stearic and oleic acids were formed by alkylation in the injection port. Again, extraction of serum with a non-polar solvent may eliminate interference by fatty acids as well as lecithin but there remains the likelihood of some loss of barbiturate[111].

Finally, reference is made to the removal of lipophilic components from serum

by means of a microprocessor-controlled, automatic centrifugal extractor[231]. Lipophilic components were extracted by means of a lipophilic resin (a polystyrene–divinylbenzene copolymer) contained in a compact cartridge, and the recovered drug(s) presented as a dry extract for subsequent analysis. Phenobarbital and other anticonvulsants were determined after on-column methylation and the use of a nitrogen-specific detector.

Interference from cholesterol has also been noted. Although its retention time is much greater than barbiturates, its removal is desirable to prevent column contamination and its slow elution during subsequent analyses. It may also produce a large negative peak, as observed during the GC of indomethacin[232] with the ECD. Cholesterol has been removed from serum with digitonin[45,56,145] but the amount of digitonin added may be critical. Thus, cholesterol was incompletely removed with insufficient amounts of digitonin but gel-formation, with attendant inclusion of drug in the gel, resulted when an excess of digitonin was used[56]. A superior approach appears to be the use of a 4-cm pre-column of 3% SP-2250, as in the separation of cholesterol from primidone, and this also improved resolution of phenobarbital from carbamazepine[223,224] when analyzed on a 2% SP-2510 column.

Proteins can interfere indirectly in the analysis of barbiturates in blood during the extraction step and formation of a protein precipitate often presents difficulty although the use of an acidic precipitant for the determination of protein-bound barbituric acids is well known[78,98,131]. During the analysis of normal plasma or serum, emulsions have usually and simply been resolved by centrifugation. In clinical studies where abnormal plasma is often encountered and intractable emulsions are frequently obtained, Horning *et al.*[111], utilised the salting-out technique involving high concentrations of an inorganic salt to promote transfer of drug from aqueous to organic phase. In this case, diluted plasma containing a small volume of isopropanol was saturated with potassium carbonate and centrifuged then the isopropanol layer containing drug and drug metabolites separated as the upper phase. Since its initial description[227], the salting-out procedure has found wide application in the GC analysis of barbiturates, extracted not only from abnormal plasma but from a range of biological fluids obtained in both healthy and diseased states. Salting-out with ammonium carbonate is preferred to potassium carbonate due to the reduced basicity of its solutions. Ammonium sulphate has also been widely used.

The use of element-selective detectors in situations where endogenous interferences have been encountered, has been of considerable advantage and has led to simplified extraction procedures. Sample volumes as low as 25[107] or 100 μl[92] of whole blood have sufficed for such analyses. However, the use of some solvents may not be compatible with certain element-selective detectors. The disturbing influence on an alkali flame ionization detector of methyl iodide and acetone used in the methylation of barbiturates, was eliminated by column-switching modules[235] which removed most of the solvent peak components prior to elution of the barbiturates[107]. Solvent-related problems have also been encountered with the electrolytic conductivity detector[89,90] during barbiturate analysis. Although halogen-, sulphur- or nitrogen-containing solvents interfered, hydrocarbon solvents were satisfactory. Extraction of barbiturates with diisopropyl ether enabled levels of approximately 2 μg/ml, to be determined both in serum and urine[89].

It would seem that despite the obvious advantages of selective detectors, the

problems of interfering substances in biological fluids cannot be disregarded, as many endogenous compounds contain nitrogen or sulphur. Furthermore, gradual accumulation of co-extracted endogenous artifacts on the column as a result of insufficient clean-up, would ultimately lead to rapid column contamination and loss of performance.

6.2 Miscellaneous sources

Notable among the few examples of interference by exogenous compounds is the oxidation of thiopental during manipulative procedures prior to GC. This reaction was prevented by direct gel chromatography of the haemolyzed blood on Sephadex G-10[81,82]. Similarly, benzene has been recommended for the extraction of thiopental[84] to avoid its degradation by impurities in solvents such as peroxides in diethyl ether. A better-known source of interference is that of plasticizers from butyl-rubber stoppers and bags used for blood collection. Tri-2-butoxyethyl phosphate, in particular was responsible for interfering peaks observed during the analysis of barbiturates in blood by GC[158,161,236].

Another example concerns the compound 5-ethyl-5-*p*-tolylbarbituric acid (EPTB) which has been suggested as an internal standard for on-column methylation of phenobarbital with TMPAH because both barbiturates decompose in a reproducible manner under identical conditions[237]. Unfortunately, co-elution of theophylline (methylated to caffeine) with EPTB on a 3% OV-17 column produced misleadingly low values for phenobarbital in serum[238].

Perhaps because there are fewer references in the literature to the extraction of barbiturates from urine than blood, the more important indicator of tissue barbiturate levels, there is less evidence of interference problems. Since relatively small amounts of most barbiturates are excreted in urine, it is useful nevertheless and certainly the biological fluid of interest in studies of their metabolites. In dealing with this fluid, extraction of barbiturates has been facilitated by the development of adsorptive columns consisting of the weakly basic anion-exchange polymer DEAE-Sephadex[240], and were described[200,201,207] during the early seventies. Again, despite high recoveries of most barbiturates [241–246] there has only been a relatively limited application of the Amberlite XAD-2 resin to barbiturate analysis by GC. In this respect, spurious responses[247] observed with some column eluates may have been more widespread than was thought and interference peaks have been attributed[247] either to impurities in the resin or to incomplete removal of endogenous compounds. The use of XAD-2 columns in the treatment of urine has, however, been widespread in drug screening programmes utilising TLC procedures[241,242,248].

More recently, the use of extraction columns (JETUBES) containing purified cotton fibres that function as an adsorptive matrix was shown to give high recoveries of several drugs, including 90–97% phenobarbital, when extracted from small volumes (15 ml) of urine[249]. A comparison of recoveries with an XAD-2 column and radiolabelled drugs claimed the superiority of the JETUBE both in extraction efficiency and working time. In another device, the removal of endogenous carboxylic acids from urine was demonstrated with pre-packed Kieselguhr columns (Merck Extrelut), prior to analysis by GC[250]. Recoveries of barbituric acids were similar to those obtained by conventional liquid–liquid extraction procedures.

7. SUMMARY

This review surveys the evolution of gas chromatographic procedures for the quantification of barbiturates as either the free acids or their derivatives obtained by direct and on-column reactions. Among the aspects discussed, some emphasis is placed on recognized and other sources of interference encountered during analyses.

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CHREV. 152

ALKYLATION WITH ALKYL HALIDES AS A DERIVATIZATION METHOD FOR THE GAS CHROMATOGRAPHIC DETERMINATION OF ACIDIC PHARMACEUTICALS

A. HULSHOFF* and A. D. FÖRCH
Department of Analytical Pharmacy, University of Utrecht, Pharmaceutical Laboratory, Catharijnesingel 60, 3511 GH Utrecht (The Netherlands)
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1. INTRODUCTION

1.1. Derivative formation in quantitative gas–liquid chromatography (GLC)

The use of analytical derivatization in gas chromatographic analysis may be desirable for various reasons. The most important of these are the necessity to increase the volatility of polar compounds; the prevention of irreversible adsorption, caused by hydrogen bonding between polar functional groups of the compounds under investigation and the free silanol groups of the column packing material and the glassware; and the need to increase the sensitivity of detection. Derivatization can also be used to improve the separation between compounds, to prevent decomposition during chromatography or to decrease the excessive volatility of compounds of very low molecular weight.

A large number of derivatization techniques are available. Most frequently applied are derivatizations by acylation, silylation or alkylation, but many other types of reactions have also been used. In an excellent review, Nicholson[1] enumerated a number of criteria to be considered when choosing a method for derivatization. Some important criteria are that the derivative must be formed rapidly and quantitatively, or at least reproducibly, with no side-reactions or structural changes and with a minimum of manipulation.

In the past few years a number of books and review papers have been published, dealing either with most aspects of derivatization in (gas) chromatography[1–8] or with selected subjects, such as silylation reactions[9], the improvement of electron-capture detector response[10–12], pyrolytic methylation in gas chromatography[13] and applications in selected fields of research[14,15].

Among the various alkylation techniques available to the gas chromatographer, the methods using alkyl halides as the alkylating agents have a prominent position. The reason is that reactions with alkyl halides combine a number of desirable properties compared with the other alkylation reactions (these other techniques are discussed briefly in section 1.2). The attractive features are the following: a wide range of derivatives can be prepared; the reaction is usually fairly rapid under mild conditions; direct injection of the reaction mixture into the gas chromatograph is frequently possible; side-reactions are rare and usually one product is formed; the derivatives are comparatively stable; the reagents used are of rather low toxicity.

The aim of this review is to discuss the various types of alkylation reactions with alkyl halides and their application in the gas chromatographic analysis of acidic compounds of pharmaceutical interest. A short treatment of the underlying reaction mechanisms is included for a better understanding of the optimal reaction conditions. Attention is paid to the incorporation of the alkylation reactions in the entire analytical procedure, particularly in relation to the analysis of pharmaceuticals in biological matrices.

1.2. Derivatization by alkylation reactions (other than with alkyl halides)

Apart from the reactions with alkyl halides, on which this review is centred, a number of other alkylation reactions exist[1–3]. All alkylation methods have in common the replacement of the active hydrogens with alkyl groups in compounds

with (mainly) OH, COOH, SH, NH, CONH and SO_2NH groups. Under certain conditions tertiary amine groups can also be alkylated to yield quaternary ammonium compounds. Of particular interest are the compounds with acidic groups (carboxylic acids, phenols, imides), as these show a strong tendency upon gas chromatographic analysis to produce badly tailing peaks in the chromatogram. Alkylation will yield less polar derivatives with better chromatographic properties.

1.2.1. Diazoalkane alkylation

The most important reagent is diazomethane, although other diazoalkanes (*e.g.*, diazoethane and phenyldiazomethane) have also been used. Diazomethane can be prepared from N-methyl-N-nitroso-*p*-toluenesulphonamide or from N-methyl-N-nitroso-N′-nitroguanidine. Diazomethane is toxic and explosive and therefore should be handled with care. The gaseous reagent can be bubbled through a solution of the compound, or a solution of diazomethane in a suitable solvent can be prepared and added to the compound to be methylated. These solutions are, however, not very stable. The methylation reaction with diazomethane is represented by

$$R\text{–}H + CH_2N_2 \rightarrow R\text{–}CH_3 + N_2$$

Excess of reagent can easily be removed by evaporation in a stream of nitrogen. Provided the choice of solvent is correct, the reaction rate can be high, but in some instances, *e.g.*, with phenobarbital, more than one product is formed.

1.2.2. Pyrolytic alkylation

Acidic compounds can react with quaternary ammonium hydroxides to form salts. Upon pyrolysis in the heated injection port (250–350°C) of a gas chromatograph a volatile alkyl derivative of the compound is produced together with a tertiary amine. The reagents that are commonly used for the deprotonation are aqueous or methanolic solutions of tetramethylammonium hydroxide (TMAH), phenyltrimethylammonium hydroxide (PTMAH) and (*m*-trifluoromethylphenyl)trimethylammonium hydroxide. Sometimes reagents such as tetrabutylammonium hydroxide (TBAH) or tetrahexylammonium hydroxide (THAH) are used.

The salt formation of an acid, ROH, with the reagent TMAH and the subsequent degradation reaction at high temperature can be written as

$$R\text{–}OH + (CH_3)_4N^+\ OH^- \xrightarrow{25°C} R\text{–}O^-\ ^+N(CH_3)_4 + H_2O$$

$$R\text{–}O^-\ ^+N(CH_3)_4 \xrightarrow{250\text{–}350°C} R\text{–}O\text{–}CH_3 + N(CH_3)_3$$

Many acidic compounds can thus be rapidly and conveniently alkylated. However, flash alkylation will frequently result in the formation of more than one product; a well known example is the thermal decomposition of phenobarbital under the conditions of high alkalinity and high temperature that are employed[16,17].

1.2.3. Alkylation with N,N-dimethylformamide dialkylacetals

A number of N,N-dimethylformamide dialkylacetals are commercially available, either as the pure reagents or in solution. Methyl, ethyl, propyl and butyl are the most commonly encountered alkyl groups. Complete alkylation can usually be achieved by heating for a short period at 60–100°C in a suitable non-aqueous solvent. If necessary, the excess of reagent can be removed by evaporation under a stream of nitrogen. The alkylation reaction for carboxylic acids is as follows:

$$R\text{–}COOH + (H_3C)_2N\text{–}\underset{\displaystyle OR'}{\overset{\displaystyle OR'}{\underset{|}{\overset{|}{CH}}}} \rightarrow R\text{–}COOR' + R'\text{–}OH + (H_3C)_2N\text{–}CHO$$

The technique has been applied to a wide range of compounds; among these are the amino acids, both the primary amine group and the carboxylic acid group of which are converted simultaneously into an N-dimethylaminomethylene derivative and an alkyl ester, respectively[18]. With this method usually one product is formed, in contrast to the results with, *e.g.*, flash alkylation.

1.2.4. Alkylation with dimethyl sulphate

Derivatization is usually achieved by heating at 60–70°C for 5–10 min the mixture of the compound(s) under investigation, aqueous potassium carbonate (5–10%) and methanol together with a small amount of dimethyl sulphate. Methylation in non-aqueous solutions (acetone) has also been described[19].

The methylation of acidic compounds is based on a base-catalysed nucleophilic substitution reaction of the conjugate base, RO^-, of the acid with the reagent:

$$R\text{–}OH + OH^- \rightarrow R\text{–}O^- + H_2O$$

$$R\text{–}O^- + (CH_3)_2SO_4 \rightarrow R\text{–}OCH_3 + CH_3SO_4^-$$

Isolation of the derivatives before chromatography is usually required. Special care should be taken in all manipulations, because dimethyl sulphate is very toxic.

1.2.5. Acid-catalysed alkylation (esterification) of carboxylic acids

Esterification of carboxylic acids with alcohols of low relative molecular mass is a well known derivatization procedure in the fields of oil and fat chemistry and in biochemistry. The esterification is catalysed by mineral acids and organic acid anhydrides. Catalysis with sulphuric acid has become outdated because of the frequently slow and incomplete reactions. Solutions of dry hydrogen chloride in the appropriate alcohol give much better results. The reagent is prepared either by bubbling hydrogen chloride through the alcohol or by the addition of acetyl chloride or thionyl chloride to the alcohol.

Methylation is usually chosen as the derivatization reaction, but in principle a wide range of different esters can be prepared. The reaction will sometimes go to completion at room temperature within a short period, but frequently the reaction

mixture has to be heated at 60–100°C for up to 2 h. Isolation of the ester before chromatographic analysis is often necessary.

Organic acid anhydrides, such as trifluoroacetic and heptafluorobutyric anhydride, have been used successfully as catalysts in the rapid esterification of a number of compounds at room temperature.

1.2.6. Other alkylation methods

Closely related to the acid-catalysed esterification is the reaction of carboxylic acids with alcohols under catalytic action of boron trifluoride or boron trichloride. The main advantage of boron trihalide-catalysed esterification over acid-catalysed esterification is its rapidity. Solutions of the gaseous boron trihalides in alcohols are comparatively stable.

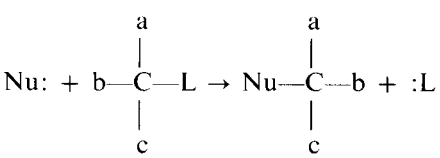
Some less commonly used alkylation reagents are the 3-alkyl-*p*-tolyltriazenes, trialkyloxoniumfluoroborates, alkylsulphonates and alkylfluorosulphonates and O-alkylisourea reagents. Up to now these reagents have found practically no application in the gas chromatographic analysis of pharmaceuticals.

2. ALKYLATION WITH ALKYL HALIDES

2.1. Reaction mechanisms[20–22]

2.1.1. S_N1 and S_N2 reactions

Alkylation reactions with alkyl halides are nucleophilic substitution (S_N) reactions at saturated carbon. A nucleophile, Nu:, displaces a leaving group, :L (halide), from the substrate (the alkyl halide):



Nu: and :L share the same general character, being anionic or neutral bases with unshared electron pairs.

Two mechanistic routes have been clearly identified for S_N reactions. One of these is

$$R\text{–}L \xrightarrow{\text{slow}} R^+ + L^-$$

$$R^+ + Nu:^- \xrightarrow{\text{fast}} R\text{–}Nu$$

The ionization to a carbonium ion intermediate is the rate-limiting step. The subsequent reaction of R^+ with the nucleophile is very fast. The reaction rate therefore depends on the concentration of the substrate (alkyl halide) and is independent of the nucleophile concentration; the reaction is unimolecular and is denoted by S_N1. First-

order kinetics are observed. The reaction rate depends heavily on the ionizing power of the solvent. Protic, polar solvents (water, methanol) stabilize the carbonium ion and the displaced leaving group by solvation of the ions. With a suitable substrate (*e.g.*, tertiary alkyl halides) the S_N1 route of reaction is favoured in these solvents. The second mechanism is the one-step direct displacement reaction:

$$Nu^-: + R\text{–}L \rightarrow [Nu \cdots\cdots R \cdots\cdots L]^- \rightarrow Nu\text{–}R + :L^-$$

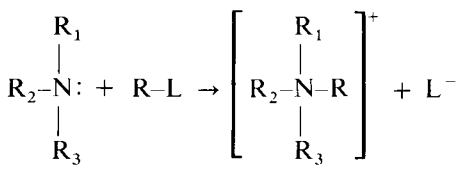
This is a bimolecular process, labeled S_N2. The species in brackets represents the transition state. Overall second-order kinetics are observed. However, derivatization reactions in GLC practice usually show pseudo-first-order kinetics owing to the large excess of the alkylating agent. The reaction is very sensitive to steric hindrance. The nucleophile must be able to approach the carbon atom of the substrate, coming in from the rear side, with its electron-pair orbital on a line with the axis of the orbital bearing the leaving group L. If the substrate (alkyl halide) carries bulky groups at the carbon atom under attack, as in tertiary alkyl halides and many branched alkyl halides, the S_N2 route becomes greatly hindered and the reaction rate will be very low. The influence of the polarity of the solvent is usually not as dramatic as in S_N1 reactions.

S_N1 reaction conditions (tertiary and secondary alkyl halides and protic, polar solvents) are generally unsuitable for alkylation reactions with alkyl halides, because of the high incidence of side-reactions, mainly the elimination of hydrogen halide from the alkyl halide. This results frequently in low yields of derivative products.

In the following sections the four main effects on the rate of S_N2 reactions, usually encountered in derivatization with alkyl halides, are discussed. These are solvent effects, type of alkyl halide, reactivity of the nucleophile and leaving group activity.

2.1.1.1. Solvent effects. In S_N2 reactions between nucleophilic anions of organic acids and alkyl halides the ionizing power of the solvent is usually of less importance than in S_N1 reactions. Aprotic solvents are the best medium for S_N2 reactions. When protic solvents, such as water and methanol, are present, the nucleophile will be stabilized, because the active electron pair of the nucleophile interacts through hydrogen bonding with the solvent molecules; these hydrogen bonds must be broken if the nucleophile is to react with the substrate, thus adding an extra energy barrier. Consequently, S_N2 reactions are favoured in aprotic solvents, such as acetone, N,N-dimethylacetamide and dichloromethane.

It should be borne in mind that not only acidic anions can act as nucleophiles; neutral amines can also be alkylated, yielding quaternary ammonium compounds:



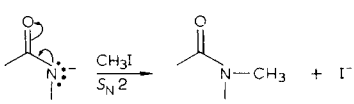
This reaction is strongly inhibited in aprotic solvents such as dichloromethane, because the ionic species which are formed cannot be efficiently solvated in these solvents. This is an important consideration when compounds with acidic groups which also carry an amine group are to be alkylated.

2.1.1.2. Structure of the alkyl halides. The order or reactivity in S_N2 reactions, with respect to the alkyl halide undergoing a nucleophilic attack, is CH_3 > primary > secondary ≫ (tertiary). Inductive effects cause the reactivity of the primary *n*-alkyl halides to be lower than that of methyl halide, because the electron density at the carbon atom under attack is increased. As mentioned above, S_N2 reactions are very subject to steric hindrance; with tertiary substrates the S_N2 displacement is virtually impossible. Resonance effects apparently play a part, although not to any great extent. This is evident from the relative reaction rates of benzyl halides and methyl halides; the benzyl halides show about a four-fold higher reaction rate.

A special case of interest is the group of alkyl halides, such as phenacyl bromide, with a carbonyl α to the reactive carbon. These are often the most reactive substrates in S_N2 reactions, apparently because the attacking nucleophile gives its charge to the carbonyl group as well as to the adjacent reaction site.

2.1.1.3. Relative reactivity of acidic anions. Acidic compounds can be alkylated (with alkyl halides) when they are present in their anionic form, provided that the nucleophilicity of the conjugate base of the acid is sufficient to displace the leaving group. Within a series of structurally related compounds nucleophilicity parallels basic strength. The following order of decreasing nucleophilicity exists for acidic anions with oxygen as the attacking atom: $C_2H_5O^-$ > HO^- > $C_6H_5O^-$ > CH_3COO^-. Although deprotonated aliphatic alcohol groups are very reactive species in nucleophilic substitution reactions, no alkylation of alcohols will take place under the comparatively mild conditions normally used in these reactions, because the aliphatic alcohols are such weak acids that deprotonation to any significant extent does not occur. Only when very strong bases and suitable solvents are used will alkylation of aliphatic alcohols be achieved.

Frequently, nucleophiles contain more than one atom bearing active electron pairs and therefore can react in different ways. An important example is represented by the barbiturates. These compounds usually contain two acidic imide groups, each of which can be alkylated at the N or O atom, depending on the reaction conditions. Under S_N2 conditions the more polarizable (larger) and less electronegative atom is preferentially alkylated. The latter preference can be enhanced by the addition of a protic solvent, which will favour hydrogen bonding of the more electronegative atom, leaving the other site free for the displacement reaction. Therefore, the less electronegative N atom of the imide group of barbiturates is preferentially alkylated under S_N2 conditions (the reverse is true under S_N1 conditions):



Steric hindrance and ion pairing are also factors which can influence the preference for one site over the other.

2.1.1.4. Relative leaving group activity. The more polarizable atoms yield the better leaving groups. For the halides the following decreasing order of leaving group activity exists: $I^- > Br^- > Cl^- \gg F^-$. Derivatizations with alkyl halides are therefore performed almost exclusively with iodides or bromides.

2.1.2. Choice of alkyl halide

Methyl iodide is usually the reagent of first choice. For reasons discussed above only primary, unbranched alkyl halides should be used under S_N2 conditions. Methyl iodide has two advantages over the other *n*-alkyl iodides: it reacts more rapidly and competing elimination reactions cannot occur owing to the absence of a β-hydrogen atom. An important reason for choosing another *n*-alkyl iodide instead of methyl iodide is the possibility of confusion of the derivative with other compounds present. For instance, upon methylation, theophylline is converted into caffeine, which is usually present in human serum and urine samples.

For the enhancement of the electron-capture detector (ECD) response the compounds to be analysed are almost invariably alkylated (in the case of a halide reagent) with pentafluorobenzyl bromide (PFB-Br). PFB derivatives are volatile with good gas chromatographic properties and provide a high response to the ECD. α-Halocarbonyl compounds, such as phenacyl bromide, are frequently used as derivatizing agents in high-performance liquid chromatography (HPLC) to enhance the UV absorption detector response. Their use in GLC has been restricted to a few isolated cases[23,24]. The application of these reagents can be expected to increase owing to the high sensitivity towards electron-capture detection of the phenacyl derivatives[23].

Another sensitive and selective detector that has won wide application in the gas chromatographic analysis of pharmaceuticals is the nitrogen- and phosphorus-sensitive detector (NPD). However, alkyl halides containing phosphorus or nitrogen for the specific aim of enhancing the NPD response have, to our knowledge, not been used.

2.2 Base-catalysed alkylation

The necessary deprotonation of the acidic compound to be alkylated can be effected by the addition of a basic agent, such as potassium carbonate or hydroxyl ions. A number of base-catalysed alkylations with alkyl halides have been described. Two main types of reactions are discernable and will be dealt with in the following sections. Sharp distinctions are not always possible, but some clearly deviating base-catalysed alkylation reactions will be discussed in Section 2.2.3.

2.2.1. Carbonate-catalysed alkylation

Kawahara[25,26] and Dünges and co-workers[19,27] introduced this method, first described by Claisen and Eisleb for preparative purposes at the beginning of the century, into gas chromatographic practice. The acidic compounds are heated in a polar, aprotic solvent (usually acetone) with an alkyl halide in the presence of carbonate. For rapid and complete alkylation the solvent should contain little or no water. The basic catalyst, usually anhydrous potassium carbonate, is almost insoluble in non-aqueous solvents and can therefore be added in large excess, thus providing a

large solid–liquid interface at which the reaction takes place. The reaction between carboxylic acids and methyl iodide, for example, proceeds as follows:

$$2\ R\text{–}COOH + K_2CO_3 \rightarrow 2\ R\text{–}COO^-K^+ + H_2O + CO_2$$

$$R\text{–}COO^-K^+ + CH_3I \rightarrow R\text{–}COOCH_3 + K^+I^-$$

The water produced during the neutralization step is bound by the excess of potassium carbonate. Many acidic compounds can be derivatized in this way: phenols, carboxylic acids and imides are often completely converted into their alkyl derivatives. Aliphatic alcohol groups and amines (except in some special cases) are not derivatized. The derivatives are usually stable in the reaction mixture. The mixture can be injected directly into the gas chromatograph, except when an ECD response-enhancing reagent has been used (PFB-Br). Concentration of the sample before injection is easily achieved by evaporation under nitrogen followed by reconstitution of the residue in a small volume of a suitable solvent, or by concentration under partial reflux[28].

2.2.1.1. The carbonate. Instead of solid potassium carbonate, other carbonates have also been used. Sodium carbonate has sometimes been applied[29–31], as well as sodium hydrogencarbonate[32]. Thio *et al.*[33] investigated the reaction times needed for the methylation of carboxylic acids in acetone (at room temperature) using the anhydrous carbonates of potassium, rubidium and caesium. The reaction rates increased with increasing size of the cation and were highest when caesium carbonate was used. The acetone–caesium carbonate mixture was found to be an excellent reaction mixture also for the derivatization of barbiturates with 2-naphthacyl bromide[34]. For the formation of the PFB derivative of tetrahydrophthalimide, a metabolite of captan, the addition of pyridine together with potassium carbonate was required[32].

Occasionally a small volume of a concentrated potassium carbonate solution in water has been used[35–37]. The introduction of water into the reaction medium will tend to slow down the reaction rate. On the other hand, more carbonate is dissolved in the mixture, which will result in more efficient deprotonation of the acidic compounds.

The addition of a crown ether to the reaction mixture with solid potassium carbonate has been proposed for derivative formation with carboxylic acids and phenols[38,39]. The potassium ion of the ion pair, potassium–acidic anion, is complexed by the crown ether, leaving the acidic anion "naked" and therefore very reactive in the acetone solution.

2.2.1.2. The solvent. Acetone is the most frequently used solvent for the reaction. A number of other solvents or solvent mixtures have been advocated. Ethyl acetate has been reported by Dünges[19,40] to be a suitable solvent for the alkylation of barbiturates (and acetone). Wu and Pearson[41] reported that the use of methanol–acetone–methyl iodide (1:1:1), rather than acetone alone with methyl iodide causes an increase in the reaction rate of the methylation of barbiturates; the reaction was complete within 10 min at 60°C. Acetonitrile[39,42,43] and 2-butanone[44,45] can also be satisfactory solvents. These solvents are less volatile than

acetone, which is helpful if one wants to prevent the loss of solvent when heating the reaction mixture.

Davis[38] investigated the usefulness of acetonitrile, benzene, ethyl acetate, dimethylformamide (DMF) and heptane for PFB derivative formation with carboxylic acids and phenols. Only when the reaction took place in benzene was the yield more than 90%. Unless the use of a strongly alkaline aqueous solution for the deprotonation of acidic compounds is necessary, water should be excluded as far as possible from the reaction mixture.

2.2.1.3. Reaction conditions. Heating the reaction mixture for some time is usually necessary for complete conversion into the derivative. In view of the volatility of the solvent the derivatization is normally performed in an air-tight, closed reaction vial. IJdenberg[42] brought the reaction mixture into a small glass tube; after purging with nitrogen the tube was fused. Alternatively, the reaction mixture can be refluxed; Dünges[46] developed an apparatus, the Microrefluxer, which allows the reflux of microlitre volumes of organic solutions[40,46].

Sometimes the derivatization is allowed to proceed at room temperature[36,47], which will lead to comparatively long reaction times. Thio *et al.*[33] reported that a reduction in reaction time was achieved by ultrasonic treatment while heating the samples at 50°C.

2.2.1.4. The alkyl halides. Methyl iodide and PFB-Br have been extensively used, the latter with the double aim of enhancing the ECD response and improving the chromatographic properties. Ethyl iodide[42,43,48], propyl iodide[42] and butyl iodide[33] have been successfully applied in derivatizations by the carbonate method. With the introduction of the method, Dünges[19] showed that methoxymethyl chloride, allyl iodide and benzyl iodide might also be useful reagents.

2.2.2. Quaternary ammonium hydroxide-catalysed alkylation

A fast and usually quantitative alkylation of acidic compounds under very mild conditions is achieved by the method introduced by Greeley[49,50] in 1974. The acidic compound is dissolved in a polar aprotic solvent (usually N,N-dimethylacetamide, DMA) and deprotonated by tetramethylammonium hydroxide in methanolic solution, or by another quaternary ammonium hydroxide. After addition of the alkyl halide, the reaction mixture is allowed to stand at room temperature; 5–10 min are usually sufficient for complete derivatization. For instance, for carboxylic acids the reaction with *n*-butyl iodide under TMAH catalysis is as follows:

$$R\text{–}COOH + (CH_3)_4N^+ OH^- \rightarrow R\text{–}COO^- {}^+N(CH_3)_4 + H_2O$$

$$R\text{–}COO^- {}^+N(CH_3)_4 + n\text{-}C_4H_9I \rightarrow R\text{–}COOC_4H_9 + (CH_3)_4N^+ I^- \downarrow$$

The alkyl iodide must be added to the reaction mixture after the base, because the hydroxyl ions necessary for the deprotonation of the acid will react immediately, also in an S_N2 reaction, with the alkyl iodide. Consequently, the derivatization of the acidic anion might not go to completion owing to insufficient deprotonation of the

acid. The excess of base is thus removed and at the end of the procedure the reaction mixture is neutral.

$$(CH_3)_4N^+ OH^- + C_4H_9 I \rightarrow (CH_3)_4N^+ I^- \downarrow + C_4H_9 OH$$

Under non-aqueous conditions the TMA iodide precipitates and centrifugation is necessary before the supernatant is injected into the gas chromatograph. The volume of the reaction mixture can be kept very small, so that concentration of the reaction mixture is usually not needed. Separation of the derivative from the solvent may be necessary, if the NPD is to be used as the detector, because a nitrogen-containing solvent such as DMA causes tremendous overload of the detector.

2.2.2.1. The quaternary ammonium hydroxide. Greeley[50] considered PTMAH to be a better organic base than TMAH, because its salts with acidic anions are highly soluble in the organic solvent system and because no precipitation of the corresponding iodide occurs. For practical reasons he chose TMAH as the base for the alkylation reactions: it is available as a concentrated methanolic solution and its solutions are considerably more stable than those of PTMAH. Further, the precipitation of TMA iodide in the derivatization can also be regarded as an advantage, as injections of quaternary ammonium salts into the gas chromatograph may lead to broader "solvent peaks" and can be detrimental to the column packing material. PTMAH has been used by a few investigators[51–53]. Von Minden and D'Amato[52] optimized the reaction conditions for the propylation of benzoylecgonine, the principal metabolite of cocaine. They found that under the prevailing conditions the use of TMAH alone gave rise to hydrolysis, whereas the use of PTMAH alone resulted in long reaction times. A mixture of TMAH and PTMAH proved to be suitable in this instance.

A few workers have reported the use of TBAH[54,55]. McCurdy *et al.*[51] used a solution of tetrabutylammonium hydrogensulphate (TBAHS) and PTMAH in methanol in the propylation reaction of N-desmethyldiazepam; the addition of TBAHS was not motivated by the authors and would appear to be superfluous.

2.2.2.2. The solvent. DMA with methanol has been used as the solvent mixture by most workers. According to Greeley[50], the actual composition of the solvent system is not critical. A solvent containing roughly 80% DMA and 20% methanol was used in his work. Greeley[50] stated that the addition of 10–20% of methanol is necessary to increase to solubility of the intermediate salts. Raisys *et al.*[53], however, used a methanol-free DMA solution to pentylate mephenytoin and desmethylmephenytoin. The methanolic solution of PTMAH was evaporated before use and reconstituted in DMA. The authors did not mention why methanol was excluded.

As usual in S_N2 reactions the presence of water was found to decrease the reaction rate, although the presence of 5% of water did not prevent the butylation of phenobarbital from being quantitative at room temperature[50].

N,N-Dimethylformamide (DMF) can serve as a substitute for DMA[50,56]. Greeley[50] found that acetonitrile was not acceptable as a solvent for the derivatization reactions; Joern[55] used acetonitrile with TBAH for the butylation of phenytoin, but at a higher temperature. The successful use of acetone[57] and butyl acetate[58] has also been reported. These solvents could be helpful when an NPD is to be applied. On the other hand, methanol, ethyl acetate and dichloromethane were found to be less suitable than DMA in the methylation of sulphinpyrazone and its metabolites[59].

2.2.2.3. Reaction conditions. When DMA is used in combination with TMAH (or PTMAH), a period of 5–10 min at room temperature is the usual time needed for the reaction. Menez *et al.*[60] studied the derivatization of barbiturates; the most effective molar ratios between TMAH, alkyl iodide and barbiturate for preparing the dialkyl derivatives proved to be about 21:150:5. Changing the solvent necessitates a change in the reaction conditions, except with DMF. For instance, phenytoin was reacted with butyl iodide in acetonitrile for 20 min at 45°C[55].

2.2.2.4. The alkyl halides. All *n*-alkyl iodides from methyl iodide to *n*-heptyl iodide have been applied in the tetraalkylammonium hydroxide-catalysed alkylation of acidic pharmaceuticals. With branched alkyl reagents such as isopropyl iodide and isobutyl iodide anomalous results were obtained[49,60], and steric hindrance totally prevents the derivatization of phenobarbital with cyclohexyl iodide[60]. We found no reports on derivatization with PFB-Br by this method.

2.2.3. Miscellaneous base-catalysed alkylation reactions

Instead of carbonate or tetraalkylammonium hydroxide a number of other bases, in conjunction with solvents ranging in polarity from very high (ethanol–water) to very low (pentane), have been used to catalyse alkylation reactions of acidic compounds.

Valproic acid has been converted into its phenacyl derivative in an acetonitrile–sodium hydrogencarbonate–crown ether system[23] and in a pentane–triethylamine solvent mixture[24]. *p*-Bromophenacyl bromide and *p*-phenylphenacyl bromide were used as alkylating agents for volatile (C_2–C_{10}) carboxylic acids[61]; the acids were neutralized in aqueous solution with potassium hydroxide and then derivatized by refluxing in ethanol–water.

The PFB derivatives of theophylline[62], pentobarbital[63] and pseudoephedrine[64] have been prepared in ethanol–water mixtures using aqueous solutions of sodium carbonate or potassium *sec.*-phosphate. Walle[65] also used a very polar solvent, methanol, for PFB derivative formation with barbiturates and phenytoin; triethylamine at a well defined concentration was chosen as the catalyst because hydrolysis of the barbiturate ring was observed with potassium carbonate as the base. Under these conditions only one (of two) acidic group of phenytoin is alkylated. On the other hand, Kogan *et al.*[66] alkylated benzoylecgonine with PFB-Br in the comparatively apolar benzene–dichloromethane mixture with pyridine as the catalyst. The PFB ester of indole-3-acetic acid was prepared in acetone with N-ethylpiperidine[67].

Davis[38] investigated the crown ether-catalysed derivatization of carboxylic acids and phenols with PFB-Br in various solvents, with potassium salts of different basicity. When potassium carbonate was used, the carboxylic acids and the phenols were both derivatized, whereas the weaker bases, potassium hydrogencarbonate, potassium acetate and potassium cyanide, allowed the derivatization of carboxylic acids but not of phenols. Volatile carboxylic acids have been alkylated with benzyl bromide in acetone after conversion of the acids into their tetrabutylammonium salts[68].

The hydantoins phenytoin and desmethylmephenytoin possess two acidic groups; one group has a pK_a value of 8.3 (ref. 69), and the pK_a of the other group is similar to that of an amide. Mephenytoin contains only the very weakly acidic amide group. The peralkylation of these compounds can be accomplished if the pH of the medium is such that even the amide group is deprotonated. Gordos *et al.*[47] added a

buffer solution of pH 13 to the acetone–methyl iodide mixture in order to methylate phenytoin; at lower pH values of the buffer the monomethylated product or no product at all is formed. De Sagher *et al.*[44] found that complete perethylation of the 5,5-disubstituted hydantoins was obtained after heating for 1.5 h at 60°C a mixture of 200 μl of acetone, containing the compound under investigation, 50 μl of ethyl iodide and 5 μl of 5 *N* potassium hydroxide in water. These authors also stated that 2-butanone can be used to replace acetone if loss of solvent during the reaction is a problem. The 2-butanone–ethyl iodide–potassium hydroxide solution system has been used for the ethylation of mephenytoin and its demethylated metabolite[45].

A curious butylation reaction for the alkylation of theophylline has been proposed by Vinet and Zizian[70]. A mixture of butyl iodide, TBAH and methanol is injected, together with a solution of theophylline in dichloromethane–isopropanol, into the injection port of a gas chromatograph at 230°C. Although the experiments performed in this study do not permit a definite conclusion, the resulting on-column butylation of theophylline is probably effected by two simultaneous reactions: the TBAH-catalysed S_N2 alkylation reaction with butyl iodide and the pyrolytic butylation with TBAH alone.

A number of investigators have used solutions of sodium hydride, potassium *tert.*-butoxide or sodium methoxide in dry dimethyl sulphoxide in order to permethylate acidic compounds. The strongly basic methylsulphinyl carbanion which is formed in these solutions deprotonates even very weak acids, which then are able to react with added methyl iodide. The reaction is fast and quantitative in a very short period at room temperature. After the reaction, water is added and the derivatives are isolated from the mixture by extraction with chloroform or another solvent. This procedure has led to successful GLC determinations of bile acids[71], nucleotides[72], insecticides[73], urea herbicides[74], 5-fluorouracil[75], 5-fluorouridine[76] and floxuridine[77]. Thompson[78] showed that the method is not well suited to the methylation of barbiturates.

The silver salts of fatty acids have been methylated in pentane with methyl iodide[79,80]. The concentration of free silver ions in the solvent was found to be important for the result of the derivatization[80].

Silver oxide[42] has been used to catalyse alkylation reactions of antiepileptic agents, such as primidone and barbiturates, by heating in acetone or acetonitrile. Ambident nucleophiles, such as primidone, can be expected to react at least partly via the S_N1 route in the presence of silver ions, resulting in O-alkylation rather than N-alkylation[22].

2.3. *Phase transfer catalysis*

Deprotonation and subsequent alkylation of acidic compounds can be achieved not only by the addition of (excess) base, but also by the transfer of the acidic anion as an ion pair with a tetraalkylammonium counter ion into a suitable aprotic solvent, such as dichloromethane, containing the alkyl halide. The phase transfer is usually from one solvent to another, and is then called liquid–liquid phase transfer catalysis; this is better known as "extractive alkylation"[81,82]. At the end of the next section, dealing with this technique, two methods deviating from the usual extractive alkylation technique, but still with strong resemblances to this method, will be discussed.

2.3.1. Extractive alkylation

The acidic compound is extracted as the ion pair formed with tetraalkylammonium from an aqueous solution at a suitable pH into an aprotic solvent, usually dichloromethane. The extracting solvent has poorly solvating properties for anions, which therefore possess high reactivity and an S_N2 reaction between the anion and the alkyl halide added to the system can then take place to give the required derivative.

For instance, in the methylation of carboxylic acids upon extraction with TBA^+ ions the reactions are as follows:

$$R\text{–}COOH_{aq} + OH^- \leftrightarrows R\text{–}COO^- + H_2O \quad (1)$$

$$R\text{–}COO^- + TBA^+ \leftrightarrows TBA^+\ R\text{–}COO^-_{org} \quad (2)$$

$$TBA^+\ R\text{–}COO^-_{org} + CH_3I \leftrightarrows R\text{–}COO\text{–}CH_3 + TBA^+I^- \quad (3)$$

Derivative formation is usually achieved by shaking the water–organic solvent system for a certain period of time (from 10 min to many hours) at room temperature.

The speed of the derivatization reaction and the yield of derivative are governed by the efficiency of extraction and the rate of the nucleophilic substitution reaction. The efficiency of extraction of the acidic anion is determined by the properties and the concentrations of the ion pair forming ions, the lipophilicity of the ion pair and the properties of the solvent[83,84].

The extraction equilibrium of an anion, X^-, with a tetraalkylammonium ion, R_4N^+, can be written as

$$X^-_{aq} + R_4N^+_{aq} \leftrightarrows R_4N^+X^-_{org}$$

The equilibrium constant is defined by

$$K_{Ex} = \frac{[R_4N^+X^-]_{org}}{[X^-]_{aq}\,[R_4N^+]_{aq}}$$

If no side-reactions occur, such as protonation of X^-, the distribution ratio of X^- is expressed by

$$D_X = \frac{[R_4N^+X^-]_{org}}{[X^-]_{aq}} = K_{Ex}[R_4N^+]$$

The distribution ratio, and therefore the extraction efficiency, is thus determined by K_{Ex} and the concentration of the counter ion, higher R_4N^+ concentrations leading to increased reaction rates[82,84,85]. The percentage of X^- extracted as the ion pair is, of course, also dependent on the phase-volume ratio of the organic extractant and the aqueous layer. The more lipophilic the ion pair, the higher will be the value of K_{Ex}. Lipophilicity is increased with increasing numbers of carbon atoms (in homologous

series). In practice, the use of quaternary ammonium ions with fewer carbon atoms than TBA^+ will generally result in too low extraction efficiencies. TBA^+, TPA^+ (tetrapentylammonium) and THA^+ (tetrahexylammonium) are the most frequently employed counter ions. With hydrophilic anions the use of a larger counter ion is indicated to achieve sufficient extraction of the ion pair. The pK_a and the partition coefficient of the acidic compound determine the minimal pH value, at which partitioning of the compound as the ion pair will occur without significant partitioning of the undissociated acid. The pH of the aqueous phase should be at least two units higher than the pK_a value of the acid, when the partition coefficient of the acid is unity. With very lipophilic acids, such as the higher fatty acids, a much higher pH value is needed; with hydrophilic acids the pH might be kept lower[82,84–86].

K_{Ex} is also very dependent on the nature of the organic extractant, which must be capable of accomodating the ion pair. Chlorinated hydrocarbons, such as chloroform, dichloromethane and dichloroethane are good solvents for ion pairs, but other solvents, such as the alcohols (C_4–C_7), methyl isobutyl ketone, and even less polar solvents such as benzene, toluene and carbon disulphide, can also be effective extractants[82–84,87–89]. The choice of the solvent is, of course, somewhat restricted because of the subsequent S_N2 reaction which has to take place. The alcohols are therefore less suitable extractants; the higher alcohols would also cause problems in concentration steps through evaporation because of their high boiling points. S_N2 alkylation reactions usually proceed rapidly in aprotic solvents such as the halogenated hydrocarbons. Dichloromethane is the most frequently used solvent in extractive alkylation procedures.

As was mentioned in Section 2.1., S_N2 reaction rates are proportional to the nucleophilicities, and consequently also proportional to the base strengths of the conjugate bases of the acids to be alkylated. A linear relationship has been observed between the logarithm of the observed rate constants and the pK_a values of the acids (in water) in some investigations related to the study of extractive alkylation processes[90,91].

The S_N2 reaction rate is also dependent on the structure and concentration of the alkyl halide. The greater reactivity of methyl iodide and PFB-Br compared with that of the *n*-alkyl iodides was confirmed in an investigation on the alkylation of sulphonamides in various solvents[87]. The use of benzyl bromide allowed very fast reactions with acetylsalicylic acid and salicylic acid[92].

In practice, the optimal conditions for extractive alkylation seldom coincide with the highest possible extraction efficiency and reaction rate. One reason is that higher selectivity can often be obtained by the judicious choice of pH, type and concentration of R_4N^+ counter ions, etc. Another reason is that the lowest detectable amount of derivative following extractive alkylation is usually not dependent on the signal-to-noise ratio but on the extent of by-product formation, the presence of contaminants and sometimes excess of reagent, causing interferences and/or tailing fronts in the chromatograms. Methyl iodide can contain dimethyl sulphate, which causes long tailing solvent fronts in the gas chromatograms[93]; distillation before use therefore is necessary. The iodide of the R_4N^+ ion formed as a by-product during alkylation can also give rise to long tailing fronts. Furthermore, transesterification reactions caused by R_4N^+ I^- have been observed upon injection of the organic reaction mixture without further purification[93].

$R_4N^+ I^-$ can be removed from the final organic solvent layer after derivatization in different ways. The organic layer can be washed with a solution of silver sulphate[75,93,94]. Another possibility is evaporation of the final organic solvent layer and extraction of the derivative from the residue with a very apolar solvent, usually an *n*-alkane, in which $R_4N^+ I^-$ is insoluble[95–98], or by taking up the residue in toluene and extracting the derivative with *n*-hexane[99]. A successful clean-up after derivatization can also be performed by an additional extraction step with diethyl ether after evaporation of the organic solvent layer and reconstitution of the residue in water[75].

In extractive alkylation procedures it is essential to use conditions that will minimize hydrolysis of the reagent and of acidic anions such as acetylsalicylate[92]. At higher pH values substantial amounts of OH^- ions can be transferred into the organic phase as ion pairs with R_4N^+ counter ions. The OH^- ions will then displace I^- or Br^- from the reagent. In particular with the use of PFB-Br problems can arise owing to the formation of by-products[100]. The OH^- transfer into the organic phase is enhanced by high pH values of the aqueous phase and by the use of R_4N^+ counter ions of increasing lipophilicity. For this reason, when optimizing extractive alkylation procedures, one should not extract at higher pH values or with larger R_4N^+ ions than is strictly necessary. The concentrations of the R_4N^+ ions and of the alkyl halide should not be higher than required and the reaction should not be unduly prolonged.

Another reason for keeping the concentration of ECD response-enhancing alkyl halides in particular as low as possible is the danger of overloading the detector. Usually an extra clean-up step is needed in order to separate the alkyl halide, PFB-Br, from the final organic solvent layer. Possible clean-up steps are evaporation of the excess of PFB-Br[101,102], extraction of the derivative into an aqueous phase[103,104], separation on a silica gel column[105] and coupling of the excess of reagent with an aminophenol (hordenine) to yield a product that can be extracted with water[106]. Another possibility is the use of a pre-column venting system[107].

The adjustment of the pH of the aqueous phase is also important in the extractive alkylation of acids with two (or more) acidic groups, such as many barbiturates and 5-fluorouracil[75,89]. Optimal conditions for ion-pair extraction exist when the acid is present in its monovalent anionic form[108]. The dialkyl derivatives are formed, probably in a stepwise fashion[75]. It seems that the monoalkyl derivative is produced first; this will have a lower pK_a value than the $pK_{a,2}$ value of the underivatized compound (compare mephobarbital and phenobarbital). The monoalkylated product is then extracted into the aqueous solution, back-extracted into the organic phase as an ion pair and subsequently derivatized to yield the dialkylated product. However, the extractive methylation of oxazepam[109] could not be explained by this (hypothetical) mechanism.

2.3.2. *Specific alkylation of phenolic compounds in a biphasic system*

An alkylation procedure closely resembling the extractive alkylation process has been proposed by Rosenfeld and co-workers[110,111] for the specific alkylation of compounds with a phenolic hydroxyl group, such as estradiol[111]. It was observed[110] that these acids can be alkylated in a biphasic system, *without the addition of R_4N^+ counter ions*, with PFB-Br or benzyl bromide. The method is specific for phenolic compounds; carboxylic acids do not react. The kinetic data suggest[111] that the usual S_N2

displacement reaction probably takes place, but there is also some conflicting evidence with respect to the type of reaction mechanism involved. There are indications that phase-transfer catalysis is not the mechanism responsible for alkylation. From a practical point of view, the specificity of the alkylation of phenols is also interesting, because it offers the advantage that one class of substrates (phenols) reacts, whereas a second class of compounds (carboxylic acids) does not.

2,3.3. Alkylation by solid–liquid phase transfer catalysis

Particularly for the alkylation of compounds sensitive to hydrolysis, the following method was found to be useful[112–114]. To dichloromethane (or another suitable solvent such as ketone[114], containing TBA hydrogensulphate, alkyl halide, RX, and the acidic compound (HA) to be alkylated, solid sodium hydrogencarbonate is added. The mixture is shaken for a certain period of time and the organic solvent phase is analysed by GLC. A three-step reaction is probably involved[113]. The hydrogencarbonate is transferred into the liquid phase through exchange with hydrogensulphate anions, followed by the simultaneous deprotonation of the acidic compound and formation of the ion pair; then the alkylation reaction takes place.

$$NaHCO_{3\,solid} + TBA^+\ HSO^-_{4\,org} \rightarrow TBA^+\ HCO^-_{3\,org} + NaHSO_{4\,solid} \quad (1)$$

$$TBA^+\ HCO^-_{3\,org} + HA \rightarrow TBA^+\ A^-_{org} + H_2CO_3 \quad (2)$$

$$TBA^+\ A^-_{org} + RX \rightarrow RA + TBA^+\ X^-_{org} \quad (3)$$

This process has been applied successfully to the analysis of indomethacin[112] and acetylsalicylic acid[113].

3. THE ALKYLATION METHOD IN RELATION TO THE ENTIRE ANALYTICAL PROCEDURE

The derivatization of the compound(s) under investigation is in practice always preceded by some kind of sample pre-treatment and followed by a gas chromatographic separation and detection. When evaluating an analytical procedure for the analysis of compounds that have to be derivatized, these three parts of the procedure should not be treated as separate entities.

The choice of the derivatization method and of the reaction conditions is generally limited. One reason is that the solvent must be compatible with the chromatographic system, and that the derivative must be sufficiently stable at the high temperature usually applied in GLC. Further, there should be a smooth connection between the sample pre-treatment and derivatization procedure, that is, the solution after the final clean-up step must be either directly suitable for the subsequent derivatization reaction or else must be exchanged for another, more suitable solvent with a minimum of manipulations. These aspects are discussed in Sections 3.1 and 3.2.

Finally, the demands made on the analytical procedure as a whole are variable and depend on the field of application of the method. This will, of course, influence the choice of the derivatization technique. For example, in emergency procedures the analysis should be as fast as possible. On-column derivatization methods are then to

be preferred[70]. In some instances the pre-column extractive alkylation technique will also be fast enough for this purpose. When large numbers of samples must be analysed with the help of an automatic sample injection system, the stability at room temperature of the final solution to be injected into the gas chromatograph is an important feature.

3.1. Alkylation method and chromatographic system

A generally accepted criterion for derivatization reactions in GLC is that the alkylated product should be stable in the solution in which it is injected at the elevated temperatures of the injection block and the column. Furosemide has been methylated to its trimethyl derivative before GLC analysis by an extractive alkylation technique[115]. When the residue of the evaporated extraction mixture, containing the furosemide derivative and $THA^+ I^-$, was reconstituted in a solvent in which $THA^+ I^-$ is soluble, one of the methyl groups of trimethyl furosemide was exchanged for a hexyl group after injection; with *n*-hexane as the solvent the degradation of the derivative was prevented. Useful exceptions to the rule of stability of the derivative in the gas chromatographic system are also known. Methylated sulphinpyrazone seems to be degraded in the injection port of the gas chromatograph, because the shape and height of the chromatographic peak change (and improve) at higher temperatures[59]. Above 270°C the peak shape and peak height were found to be constant and reproducible, apparently owing to the quantitative degradation of methylsulphinpyrazone. In fact, this can be regarded as the on-column derivatization of a pre-column derivatized compound.

The most frequently and broadly applied detection methods in the GLC analysis of drugs are FID, NPD and ECD. With FID, extra clean-up steps after the alkylation reaction are generally not needed. There is little danger of overloading the detector, provided that the differences in retention (volatility) between the derivative(s) formed and the solvent with excess of reagent (alkyl halide) are large enough. The FID response for carbon disulphide is very low. Ehrsson[89] described a procedure for the extractive alkylation of barbiturates with carbon disulphide as the organic extractant. Owing to the reduced solvent front compared with those obtained after the injection of the more common organic solvents, the sensitivity of detection could be significantly improved. The FID is an almost universal detector for organic compounds; therefore, the selectivity of GLC–FID procedures depends entirely on the column and the sample pre-treatment. Elaborate sample clean-up procedures are therefore occasionally necessary.

The main goal of derivatization in GLC–FID analysis is the improvement of the chromatographic behaviour of compounds. The gain in sensitivity on introducing very large alkyl groups into the molecules of the acidic compounds is marginal and problems may arise because of the reduced volatility of the derivatives. NPD allows the sensitive and selective detection of nitrogen- and phosphorus-containing compounds. As with FID, the main objective for derivatization in GLC with NPD is the improvement of chromatographic behaviour. To prevent detector overloading the final solution to be injected should not contain large amounts of nitrogen- (or phosphorus-) containing components. The reaction mixture obtained after alkylation in acetone with carbonate catalysis is therefore suitable for direct injection into the

GLC–NPD system. After a $R_4N^+ OH^-$-catalysed alkylation in DMA, however, an extra clean-up step is necessary to remove the DMA and dissolved $R_4N^+ I^-$. The obvious remedy would be to replace DMA with a nitrogen-free solvent, in which the $R_4N^+ I^-$ formed during the reaction is insoluble. No reports on the successful application of such a system in the GLC–NPD analysis of pharmaceuticals could be found.

The alkylation of acidic compounds can serve a single or a double purpose when ECD is chosen as the method of detection. With compounds that possess electron-capturing properties, the main objective of derivatization is again improvement of the chromatographic behaviour of the compounds. More often, lowering the detection limit of non-electron-capturing compounds is the principal reason for derivatization, and the necessity to improve the chromatographic properties of the compounds is the second one. In either instance, the excess of alkyl halide, and very often also the organic solvent of the extractive alkylation procedure, must be removed in order to prevent detector overloading. Only when the concentration of the reagent is kept at a minimum and when the greatest sensitivity is not required, can the reaction mixture after derivatization be injected directly into the GLC–ECD system[23]. Some of the problems encountered in the use of GLC–ECD following alkylation reactions, particularly with the extractive alkylation technique with PFB-Br as the alkyl halide, have been discussed in Section 2.3.

3.2. Sample pre-treatment and alkylation method

When the acidic pharmaceutical is present in a solid matrix such as a powder or a tablet, a very simple clean-up will generally be sufficient. The compound(s) of interest can then often be extracted with an organic solvent, in which the derivatization reaction is carried out either straight away or after evaporation of the solvent and reconstitution of the residue in the reaction medium.

Aqueous biological matrices, such as plasma or serum, urine and saliva, contain a multitude of naturally occurring compounds, often in much higher concentrations than the drug or drug metabolite(s) to be analysed. A more involved clean-up procedure of the sample is then very often required. The presence of proteins in plasma and serum samples is another complicating factor. A conceivable approach for the analysis of acidic drugs in urine and saliva would be to derivatize the drug directly by mixing an aliquot of the sample with a sufficiently large volume of a solution of an alkyl iodide in a water-miscible solvent such as acetone or acetonitrile, with the addition of a basic catalyst. No reports on such an approach with protein-free samples were found.

Chan[23] precipitated the proteins in serum samples containing valproic acid with acetonitrile. After centrifugation the acid in the supernatant was directly alkylated with phenacyl bromide.

The extractive alkylation technique in principle allows the simultaneous extraction and alkylation of acidic compounds when dealing with aqueous biological samples; many examples of this approach have been reported (*e.g.*, refs. 92, 93, 97, 107 and 116–120). Frequently, however, extra clean-up steps will have to be included, in order to prevent the appearance of interfering peaks in the chromatograms. These steps can be the preliminary extraction of the acid from the sample with an organic

solvent (*e.g.*, refs. 88, 109, 112 and 121) or the extraction of the acid from the sample followed by back-extraction into an alkaline aqueous phase (*e.g.*, refs. 95, 104, 121 and 122). In some instances a more extensive clean-up procedure proved to be necessary[123,124].

Extraction of the aqueous samples with an organic solvent is the most frequently applied clean-up step prior to base-catalysed alkylation reactions, Dünges and co-workers[28,40] developed an assay procedure that is generally applicable to the gas chromatographic determination of acidic drugs, such as barbiturates, in whole blood. The samples (20 μl) are extracted by stirring with 50-μl portions of acetone–diethyl ether (1:1). The combined extracts are dried with activated molecular sieve and concentrated under partial reflux. After the addition of alkyl iodide and potassium carbonate the alkylation reaction is performed with a microrefluxer. The need for strict adherence to the experimental conditions was stressed by the author[40,28], otherwise unsatisfactory results are obtained.

Double extraction procedures, preceding base-catalysed alkylation reactions, have also been applied frequently. A double extraction–concentration procedure, which is very suitable for a subsequent TMAH-catalysed alkylation, has been reported[125–128]. The acidic drug is extracted with toluene and the toluene layer is back-extracted with a small volume (20–50 μl) of TMAH in methanol. The TMAH layer is then used for the alkylation reaction, which is effected by the addition of DMA and an alkyl iodide. When the acidic drug is not efficiently extracted with toluene, a more polar solvent such as a chloroform–isopropanol mixture can be used; after evaporation of the resulting extract the residue is taken up in a toluene–TMAH in methanol system, etc. Very clean chromatograms from blank plasma samples are thus obtained and the method is applicable to many acidic pharmaceuticals[128].

Occasionally separation of the acid from the aqueous matrix is achieved by the addition of a solid adsorbent, such as charcoal[53,96]. Sample clean-up procedures with thin-layer chromatographic methods[101,129,130] or by column chromatography have also been reported[72,76,77,123].

4. APPLICATIONS OF THE DERIVATIZATION WITH ALKYL HALIDES IN THE ANALYSIS OF ACIDIC PHARMACEUTICALS

The desirable properties of the derivatization with alkyl halides, as mentioned in Section 1.1, have resulted in the widespread use of (the earlier discussed variants of) this alkylation method in GLC practice. In this section an up-to-date survey, in the form of tables, is presented of the manifold applications of the alkylation with alkyl halides in the gas chromatographic analysis of acidic drugs, especially with respect to the quantitative determination of drugs and/or drug metabolites in biological matrices. Tables 1–7 cover the following groups of drugs: 1, barbiturates; 2, anticonvulsants (with the exception of benzodiazepine and barbiturate anticonvulsants); 3, benzodiazepines; 4, xanthines; 5, sulphonamides; 6, analgesic (narcotic and non-narcotic) and anti-inflammatory drugs; and 7, miscellaneous drugs.

4.1. Key to the tables

Under "Compound(s)" the name of the drug or group of drugs is given, for which the procedure referred to was originally designed. In some instances a single compound can be regarded as a model compound for a group of structurally related drug molecules.

"Sample" refers to the type and required volume of the biological sample (*e.g.*, blood, plasma, serum, urine, saliva) for which the method is suitable.

Many differences are to be found in the details of the clean-up procedures, which are performed when analysing drugs in biological materials. It is possible, however, to give a classification based on the type and number of clean-up steps in the pre-chromatographic sample treatment. Simple washings of samples or extracts, in which the compound of interest does not move into the other phase, are not considered as clean-up steps here.

The symbol "a" in the third column denotes some form of protein removal from the biological material, which precedes any further sample clean-up. Proteins can be removed by ultrafiltration or by precipitation through the addition of some reagents (*e.g.*, sodium tungstate, zinc sulphate, ammonium acetate and ammonium sulphate). One example has been found in which protein denaturation by the addition of acetonitrile forms the sole sample treatment before derivatization.

The letter "b" stands for a single pre-chromatographic extraction step. Derivatization is performed either directly in the separated organic phase, following solvent extraction of the aqueous sample with an organic solvent, or after evaporation of the organic solvent and reconstitution of the residue in a more suitable solvent.

The symbol "c" is used when more than one clean-up step is involved, *e.g.*, when the acidic drug molecule is back-extracted from the initial organic extract into an alkaline phase (*e.g.*, methanolic TMAH). Derivatization sometimes can be performed using this alkaline layer after phase separation. In other instances the drug to be derivatized is re-extracted from the alkaline phase with an organic solvent after phase separation and acidification. The symbols "b" and "c" are used only when some form of base-catalysed alkylation follows.

The letter "d" denotes any form of sample clean-up other than solvent extraction (*e.g.*, by thin-layer or column chromatography), whereas the letter "e" is used when the initial isolation of the drugs to be analysed is effected by adsorption on to a solid adsorbent (in the two instances mentioned, charcoal is used as the adsorbent).

In extractive alkylation procedures the simultaneous extraction and derivatization of the compounds under investigation could be regarded as a clean-up step by itself. When this is the only sample clean-up involved, as is the case in the many instances in which extractive alkylation is performed directly on the biological sample, the symbol "f" is used.

When a single extraction step precedes the extractive alkylation procedure, "g" is used, whereas "h" stands for methods in which more than one clean-up step preceding the extractive alkylation is included. The convenient procedure involving back-extraction of the drugs to be analysed from the initial organic extract with an aqueous alkaline layer, which, after phase separation, is submitted to the extractive alkylation by the addition of a quaternary ammonium ion and the organic extractant containing the alkyl halide, is thus denoted by "h".

TABLE 1

BARBITURATES

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
(a) Based on carbonate method						
Barbiturates	–	–	Acetone or ethyl acetate, alkyl iodide (methyl, allyl or benzyl)/K_2CO_3	3% OV-225	FID	19
Barbiturates	–	–	Acetone, CH_3I/K_2CO_3	3% OV-225	FID	27
Barbiturates	–	–	Acetone, methanol, CH_3I/K_2CO_3	Capillary, 15-m OV-17	FID	41
Barbiturates	Blood, 20 µl	b	Acetone, CH_3I/K_2CO_3	3% OV-225 3% OV-17	FID NPD	28
Phenobarbital, mephobarbital	–	–	Acetone or acetonitrile, alkyl iodide/K_2CO_3	3.8% SE-30	FID	42
Pentobarbital	Serum, 0.1 ml	a + b	Ethanol, PFB-Br, Na_2CO_3 in H_2O	3% OV-17	ECD	63
Pentobarbital	Serum, 0.1 ml	b	Acetone, CH_3I/Na_2CO_3	2% OV-17	NPD	31
Thiopental	Plasma, 1 ml	c	Acetone, CH_3I/Na_2CO_3	3% OV-17	NPD	30
(b) Based on tetra-alkylammonium hydroxide catalysis						
Barbiturates	–	–	DMA, TMAH or PTMAH, alkyl iodide	2% OV-17 1.5% SP-2250	FID	49

Barbiturates	–	–	DMA, TMAH, alkyl iodide	3.08% OV-101 1.82% Dexsil 300 GC 3% SP-2250 1.57% OV-7	FID	60
Barbiturates	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-17	FID	126
Barbiturates	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Pentobarbital	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-17	FID	126
Phenobarbital	Serum, 50 µl	c	DMA, TMAH, CH_3I	3% OV-1	NPD	125
Phenobarbital, mephobarbital, heptobarbital	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Phenobarbital, mephobarbital	Plasma, 0.1–1 ml	b	DMA, TMAH, C_3H_7I	3% OV-101	MS (SIM)	131
(c) Other base-catalysed methods						
Barbiturates	–	–	Methanol, PFB-Br, triethylamine	3% OV-1 3% OV-17 3% NPGSe	ECD	65
(d) Extractive alkylation						
Barbiturates (pento- and pheno-barbital)	–	–	Buffer pH 10, THA^+/CS_2, CH_3I	3% SE-30	FID	89
Barbiturates	Plasma, 0.5 ml	e + f	1 *M* NaOH, TBA^+/CH_2Cl_2, C_2H_5I	Capillary, 43-m SE-30	FID	96
Phenobarbital	Saliva, 100 µl	f	Buffer pH 9, TBA^+/CH_2Cl_2, PFB-Br	3% OV-17	ECD	107

TABLE 2

ANTICONVULSANTS (WITH THE EXCEPTION OF BENZODIAZEPINE AND BARBITURATE ANTICONVULSANTS)

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
(a) Hydantoins						
Phenytoin	–	–	Acetone or acetonitrile, alkyl iodide/K_2CO_3	3.8% SE-30	FID	42
Phenytoin	Serum, 50 µl	c	DMA, TMAH, CH_3I	3% OV-1	NPD	125
Phenytoin, mephenytoin	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Mephenytoin, desmethyl-mephenytoin	Serum, 1 ml	e	DMA, PTMAH in DMA, $C_5H_{11}I$	3% OV-225	FID	53
Phenytoin	Plasma, (serum), saliva, 100 µl–1 ml	b	Acetonitrile, TBAH, C_4H_9I	3% OV-17	NPD	55
Phenytoin	–	–	Methanol, PFB-Br, triethylamine	3% OV-1 3% OV-17 3% NPGSe	ECD	65
Phenytoin	Plasma, 100 µl	c	Acetone, CH_3I, buffer pH 13	3% OV-225	FID	47
Phenytoin, mephenytoin, desmethyl-mephenytoin	–	–	Acetone, C_2H_5I, KOH soln.	3% OV-225 capillary, 37-m SE-30	FID	44
Mephenytoin, desmethyl-mephenytoin	Plasma, 1 ml	c	2-Butanone, C_2H_5I, KOH soln.	2% OV-101	MS (SIM)	45
(b) Ethosuximide						
Ethosuximide	–	–	Acetone or acetonitrile, alkyl iodide/K_2CO_3	3.8% SE-30	FID	42

Ethosuximide	Plasma, serum, 0.5 ml	c	DMA, TMAH, C_4H_9I	3% OV-1	FID	132
Ethosuximide	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
(c) Primidone						
Primidone	–	–	Acetone or acetonitrile, alkyl iodide/Ag_2O	3.8% SE-30	FID	42
Primidone	Serum, 50 µl	c	DMA, TMAH, CH_3I	3% OV-1	NPD	125
Primidone	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Primidone + metabolites	Serum, urine, saliva, breast milk, tissues, 5–100 µl	a + b	DMA, TMAH, C_2H_5I	3% OV-17	MS (SIM)	133
(d) Valproic acid						
Valproic acid	–	–	Acetone or acetonitrile, alkyl iodide/K_2CO_3	3.8% SE-30	FID	42
Valproic acid	Serum, plasma, 50 µl	c	DMA, TMAH, C_4H_9I	3% OV-17	FID	127
Valproic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Valproic acid	Serum, 100 µl	f	Buffer (pH 8), TBA^+/CH_2Cl_2, CH_3I	2% SP-1000	FID	116
Valproic acid	Plasma, 0.25 ml	b	Pentane, phenacyl bromide, triethylamine	3% OV-17	FID	24
Valproic acid	Serum, 100 µl	a	Acetonitrile, phenacyl bromide, crown ether, sat. $NaHCO_3$ solution	3% PC-3210	ECD	23
(e) Other anticonvulsants						
(Neo)sulphalepsine + metabolites	Blood, 0.2 ml; plasma, urine, 1 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% OV-101	ECD	122

TABLE 3

BENZODIAZEPINES

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
Clonazepam	Serum, CSF, 0.2 ml	b	Acetone, C_2H_5I/K_2CO_3	4% OV-101	ECD	48
Bromazepam	Plasma, 0.5–1 ml	b	0.1 *M*. NaOH, TBA^+/diethyl ether, CH_3I*	0.5% OV-17	ECD	134
Desmethyl-diazepam	Plasma, 0.5 ml	b	DMA, TBAH, C_4H_9I	3% OV-17	ECD	54
Desmethyl-diazepam	Blood, 2 ml (bile, tissue)	c	DMA, 0.025 *M* TBAHS in 0.2 *M* PTMAH, C_3H_7I	3% OV-1	NPD	51
Nitrazepam	Plasma, 0.5 ml	g	0.4 *M*. NaOH, TBA^+/benzene, CH_3I	5% OV-17	ECD	88
Clonazepam, nitrazepam	Serum, blood, plasma, 0.1–1 ml	a + g	0.1 *M*. NaOH, TBA^+/benzene–CH_2Cl_2 (9:1), CH_3I	3% OV-17	ECD	121
Demoxepam	Serum, blood, plasma, 0.1–1 ml	h	0.1 *M* NaOH, TBA^+/benzene–CH_2Cl_2 (9:1), CH_3I	3% OV-17	ECD	121
Oxazepam	Serum, 2 ml	g	pH 13, THA^+/CH_2Cl_2, CH_3I	3% OV-225	ECD	109, 135

* The reaction is not an extractive alkylation procedure, because the undissociated bromazepam molecules partition into the other phase; the reaction is probably catalysed by OH^- ions present in the ether layer.

TABLE 4

XANTHINES

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
Acephylline	Urine, 1 ml	b	Acetonitrile, C_2H_5I/K_2CO_3	3% OV-17	FID	43
Theophylline	Serum, 0.1 ml	b	Ethanol, PFB-Br, Na_2CO_3 in H_2O	5% OV-225	ECD	62
Theophylline	Serum, saliva, 1 ml	c	DMA, TMAH, C_4H_9I	3% SP-2250	FID	136
Theophylline	Serum, 1 ml; saliva, 1 ml	c b	DMA, TMAH, $C_5H_{11}I$	3% OV-17	FID	137
Theophylline	Plasma, serum, saliva, 20 µl	b	DMA, TMAH, $C_5H_{11}I$	3% OV-17	NPD	138
Theophylline	Blood, 0.2–2 ml	a + b	DMA, TMAH, C_4H_9I	3% OV-17	MS	139
Theophylline	Serum, 50 µl	b	DMA, TMAH, $C_5H_{11}I$	3% OV-17	NPD	140
Theophylline	Plasma, 0.05–1 ml	b	DMA, TMAH, $C_5H_{11}I$	Capillary, 70-m SE-30	NPD	141
Theophylline	Serum, 1 ml	c	DMA, TMAH, C_4H_9I	3% SP-2250 DB	FID	142
Xanthines (+ metabolites)	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Theophylline	Serum, 100 µl	b	Methanol, TBAH, C_4H_9I ("on-column")*	3% OV-17	NPD	70
Xanthine, hypoxanthine	Serum, 0.5 ml; urine (10 × diluted)	a + b	DMA, TBAH, C_4H_9I	3% OV-17	MS	143
Theophylline	Plasma, 100 µl; rat brain	h	1 *M* NaOH, TPA^+/CH_2Cl_2, PFB-Br	3% XE-60	ECD	104
Theophylline	Serum, saliva, 25 µl	f	0.1 *M* NaOH, THA^+/CH_2Cl_2, $C_5H_{11}I$	3% OV-17	NPD	97
Theophylline	Plasma, 50 µl	f	Buffer (pH 10), TBA^+/CH_2Cl_2, C_2H_5I	Capillary, 25-m OV-225	MS (MIM)	117

* See Section 2.2.3.

TABLE 5

SULPHONAMIDES

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
(a) Sulphonamide diuretics						
Dichlorphenamide	Serum, 0.5 ml; eye liquor	c	DMA, TMAH, CH_3I	3% OV-17	ECD	144
Acetazolamide	Serum, 0.1 ml	f	0.5 *M* NaOH, TPA^+/CH_2Cl_2, CH_3I	1% SE-30	ECD	93
Bendrofluazide	Blood, 1 ml	h	1 *M* NaOH, TBA^+/benzene, CH_3I	1% OV-1	ECD	145
Chlorthalidone	Plasma, serum, urine, 2 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% JXR	ECD	95
Chlorthalidone	Blood, plasma, urine, 1 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% OV-101	ECD	122
Chlorthalidone	Plasma, urine, 1 ml; erythrocytes	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% SE-30	NPD	146
Furosemide	Plasma, 1 ml	g	0.2 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% JXR	ECD	115
Hydrochlorothiazide	Plasma, urine 1 ml; blood cells	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	1% SE-30	ECD	147
Hydrochlorothiazide	Plasma, 2 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	1% OV-225	ECD	98

Mefruside	Blood, urine, plasma, erythrocytes, 1–2 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% SE-30	NPD	148
Mefruside metabolites	Blood, urine, plasma, 1–2 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% SE-30	ECD	149
Sulphonamide-diuretics (as a group)	–	–	0.2 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	1% SE-30 (3% OV-17, 3% QF-1)	ECD	93
(b) Antibacterial sulphonamides						
Sulphonamides (model compounds)	–	–	0.2 *M* NaOH, TBA^+/CH_2Cl_2, PFB-Br	3% and 5% OV-17	ECD	150
Sulphonamides	–	–	pH 10, TBA^+/different organic solvents and alkylating agents	5% OV-17	ECD	87
Sulphapyridine, N-acetylsulphapyridine	Serum, 0.1 ml	f	Buffer (pH 10), TBA^+/CH_2Cl_2, CH_3I	5% OV-17	ECD	118
(c) Other sulphonamides						
Sulphonylureas	–	–	Buffer (pH 6.9), TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	119
Glipizide	Plasma, 0.5 ml	f	Buffer (pH 6.9), TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	119
Tolbutamide	Plasma, 0.1 ml	f	Buffer (pH 6.9), TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	119
(Neo)sulphalepsine			See Table 2, anticonvulsants			122
Saccharin			See Table 7, miscellaneous drugs			151

TABLE 6

ANALGESIC (NARCOTIC AND NON-NARCOTIC) AND ANTI-INFLAMMATORY DRUGS

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
(a) Acetanilide derivatives						
Acetaminophen	Serum, saliva, 1 ml	c	DMA, TMAH, $C_7H_{15}I$ (+ flash methylation on nitrogen)	3% OV-17	FID	152
Acetaminophen	Plasma, urine 0.5 ml	a + b	Acetone, PFB-Br/K_2CO_3	3% SP-2100	ECD	153
Acetanilide derivatives	Liver tissue homogenate	b	Acetone, TMAH, alkyl iodide	3% XE-60	MS (SIM)	57
(b) Anthranilic acid derivatives						
Mefenamic acid	Serum, 2 ml	b	DMA, TMAH, C_4H_9I	3% SP-2250 DA	FID	154
Flufenamic acid, mefenamic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
(c) Indomethacin						
Indomethacin	Plasma, urine, 1 ml	b	Acetone, PFB-Br/K_2CO_3	2% Dexsil 300	ECD	155
Indomethacin	Plasma, 0.5–1 ml	g	TBAHS, CH_2Cl_2, C_3H_7I/$NaHCO_3$ (SLPT catalysis)	3% OV-1 3% XE-60	ECD	112
Indomethacin	Plasma, 0.5–1 ml	g	pH 7, TPA^+/CH_2Cl_2, C_3H_7I	3% OV-1 3% XE-60	ECD	112
Indomethacin	Serum, 0.5 ml	f	THAHS/CH_2Cl_2, C_2H_5I	3% E-350 (SE-52)	ECD	120
(d) Narcotic analgesics and related drugs						
Morphine, nalorphine	Plasma, 1 ml	f	0.2 *M* NaOH, TBA^+/ethyl acetate, PFB-Br	2% OV-17	MS	156
Naloxone	Blood, plasma, 1 ml	h	NaOH soln., TBA^+/CH_2Cl_2, PFB-Br	3% OV-17	ECD	157
Pentazocine	Plasma, 0.5 ml	h	NaOH soln., TBA^+/CH_2Cl_2, PFB-Br	5% OV-17	ECD	103
Pentazocine	Blood, plasma, urine, 0.2–1 ml	h	NaOH soln., $TBA^+/C_2H_4Cl_2$, PFB-Br	3% Dexsil 300	ECD	158

Pethidinic acid, norpethidinic acid	Urine, 1 ml	i	pH 9.5, TPA^+/CH_2Cl_2, PFB-Br	3% and 5% OV-17	ECD MS	123
(e) Phenylacetic and phenylpropionic acid derivatives						
Diclofenac metabolites	urine, 1 ml	g	2.5 *M* NaOH, TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	159
Flurbiprofen	Plasma, 1 ml	b + d	Acetone, PFB-Br/K_2CO_3	3% OV-17	ECD	101
Ibuprofen	Serum, 0.1 ml	b + d	Acetone, PFB-Br/K_2CO_3	10% 3-cyano-propylsilicone	ECD	130
Ketoprofen	Plasma, 400 µl	f	Buffer (pH 7.3), TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	160
Phenoprofen	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
(f) Pyrazolinone derivatives						
Phenylbutazone, oxyphenbutazone	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Sulphinpyrazone + metabolites	Plasma, serum, urine, 1 ml	c	DMA, TMAH, CH_3I	2% OV-17 3% OV-225	NPD ECD MS	59
(g) Salicylic acid derivatives						
Salicylic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Acetylsalicylic acid	–	–	TBAHS, CH_2Cl_2, alkyl iodide or PFB-Br/$NaHCO_3$ (SLPT catalysis)	3% OV-17	FID ECD	113
Acetylsalicylic acid	Plasma, 100 µl	f	pH 6.5., TPA^+/CH_2Cl_2, benzyl bromide	1.5% OV-17	MS	92
Methylsalicylate	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
(h) Miscellaneous						
Niflumic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Tolmetin	Plasma, 25–100 µl	c	Ethyl acetate, PFB-Br/1 *M* K_2CO_3	3% XE-60	ECD	37

TABLE 7

MISCELLANEOUS DRUGS

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
Acenocoumarin	Plasma, 2 ml	a + b	Acetone, PFB-Br/Na_2CO_3	3% OV-17	ECD	29
Benzoic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Benzoic acid	Plasma, urine, 1 ml	b	Acetone, PFB-Br/K_2CO_3	3% OV-17	ECD	161
Benzoic acid	–	–	TBAHS, CH_2Cl_2, alkyl iodide or PFB-Br/$NaHCO_3$ (SLPT catalysis)	3% OV-17	FID ECD	113 114
Benzoylecgonine	Urine, 5 ml	b	DMA, TMAH + PTMAH, C_3H_7I	3% SP-2250 DA	FID	52
Benzoylecgonine	Plasma, 0.5 ml; urine, 1 ml	b	CH_2Cl_2, PFB-Br, pyridine in benzene	3% OV-225	ECD	66
Benzoylecgonine	Urine, 2 ml	f	NaOH, pH 12, THA^+/CH_2Cl_2, C_2H_5I	3% OV-17	MS	99
p-Chlorophenoxy-isobutyric acid (metabolite of clofibrate)	Serum, 25 µl; saliva, 500 µl	b	DMA, TMAH, C_4H_9I	3% OV-17	FID	162
Chloroquinaldol	Plasma, urine, 0.1–0.5 ml	f	Buffer pH 11, TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	94
Chloroquinaldol	Plasma, 0.1–0.5 ml	f	NaOH soln., THA^+/CH_2Cl_2, CH_3I	3% JXR	ECD	122
Clioquinol	Plasma 0.1–0.5 ml	f	NaOH soln., THA^+/CH_2Cl_2, CH_3I	3% JXR	ECD	163
Clioquinol	Plasma, urine 0.1–0.5 ml	f	Buffer pH 11, TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	94
Clonidine	Plasma, 5 ml	c	Acetone, PFB-Br/K_2CO_3	Capillary, *e.g.*, 25-m SE-30, OV-17, SP-1000	ECD	164
Floxuridine	Plasma, 1 ml	d	DMSO, CH_3I, potassium *tert.*-butoxide	3% OV-17	NPD	77
5-Fluorouracil	–	–	(1) pH 10, TPA^+ or THA^+/CH_2Cl_2, CH_3I (2) DMSO, CH_3I, methylsulphinylcarbanion (3) DMA, TMAH, C_4H_9I	2% and 3% SP-2250, 5% XE-60 5% OV-1	FID	75

5-Fluorouridine	Urine, 1 ml	a + d	DMSO, CH_3I, potassium *tert.*-butoxide	Capillary 15-m FFAP	NPD	76
Glutethimide	Plasma, 0.5 ml	e + f	1 *M* NaOH, TBA^+/CH_2Cl_2, C_2H_5I	Capillary, 43-m SE-30	FID	96
Mandelic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
6-Mercaptopurine	Plasma, 1 ml	a + f	0.5 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% OV-225	MS (DID)	165
Methimazole	Plasma, 1 ml	f	Buffer (pH 10), TBA^+/CH_2Cl_2, PFB-Br or benzyl chloride	Capillary, 20-m UCON HB 5100	MS (MIM)	166
Nalidixic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	10% OV-17	FID	167
Nalidixic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17	FID	128
Niclosamide	Urine, 25 ml	h	NaOH soln., TBA^+/CH_2Cl_2, CH_3I	4% and 5% OV-101	ECD	168
Nicotinic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Nucleotides (6-mercaptopurine)		a + i	(1) DMSO, $CH_3I/NaOCH_3$ (2) DMSO, CH_3I, methylsulphinylcarbanion	3% OV-17 3% OV-101	MS	72
Oxyphenonium	Plasma, 1 ml	i	pH 7, TPA^+/CH_2Cl_2/PFB-Br	3% OV-17	ECD	124, 169, 170
Palmitic acid (fatty acids)	Serum, 10–50 μl	h	0.2 *M* NaOH, TBA^+/CH_2Cl_2, PFB-Br	3% and 5% OV-17	ECD	106
Pemoline	Serum, urine, 1 ml	f	0.1 *M* NaOH, TPA^+/CH_2Cl_2, CH_3I	5% FFAP	NPD	171
Phenprocoumon	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17	FID	128
Probenecid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Pseudo-ephedrine	Serum, 1 ml	c	Ethanol, PFB-Br, K_2HPO_4 soln.	5% OV-225	ECD	64
Saccharin	Urine, 4 ml	f	Buffer (pH 7.4), TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	151
Tranexamic acid	Plasma, 200 μl urine	f	pH 9.5, TBA^+/CH_2Cl_2, C_2H_5I (+ derivatization of amino group)	1% OV-225	ECD	172
Warfarin	Plasma, 1 ml	b + d	Acetone, PFB-Br/K_2CO_3	1% OV-17	ECD	129
Warfarin	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17	FID	128

Clean-up procedures that do not fit very well in one of the aforementioned categories are denoted by the letter "i".

Under the heading "Derivatization" the major conditions of the alkylation procedure are given.

The next two columns mention the stationary phases on which the derivatives can be chromatographed and the type(s) of detection used in the analysis, respectively.

The last column refers to the original publication in which the method was presented and where more detailed information concerning the entire analytical procedure can be found.

5. SUMMARY

The various types of alkylation reactions with alkyl halides and their application in the gas chromatographic analysis of acidic compounds of pharmaceutical interest are reviewed. An extensive survey of the use of these methods for the analysis of various (classes of) compounds is given, with special reference to their determination in biological matrices.

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LIQUID CHROMATOGRAPHY OF SUGARS ON SILICA-BASED STATIONARY PHASES

L. A. Th. VERHAAR* and B. F. M. KUSTER
Eindhoven University of Technology, Department of Chemical Engineering, P.O. Box 513, 5600 MB Eindhoven (The Netherlands)

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1. INTRODUCTION

The use of liquid chromatography for the qualitative and quantitative analysis of organic chemicals has made stormy progress in the last 10 years. Using a very finely particulated solid phase, high eluent flow-rates could be applied without loss of separation efficiency. Because high eluent pressures are necessary under such circumstances, the solid material used must be very pressure resistant.

Nowadays, high-performance liquid chromatography (HPLC) is generally performed with porous silica as the solid phase. This silica can be used either pure or in a modified form. Derivatization of the surface hydroxyl groups with organic silyl compounds results in stationary phases with a wide range of different chemical and physical properties. Because the mobile phase can also have different compositions, a

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TABLE 1

SILICA-BASED STATIONARY PHASES USED IN LIQUID CHROMATOGRAPHY OF SUGARS

Trade name	*Supplier*	*Base material*				*Modification*				*Used by (ref.)*
						Chemical				
		Irregular or spherical	*Pore diameter (Å)*	*Surface area (m^2/g)*	*Pore volume (ml/g)*	*Alkylamine*	*Alkylnitrile*	*Octadecyl*	*Dynamical by adding amine to the eluent*	
μBondapak carbohydrate	Waters Assoc.	I	125			+				1,2,4,9,10,18,24,29,30,35,36
LiChrosorb NH_2	Merck	I	60	500	0.75	+				26,33,40
Micropak NH_2	Varian	I	60	500	0.75	+				8
Chromosorb NH_2	Johns-Manville					+				33
Partisil-10 PAC	Whatman	I	60	400			+			7
Chromosorb LC 9	Johns-Manville					+				28
No. 71120	Macherey, Nagel & Co.					+				32
LiChrosorb Si 100	Merck	I	100	250	1.0	+				12
Partisil-5	Whatman	I	60	400		+				15
Silasorb	Lachema	I		260		+	*			39
				600		+				39
LiChrosphere Si 100	Merck	S	100	250	1.2	+				3,11,14
LiChrosorb Si 60	Merck	I	60	500	0.75				+	20,31,34,41,42
?	?	I	130	320					+	23
μBondapak C_{18} Corasil	Waters Assoc.	S	(pellicular)					+		25,37,38
LiChrosorb Si 60	Merck	I	60	500	0.75					5,13,14,16,19,22
Partisil	Whatman	I	60	400						17
μPorasil	Waters Assoc.	I		400						21,27
Corasil II	Waters Assoc.	S	(pellicular)							6

* Own modification.

wide variety of phase systems is the result and most separations can be effected in a short time.

The components to be analysed remain in the liquid phase and, therefore, the method is very well suited to the analysis of thermally unstable compounds such as sugars. It is not surprizing that, after the first publications of Linden and Lawhead[1] and Palmer[2] on HPLC for sugar analysis, many others followed[3–42]. In order to obtain rapidly an idea of how to analyse a given sugar mixture, we made a survey of these publications. The different types of silica and the mobile phases used are mentioned, as well as the method of column preparation, possible pre-column derivatization and the detection method applied. Tables are presented that give an overview of the different sugars and sugar-containing samples analysed by the authors with the methods concerned.

2. COLUMN MATERIAL AND PREPARATION

Silica-based HPLC of sugars is carried out with a variety of silicas, as shown in Table 1. However, for both modified and unmodified materials a base quality is used with pore diameter 60–130 Å and surface area 250–600 m^2/g. Chemical modification is generally performed by the manufacturer, introducing alkylamine or octadecyl groups at surface hydroxyl groups through silyl ether bonds. However, the manufacturers do not give exact details.

Schwarzenbach[3,11,14] and in a similar way Hunt *et al.*[12], Jones *et al.*[15] and Kahle and Tesařík[39] carried out their own modification using 3-aminopropyltrieth-oxysilane. Because their chromatographic results are very similar to those obtained using prefabricated amine columns, the manufacturers' procedures will not be very different.

Apart from chemical modification, physical modification is also applied following the publications of Aitzetmüller and co-workers[20,31,42]. A small amount of amine is added to the eluent and the initially pure silica column acquires an amine coating due to simple adsorption.

Table 2 shows that in half of the references the authors prefer pre-packed columns. For the self-made columns the slurry method and the balanced density method are frequently used.

As shown in Table 3, amine-modified silica columns are almost exclusively used with an acetonitrile (ACN)–water eluent, and the sugars are analysed without derivatization. Pure silica is mainly applied for the separation of sugar derivatives using other eluents; it will be mentioned in section 3.

2.1. Chemically modified amine columns

Jones *et al.*[15] investigated the influence of the silica:silane ratio, the reaction temperature and the reaction time on the amine load using aminopropyl reagent. He found that the amine load was virtually independent of the temperature and time of reaction but was strongly dependent on the amount of reagent added. Too much silane leads to "strand structures" of several silica particles owing to reagent polymerization, especially if the silica has not been sufficiently well dried. Such material cannot be packed properly and columns with low separation efficiency are the result.

TABLE 2

SILICA-BASED HPLC COLUMNS FOR SUGAR ANALYSIS

Column preparation method	*Used by (ref.)*		
	Unmodified silica gel	*Alkylamine modification*	*Octadecyl modification*
Slurry packed	14,20,31, 34,41,42	3,11,12,14, 26,28,39	
Balanced-density packed	13,16,19, 22	7,15,32,33, 40	
Pre-packed	6,17,21,27	1,2,4,8,9,10, 18,24,29,30, 35,36	25,37,38

According to Woidich *et al.*[26], LiChrosorb NH_2 columns are serviceable for approximately 3 months when in permanent use. The amine groups bound by silyl ether are stripped off gradually, and the silica is also slowly dissolved by the eluent; decreased retention and resolution result. Although decreased retention can be compensated for by a lower water content of the ACN–water eluent, the column will finally become of inferior quality. Continuous addition of amine to the eluent was tried in order to overcome this problem.

TABLE 3

PHASE SYSTEMS USED FOR CHROMATOGRAPHIC SEPARATION OF SUGAR COMPOUNDS

Mobile phase		*Stationary phase*			*Separation*		*Ref.*
Acetonitrile–water	*Other composition*	*Silica gel, pure*	*Silica gel, chemical modification*		*Directly*	*After derivatization*	
+			+	Amine	+		1–4,8–12,14,15,18,24, 26,28–30,32,33,35,36, 39,40
+			+	Amine (physically)	+		20,23,31,34,41,42
+			+	Nitrile	+		7
+			+	Octadecyl		+	25
+		+			+		17,22
+		+				+	17
	+		+	Amine	+		32,40
	+		+	Octadecyl	+		37,38
	+	+			+		5
	+	+				+	6,13,14,16,17,19,21,27

2.2. Physically modified amine columns

Aitzetmüller[20] used a polyfunctional amine (*A*mine *M*odifier *I*) of concentration 0.01 % together with LiChrosorb Si 60, and obtained results similar to those with chemically modified columns, if slightly higher water contents in the eluent were applied. This higher water content seems to be in contradiction with the experience of Wheals and White[23], who investigated the applicability of several amines and found that retention ability decreased in the order chemically bonded aminopropyl (and no amine added to the eluent), polyamine, diamine, amine. According to White *et al.*[41], 1,4-diaminobutane is better than other amines, including polyamines, if a series of glucose polymers have to be separated. Apparently the proper choice of amine is dependent on the type of sugars to be analysed. Aitzetmüller *et al.*[31] also tested silicas other than LiChrosorb Si 60 and obtained similar column qualities.

Aitzetmüller, Wheals and White and White *et al.* used an amine concentration of 0.01 %. If one starts with an amine-free column it takes a long time to reach an equilibrium state using this concentration. According to Aitzetmüller[42], the procedure can be speeded up by using a 0.1 % concentration overnight, subsequently changing it to 0.01 %.

Aitzetmüller also considers the use of a pre-column to be important. If this column is packed with silica of similar quality, the silica in the analytical column will not hydrolyse owing to the high eluent pH. The pre-column should be mounted before the injection valve in order to cause no increase in peak widths due to the dead volume created on silica dissolution.

The following advantages and disadvantages of physically modified amine columns were mentioned by Aitzetmüller[42]:

Advantages:

constant retention after prolonged use;

cheap material compared with the expensive modified silica;

easy modification;

the possibility of using eluents with a higher water content, which results in higher sugar solubility and lower peak tailing;

an increased isocratic range, which means that a relatively wide range of sugars with different molecular weights can be separated with an eluent of constant composition;

less sensitive to pollution by samples.

Disadvantages:

preparative chromatography can be difficult owing to the presence of amine in the eluent;

certain detection methods give problems when using amine in the eluent, *e.g.*, UV and moving-wire detection;

acid-containing samples, *e.g.*, from citrus-drinks, cause baseline disturbance owing to the variable amine delivery.

The changed sensitivity to the refractive index (RI) detector as a result of the higher water content of the eluent was considered to be a disadvantage by Aitzetmül-

ler, whereas Woidich *et al.*[26] considered it to be an advantage because the sensitivity for sugars increases.

2.3. *Octadecyl columns*

To separate an acetylated starch hydrolysate, Wells and Lester[25] used a RP-18 column. On changing the ACN content in an ACN–water eluent during the analysis from 10 to 70% a good separation is obtained from DP 1 up to DP 35 (DP = degree of polymerization).

Heyraud and Rinaudo[37] and Fonknechten *et al.*[38] analysed underivatized sugars on an octadecyl column with pure water as the eluent. Both groups applied a low eluent flow-rate (0.1 and 0.33 ml/min, respectively, using 4 mm I.D. columns). Heyraud and Rinaudo used a column temperature of 3.5°C: they stated that the separation efficiency decreases with increasing temperature and with increasing eluent flow-rate.

2.4. *Silica columns*

As mentioned before, pure silica is used almost exclusively for the separation of derivated sugars. Binary or ternary mixtures of organic solvents of different polarity are used as eluents.

Rocca and Rouchouse[5] eluted underivatized sugars on a LiChrosorb Si 60 column with a mixture of ethyl formate, methanol and water. McGinnis and Fang[17] mentioned a separation of underivatized sugars with ACN–water (9:1) as the eluent, whereas Van Olst and Joosten[22] used ACN–water (99.9:0.1).

3. ELUENT SYSTEMS AND ELUTION MECHANISM

3.1. *Chemically and physically modified amine columns*

A mixture of acetonitrile and water is the most commonly applied eluent with this type of column. The water content is usually between 10 and 40% (v/v), depending on the sample composition. An increase in the water content decreases the retention without changing the elution sequence. Fig. 1 shows a plot of elution times of sugars obtained by Meagher and Furst[4] against the number of carbon atoms in the molecule. As can be seen, the elution sequence follows the order of molecular size. Identical results are obtained with similar data from other references. On the one hand it is impossible to change the elution sequence, which is a limitation, but on the other hand the elution time gives qualitative information about the molecular size of the sugar.

The separations are carried out at room temperature. Hunt *et al.*[12] found that elution times decreased with increasing temperature, but at the same time the noise level of the RI detector increased.

Different elution mechanisms are proposed in the literature. Meagher and Furst[4] considered it to be reversed-phase chromatography, probably because of the organic modification of the silica. Because an increased water content in the eluent or an increased polarity speeded up the elution, Rabel *et al.*[7] and Hettinger and Majors[8]

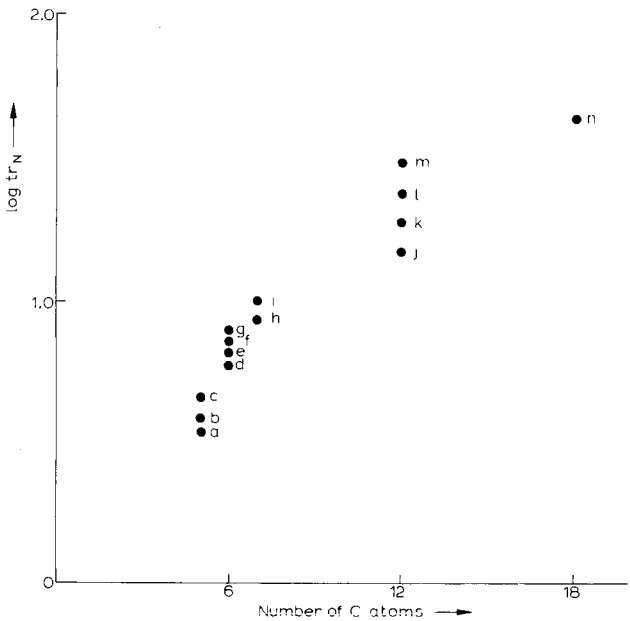


Fig. 1. Plot of elution times (tr_N) of sugars *versus* carbon number (data from Meagher and Furst[4]). Stationary phase, μBondapak carbohydrate; mobile phase, acetonitrile–water. Compounds: (a) ribose; (b) xylose; (c) L-arabinose; (d) fructose; (e) mannose; (f) glucose; (g) galactose; (h) mannoheptulose; (i) glucoheptose; (j) sucrose; (k) maltose; (l) lactose; (m) melibiose; (n) raffinose.

concluded the process to be normal-phase chromatography. Hettinger and Majors assumed that a competitive interaction of water and sugar with the bonded phase caused the retention. Wong-Chong and Martin[29] stated it to be adsorption chromatography, where the eluent polarity is important. Binder[32] explained retention by hydrogen bonding between sugar hydroxyls and amine groups in the solid phase. With more hydroxyl groups, there is more hydrogen bonding and, therefore, increased retention. D'Amboise *et al.*[33] gave the same opinion; further, he stated that retention increases if the hydroxyls are one-sided in the sugar molecule. This explanation seems to be supported by the results of Jones *et al.*[15], who found a linear relation between k' values of several sugars and the amine load. At a high load the k' values become constant, which Jones *et al.* ascribed to phase polymerization after a monolayer of amine has been formed. Verhaar and Kuster[43] measured a higher water content in the stagnant liquid phase in comparison with the eluent using a LiChrosorb NH_2–ACN–water system. This is probably caused by the hydrophilic nature of the amine. Sugars preferred a water-rich phase and so retention occurred.

Attempts have also been made to use alternative eluents with chemically modified silica columns. Rabel *et al.*[7] obtained poor results with methanol–water as the eluent, which they ascribed to the relatively poor solution of the bonded phase in methanol. The results obtained with an ethyl acetate–ethanol–water mixture, as applied by Binder[32], are also less satisfactory than those obtained with ACN–water as the eluent. Similar results to those obtained with the latter eluent can be obtained with a mixture of acetone, ethyl acetate, and water, as reported by Müller and

Siepe[40]. This eluent has the advantage of the absence of poisonous acetonitrile; an increase in the water content has a similar influence on the elution pattern to that in the ACN–water system.

Because elution patterns can hardly be changed, data from different references could be combined, and relative retention times for some common sugars are presented in Table 4.

TABLE 4

RELATIVE RETENTION TIMES OF UNDERIVATIZED SUGARS ON AN ALKYLAMINE-MODIFIED SILICA COLUMN WITH ACETONITRILE–WATER (80:20) AS ELUENT

Sugar	*Relative retention time**	*Sugar*	*Relative retention time**
Ribose	60	Psicose	55
Lyxose	65	Fructose	80
Xylose	70		
Arabinose	80		
Glucose	100	Rhamnose	65
Mannose	90	Fucose	70
Galactose	110		
Maltose	180	Glycerol	40
Cellobiose	180	Xylitol	75
Lactose	200	Arabinitol	80
Saccharose	150	Glucitol	95
Melibiose	240	Mannitol	105
Raffinose	350	Galactitol	105

* Glucose = 100

Normally, amine columns do not separate anomeric forms of reducing sugars. Verhaar and Kusters[43] showed this to be caused by the catalytic activity of the bonded amine for the mutarotation reaction. At high reaction rates the anomeric forms have the same average residence time and a single symmetrical peak is the result, and interpretation of the chromatogram is simplified, as reported by Cerny *et al.*[36]. The addition of acids or salts to the eluent, investigated by Rabel *et al.*[7], influences the peak quality, but no clear mechanism could be given. By converting an amine column into the sulphate form, Kahle and Tesarik[39] succeeded in separating anomeric forms using ACN–water as the eluent and a column temperature of 0°C. For all D-sugars investigated the α-form elutes before the β-form, which should be due to the larger number of equatorial hydroxyl groups for the β-form, and these groups mainly defined the retention.

3.2. Octadecyl columns

As mentioned before, an excellent separation of acetylated starch hydrolysate was obtained by Wells and Lester[25] by applying a negative water gradient in an ACN–water eluent. Poor results were obtained by Heyraud and Rinaudo[37] using pure water as the eluent for the separation of mono- and disaccharides. The elution

volume of the first and the last sugars in a series of 17 differed by only 1 ml, whereas for the baseline separation of two sugars a difference of 0.4 ml is necessary. According to Heyraud and Rinaudo[37] and Fonknechten *et al.*[38], this system can be better applied to the separation of sugars with different numbers of monomeric units. In about 30 min an almost complete separation can be obtained from DP 1 up to DP 10. Especially at low column temperatures double peaks occur for the anomeric forms because this system lacks a mutarotation catalyst, in contrast to the system mentioned previously.

3.3. Silica columns

Derivatization of sugars with acetyl-, benzoyl- or nitrobenzoate compounds leads to increased retention with increasing molecular weight when using silica columns and a mixture of organic solvents as the eluent. Generally, separate peaks for the anomeric forms are obtained on acetylation and also on benzoylation with benzoyl or nitrobenzoyl chloride. Single peaks are obtained if derivatization is accompanied by ring opening, as carried out by Thompson[21] using as the reagent benzoyl chloride and benzyloxyamine. Because more peaks need more space in the chromatogram, the number of sugars that can be separated is decreased by the occurrence of anomeric forms and, according to Cerny *et al.*[36], quantitative interpretation is also hampered.

Thiem *et al.*[19] analysed acetylated sugars with acetone–hexane as the eluent, and reported a k' value that increased with decreasing eluent pressure or eluent flow. However, in an earlier publication Thiem *et al.*[16] reported the opposite effect.

4. DETECTION METHODS

The different detection methods applied are presented in Table 5. The refractive index detector is most generally used, and almost solely for underivatized sugars, as is clear on comparing the data in Tables 5 and 3. Derivatized sugars are preferably detected with UV absorption. Apart from the methods mentioned in Table 5, the applicability of a micro-polarimeter and mass detector will also be discussed.

4.1. Refractive index detector

This method is universally applicable, only moderately sensitive and very sensi-

TABLE 5

DETECTION SYSTEMS USED IN SILICA-BASED HPLC

Method of detection	*Ref.*
Refraction index	1–12,14,15,17–20,22–24,26, 28–32,34–42
Ultraviolet	6,8,13*,16*,19,21*,27*,32
Visible light	28**, 33**
Moving wire	25

* After pre-column derivatization.

** After post-column derivatization with tetrazolium blue.

tive to temperature fluctuations. The refractive index of the compounds to be detected should differ, of course, from that of the eluent. The sensitivity of the RI detector towards a given compound is dependent on the eluent composition and therefore the method is not suitable for gradient systems. According to Woidich *et al.*[26] and Black and Glover[35], the response for sugars increases with increasing water content of an ACN–water eluent. Johncock and Wagstaffe[34] considered both long-term and short-term instability to be due to temperature fluctuations in the detector cell. Hunt *et al.*[12] mentioned increased noise when using higher column temperatures. Our experience is that at reasonably constant room temperature no special temperature control is necessary.

The detection limit depends on the retention time and the column quality. Minimal detectable amounts of 20 μg of sugars were reported by Palmer[2], whereas Cerny *et al.*[36] mentioned a value of 3 μg at a signal-to-noise ratio of 5. Schwarzenbach[14] reported that the minimal detectable amount can be decreased by a factor of several thousand by using nitrobenzoate derivatization in a pre-column mode.

4.2. *Ultraviolet detector*

This method is suitable only for UV-absorbing compounds, it is very sensitive and is only moderately influenced by temperature fluctuations. Sugars can be detected directly in the range 185–195 nm. Using an ACN–water eluent, the ACN must be of analytical-reagent grade because impurities generally give strong UV absorption. Derivatized sugars can be detected at higher wavelengths and generally their detectability is improved drastically.

Hettinger and Majors[8] and Binder[32] used 192 and 188 nm, respectively, for direct detection. According to Hettinger and Majors, for glucose and fructose the sensitivity is approximately 10 times higher than that in RI detection. For sugars of higher molecular weight the sensitivity decreased to the values for RI detection. Binder analysed a number of monosaccharides and found almost equal sensitivity for the two methods.

Thiem and co-workers[16,19] detected acetylated sugars at 220 nm. In the eluent used, acetone–*n*-hexane, only a small amount of acetone can be used because of its own absorption. Lehrfeld[6] and White *et al.*[27] detected benzoyl esters of sugars at 254 nm, a commonly available fixed wavelength in UV detectors, and obtained a 1000-fold increase in sensitivity. According to Nachtmann and Budna[13], an even higher sensitivity can be achieved by esterification with nitrobenzoate, so this could be a good method in trace analysis.

A disadvantage of derivatization, especially with large ester groups, could be the reduction in the differences in molecular structure, through which the separation of hexoses, for example, could become more difficult. However, for sugars with a different number of hydroxyl groups such as starch hydrolysate products, separation could be more efficient. Using an eluent composition that does not absorb UV light at the choosen wavelength, a gradient system can be used, as shown by Lehrfeld[6].

4.3. *Visible light–fluorescence detection*

In comparison with RI detection, an increased sensitivity for sugars can be

TABLE 6

APPLICATION OF SILICA-BASED CHROMATOGRAPHIC MATERIALS TO SUGAR ANALYSIS IN DRINKS, FOODS, PROCESS AND PLANT MATERIALS AND OTHER SUGAR-CONTAINING SAMPLES

Material	*Ref.*	*Material*	*Ref.*
Drinks:		*Proces materials:*	
Apple cider	2,9	Cellulose hydrolysate	2,9,37,43
Beverage	7	Dextran	43
Breakfast drinks	26	Fructose isomerization	24
Fruit drinks	9,11,14, 36,40,42	Glucose isomerization	22
		High fructose syrup	9,22
Lemonades	11,42	Inulin hydrolysate	33
Milk	7,9,30	Polyglycerol	9
		Starch syrup	3,7,9,25,28,37,42
Foods:		Xylan	28,43
Breakfast cereals	15		
Carrot purée	40	*Plant materials:*	
Cocoa	9	Cotton seed	10
Confectionery	34	Maple sugar	7
Corn products	40	Soybeans	7,9,10,15,31,35
Fruit purée	40	Sugar cane	29
Honey	9,11,42	Sun flowers	10
Ice cream	15,36	Tomato cell wall	9
Jam	42	Wheat straw	18
Kidney beans	9	Wood	28
Peanuts	10		
Sauces	40	*Others:*	
Toffees	12	Intravenous high calorie electrolyte solution	7
		Narcotics	23
		Pharmaceutical syrup	13
		Rat urine	4
		Tobacco humectant	14
		Toothpaste	14

TABLE 7

METHODS OF SUGAR DERIVATIZATION FOR HPLC ANALYSIS

Derivatization method	*Ref.*
Methylation	17
Acetylation	16,17,19,25
Benzoylation:	
With benzoyl chloride	6,27
With 4-nitrobenzoyl chloride	13,14,27
With benzyloxyamine hydrochloride	21

obtained by post-column derivatization, already used in sugar analysis with ion exchangers and borate buffers[44]. In HPLC narrow peaks are obtained, so the continuous flow reaction system in wide-bore glass tubes is replaced by a reaction system in capillary tubes having a much lower peak broadening. Because these tubes have a

TABLE 8

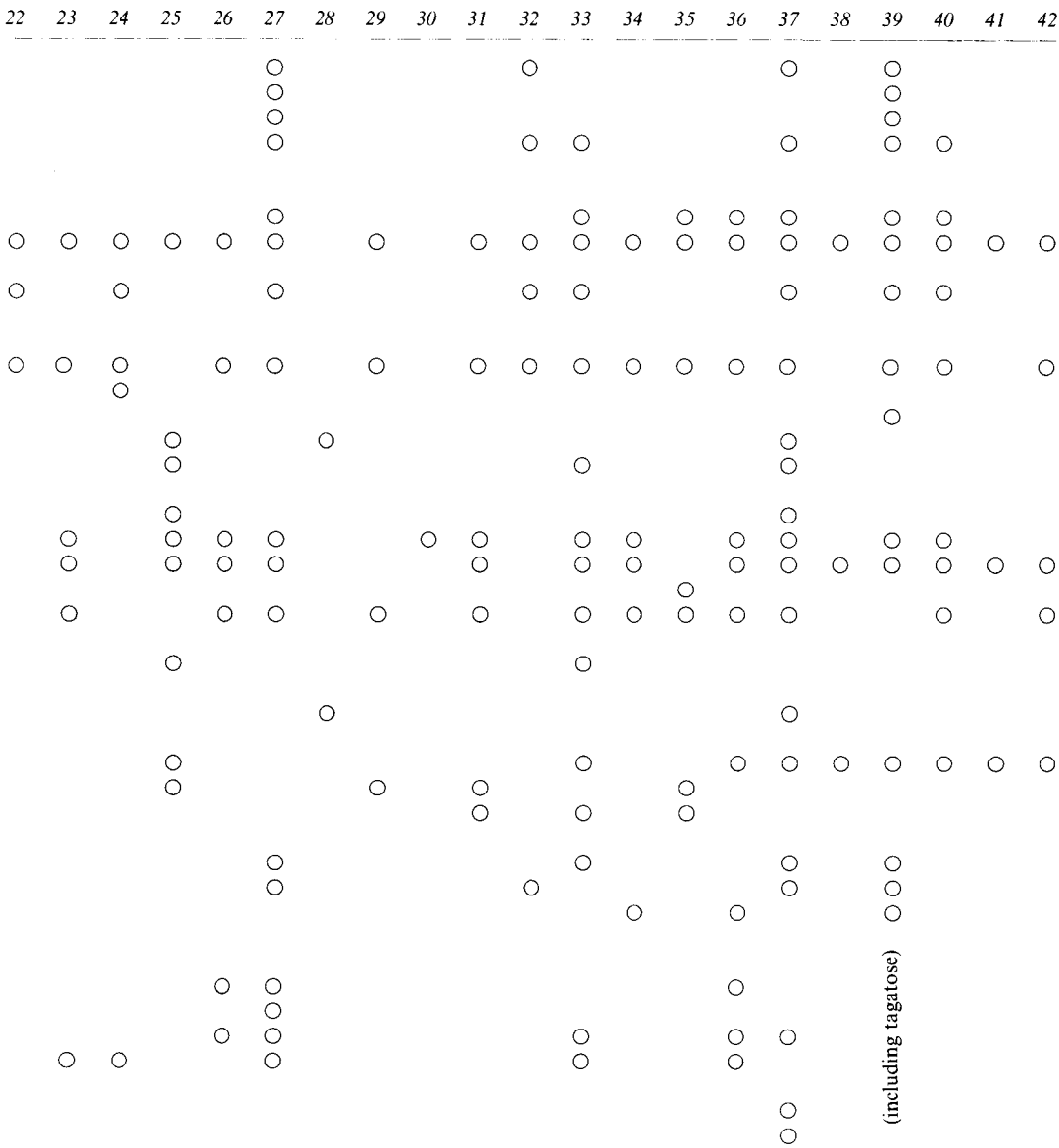
SUGAR COMPOUNDS CHROMATOGRAPHED

Sugar compounds	*Ref.*																				
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*
Arabinose		○	○	○	○		○	○	○								○	○	○		○
Lyxose							○												○		
Ribose		○	○	○	○		○	○				○					○		○		○
Xylose		○	○	○	○	○	○	○	○								○	○	○		○
Allose																○			○		
Galactose	○	○		○	○	○	○	○	○							○	○		○		○
Glucose	○	○	○	○	○	○	○	○	○	○	○	○	○		○	○	○	○	○	○	○
Idose																○			○		
Mannose		○		○		○	○		○							○	○	○	○		
Tallose																○			○		
Fructose	○	○	○	○	○		○	○	○		○	○	○		○		○	○		○	○
Psicose									○												
Sorbose					○																
Cellobiose		○			○		○		○								○	○	○		
Gentiobiose									○										○		
Isomaltose																					
Lactose		○	○	○	○	○	○	○	○			○	○		○				○	○	○
Maltose	○	○	○	○	○	○	○		○		○				○				○	○	○
Melibiose	○			○			○														
Sucrose	○	○	○	○	○	○	○	○	○	○	○	○	○		○				○	○	○
Trehalose					○			○		○											○
Cellotriose (and higher)		○																○			
Maltotriose (and higher)	○		○			○	○		○												
Raffinose	○			○	○		○	○	○	○										○	
Stachyose									○	○											
Fucose		○		○			○														
Rhamnose		○	○	○					○										○		
Glycerol			○	○		○			○		○		○							○	
Erythritol																	○				
Arabinitol									○					○			○				
Xylitol			○											○							○
Galactitol					○				○												
Glucitol			○		○				○		○		○	○						○	
Mannitol					○				○		○			○							○
Cellobitol																					
Maltitol																					

(including rare sugars)

(including altrose)

high flow resistance, an additional pump is necessary which can deliver a liquid flow at the proper pressure. Post-column derivatization with tetrazolium blue and absorption measurement at 530 nm was used by Noël *et al.*[28] and D'Amboise *et al.*[33]. Another type of post-column derivatization is the reaction of sugars with cerium(IV),



as mentioned by Katz and Pitt[45]. This permits fluorescence measurement, as reported by Mrochek *et al.*[46].

4.4. *Moving-wire detection*

In this method, underneath the column exit a clean platinum wire is moved continuously and becomes moistened with the pure eluent or with eluent containing the component to be detected. The eluent is evaporated and the residual material is combusted to carbon dioxide in a pyrolysis oven. This carbon dioxide is reduced to methane, and the methane is detected with a flame-ionization detector (FID). The method can be combined very well with a gradient system, as reported by Wells and Lester[25]. However, the manufacturer, Philips–Pye Unicam, took this detector out of production in 1978.

4.5. *Polarimetric detection*

The development of a micro-design of the polarimeter made this instrument applicable in HPLC, as shown by Yeung *et al.*[47] and Böhme[48]; the latter applied it to the detection of sugars. Because the anomeric forms give a different response, an additional problem occurs in quantitative analysis. If anomers elute in the same time, for accurate quantitation the anomer ratio should be constant or known. However, the polarity of the response can give additional qualitative information. The applicability to gradient elution using optically inactive eluents is another advantage. The sensitivity for sugars is lower than that in direct RI or UV detection.

4.6. *Mass detection*[49]

In the mass detector, designed by Applied Chromatography Systems Ltd., the eluent stream containing the solute is nebulized and carried by an air stream through a heated column. The eluent evaporates, leaving a fine mist of solute particles which pass through a light beam. Light scattering occurs and is detected by a photomultiplier. Except with eluents to which salts have been added, the method can also be used for sugar detection and the minimal detectable amount is about 500 ng.

5. MISCELLANEOUS

5.1. *Sample pre-treatment*

Before injection on to the chromatographic column can be carried out, the sample generally needs pre-treatment. Sugars often occur in natural substances together with proteins and fats, which interfere in the analysis. According to Meagher and Furst[4], proteins can cause considerable pressure resistance in the column if not removed, which can be effected by ultrafiltration or dialysis. Because the pre-treatment is closely related to the origin of the sample, a survey of sugar-containing products analysed by different authors is given in Table 6. In the publications concerned, good descriptions of the pre-treatments are generally given.

Another pre-treatment is derivatization, as mentioned before. Table 7 lists the different methods used.

5.2. *Choice of chromatographic system*

The choice of the chromatographic system depends on a number of factors, such as available apparatus and sugar concentration in the sample. Because it is impossible to mention all factors, Table 8 lists many sugars that have been analysed. This table makes it easy to find information about the analysis of a particular sugar.

6. SUMMARY

A review is given of sugar analysis by liquid chromatography using silica columns. Aspects covered are column materials and preparation, chemically and physically modified amine columns, octadecyl- and unmodified silica columns; eluent composition and elution mechanisms for the different types of columns used; detection methods, RI and UV detectors, visible light, fluorescence, moving-wire, polarimetric and mass detection; and sample preparation and origin of samples.

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This book provides the first comprehensive coverage of all aspects of the theory, design, operation and applications of the electron capture detector (ECD) from the chromatographer's point of view. In addition, an up-to-date look at the ancillary techniques of selective electron-capture sensitization, atmospheric pressure ionization and plasma chromatography has been included. ECD users will find the solutions to instrumental and technical problems which arise during practice particularly valuable. These have been derived from the experiences of the internationally distinguished team of authors.

Each chapter has been prepared by experts in their field and provides an in-depth coverage of its topic. The basic theory of the mechanisms of electron capture detection is included. Practical sections form the bulk of the book and are devoted to such topics as the construction and operating principles of the detector, including the establishment of instrument design criteria, and the different methods of derivatization. A more personal touch is provided by the inventor of the ECD, J.E. Lovelock, in his review of the development of the technique. Other chapters illustrate the importance of ECD in trace analysis in environmental and biomedical research. A unique feature is the extensive tabulation of all the pertinent data concerning the use of ECD in gas and liquid chromatography.

For those analytical chemists who use chromatography in their research, this book should become a standard text.

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