The Analyst

Supercritical fluid extraction of pesticides from meat: a systematic approach for optimisation

René K. Juhler†

Institute of Food Research and Nutrition, Danish Veterinary and Food Administration, Mørkhøj Bygade 19, 2860 Søborg, Denmark

A method for quantification of pesticide residues in meat and fatty matrices was developed using supercritical fluid extraction (SFE). The SFE method allows selective extraction of residues and subsequent gas chromatography analysis without further clean-up. Quantification was done by GC using nitrogen–phosphorus detection and electron capture detection. Initial method development was made using organophosphorus pesticides (OPPs). The dependence of fat and OPP residue recovery on supercritical fluid density, temperature, flow rate and extraction time was investigated through a reduced factorial design. Since temperature and density were found to have pronounced effect on the recovery of OPPs these extraction parameters were studied using a new arbitrary measure for co-extractivity. An optimisation score was established as relative pesticide recovery subtracted by relative fat recovery. Using this algorithm a response plane was modelled varying the primary factors temperature and density. The applicability of this approach and the algorithm was verified. The polarity range covered by the SFE method was demonstrated using OPPs: chlorpyrifos, chlorpyrifos-methyl, malathion, pirimifos-methyl and prothiofos. Additionaly the final method was evaluated using four pesticides that are not OPPs: carbofuran, phorate, procymidone and vinclozolin. All pesticides showed good recovery (78–95%), and limits of detection (0.01–0.03 mg kg^{-1}) and limits of determination (0.01–0.05 mg kg^{-1}) meet the requirements set by the European Council (Directive 96/33/EEC). Compared to traditional methods based on organic solvent extraction, the SFE method is fast, less labour intensive, uses smaller amounts of potentially harmful solvents and has the potential to be fully automated.

Keywords: *Factorial design; fat; food; monitoring; optimisation; organophosphorus pesticides; residues; supercritical fluid extraction*

An extension of monitoring programs for pesticide residues can be foreseen in years to come. More samples are to be analysed for more pesticides at still lower levels. To a great extent this is the consequence of increased consumer concern about food quality and the establishment of numerous and lower maximum residue limits (MRLs). Consequently, government and industry laboratories need fast yet sensitive methods for residue analysis. Analysing pesticides in fatty matrices by supercritical fluid extraction (SFE) may prove superior to traditional, organic solvent based methods. Selectivity and swiftness of the extraction process coupled with the potential for fully integrated and automated protocols are characteristics of SFE. Using selective extraction off-line clean-up can be omitted, and the very same SFE method can be used for both screening and quantification. Furthermore, SFE using carbon dioxide possesses well-known attributes such as environmentally benign nature coupled with low cost and wide availability of carbon dioxide.

The supercritical state has been known for more than a century, yet the physical properties, kinetics, and thermodynamics underlying SFE still remain to be fully elaborated. Additionally, several factors in sample preparation, extraction, and trapping can be varied to improve performance of a SFE method.[1,2] Developing SFE methods for residue analysis therefore encompass some intuition. Part of the missing knowledge is compensated for using models describing the processes underlying SFE. Typically, these models are based upon simplified and idealised systems that are adjusted to experimental observations. Insight into solubility phenomena,[3] diffusion theory,[4] and chromatographic processes[5] provides the basis of most models.[6–8] These also cover aspects on solute accessibility and cosolvent effects in relation to effects of active matrix sites.[9–11] Models are refined as further data describing the supercritical state emerges,[12] and techniques such as computerised neural networks and molecular modelling of solute–solvent interactions[13] are used.

As a consequence of high fat solubility in supercritical CO_2, SFE has been evaluated by several groups for the determination of composition and total fat content of food matrices.[8,14–16] SFE has also been used for removal of lipophilic compounds, oil and cholesterol from food.[17,18] Yet, using SFE for determination of lipophilic residues, the readily extracted fat must be considered an undesirable matrix contamination. Thus, coextracted fatty substances cause problems using nitrogen–phosphorus detection (NPD) and electron capture detection (ECD) for determination of compounds such as organophosphorus pesticides. Optimising SFE for extraction of lipophilic compounds from fatty matrices thus implies the establishment of conditions allowing residues to be extracted while fatty matrix substances remain in the matrix. Factorial experimental designs in combination with simplex methods and response surface plotting have been used for optimising the SFE of environmental pollutants.[19] Compounds were extracted from spiked C_{18}-cartridges wherefore matrix interaction was not considered. However, the selective extraction of contaminants from fatty matrices was studied by Gere *et al.*,[20] who also described 'the fatband', *i.e.*, provided tabulated data which can be used for predicting SFE conditions which are likely to result in high fat recoveries. SFE has been employed for extraction of pesticides and chlorinated compounds such as PCBs from fatty matrices.[2,6,21] Characteristically, the end determination is made by GC with ECD or NPD, and clean-up of extracts is often required. Clean-up is typically performed off-line whereby the potential for automation is reduced. It has been accomplished by conventional sample preparation procedures such as adsorption chromatography.[22] *In situ* clean-up of SFE extracts has been made using neutral alumina for adsorption of lipids[23] when extracting sulfonamides from chicken tissue. Extracting organochlorine pesticides from fat and lard supercritical fluid based clean-up was obtained by introducing either a silica or neutral alumina column situated after the extraction vessel.[24]

† Present Address: Department of Geochemistry, Geological Survey of Denmark and Greenland, GEUS, Thoravej 8, 2400 Copenhagen NV, Denmark.

Considering supercritical fluid chromatography (SFC) hyphenated techniques such as on-line SFE–SFC–GC have been used for analysis of organochlorine and OPPs in fatty food[25] and using SFE–SFC–MS it was possible to extract carbamate pesticides from chicken and bovine tissue,[26] the fatty acids being retained on a mixture of diol and C_{18} sorbents within the extraction vessel. Being a supercritical technique SFC is very suitable for combination with SFE. However, at present SFC cannot be considered a common methodology for routine laboratories. SFE combined with immuno and enzyme inhibition assays has been used for developing screening methods for pesticides in fat and meat products.[27,28] Yet enzyme assays tend to be less versatile, cross-reactivity and matrix effects are critical[29,30] and sample clean-up is necessary when using SFE based enzyme immunoassays.[28] In a recent work Hopper has been able to extract organochlorine and organophosphorus pesticides from fats using SFE.[21] Evaluating numerous pesticides and food matrices SFE was found to be comparable to the traditional method used in the United States Department of Agriculture (USDA) Total Diet Study. Still, Florisil clean-up of SFE extracts was required and as presented the methodology cannot readily be totally automated.

The aim of the present study was to optimise SFE conditions for selective extraction of lipophilic pesticide residues allowing direct GC analysis of extracts. Extraction parameters were evaluated to exploit the selectivity of SFE for the high level recovery of OPPs from spiked bovine tissue without resorting to more involved procedures requiring the use of clean-up stages. The main novelty of this work lies in the development and successful utilisation of a co-extractability parameter for method development. The outcome of the present work is an optimised SFE–GC–NPD method. Compared to traditional methods based upon organic solvent extraction, it is fast and less laborious and uses smaller amounts of potentially harmful solvents. Also it has the potential for fully automated screening and quantification of lipophilic pesticides residues in meat and fatty matrices.

Experimental

Chemical reagents

Acetone, acetonitrile, cyclohexane, ethanol, ethyl acetate, heptane, methanol, and tetrahydrofuran were purchased from Rathburne (Walkerburn, UK) whereas propan-2-ol was purchased from Merck (Darmstad, Germany). All organic solvents were HPLC grade. Chem Tube-Hydromatrix was purchased from Varian (Harbor City, CA, USA). Pesticide standards of carbofuran (99.1%), chlorpyrifos (99.7%), chlorpyrifos-methyl (98.3%), malathion (96.3%), parathion (99.7%), phorate (95.3%), pirimifos-methyl (99.0%), procymidone (99.7%), prothiofos (93.2%) and vinclozolin (97.0%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Glass only was used for handling extracts and standards in order to reduce the risk of residue loss due to adsorption. Nitrogen (99.996%) was used for GC and solvent evaporation. For GC hydrogen (99.995%) was used as make-up gas and helium (99.999%) as carrier gas.

Sample preparation and meat characterisation

Beef was purchased from a local market, ground in a food chopper and stored at −20 °C until analysis. The characteristic meat particle size was 1–2 mm. For characterisation moisture was measured[31] by drying five samples of each 2 g for 17 h at 102 °C and the pH was determined[32] by thoroughly mixing 8 g of sample in 12 ml deionized water and measuring using a Radiometer PHM 82 (Copenhagen, Denmark). A SFE sample was prepared using thawed meat. For recovery experiments a pesticide mixture for spiking was made using stock solutions of the individual pesticide (2–20 μg ml^{-1}) dissolved in ethanol. Meat samples of 1.0 g were spiked with 10–50 μl of a pesticide mixture dissolved in ethyl acetate–cyclohexane (1 + 1 v/v). Samples of 1.0 g were transferred to extraction thimbles whereafter the void volume was filled with Hydromatrix material.

SFE extraction of meat samples

SFE was performed using a 7680T Hewlett-Packard (Waldbronn, Germany). In the optimised method samples were extracted in a randomised sequence with pure CO_2 at a flow rate of 2 ml min^{-1} for 2 h at 0.40 g ml^{-1} and 95 °C. Extracted compounds were collected on a Florisil trap at 35 °C. To collect the pesticide residues trap temperature was raised to 50 °C and eluted with 1.5 ml of heptane (Fraction 1) and subsequently with 1.5 ml of acetone (Fraction 2). The SFE apparatus was cleaned using 10 ml of heptane and 5 ml of acetone.

Gas chromatography

Pesticide contents of the extracts were analysed using a Hewlett-Packard 5890A GC using a deactivated fused silica capillary tubing as injection port liner and a Hewlett-Packard 7673A automatic injector. Splitless injection of 2 μl was used and the injector temperature was 260 °C. The column was a DB1701 (J&W Scientific, Folsom, CA, USA) capillary coated with a phenyl-cyanopropyl-methyl phase (30 m × 0.32 mm id, 0.5 mm od, 0.25 μm film thickness). Helium (1 ml min^{-1} on column) was used as carrier gas. Detection was by NPD using hydrogen as make up gas and a temperature set point of 250 °C. The temperature programme was: 1 min at 70 °C followed successively by a 30 °C min^{-1} increase to 180 °C and 4 °C min^{-1} to 260 °C maintaining this final temperature for 20 min. Total GC analysis time was 45 min. Quantification was performed using standards in ethyl acetate–cyclohexane (1 + 1 v/v). To sample extracts and calibration standard 100 μl of 3 μg ml^{-1} parathion in ethyl acetate–cyclohexane (1 + 1 v/v) was added. Quantification was performed by combining the results of the GC analysis for solvent rinse Fraction 1 and 2 using parathion as internal standard.

Fat determination

Using a stream of N_2 the solvent was evaporated from the vials after GC analysis. Subsequently vials were heated for 1 h at 70 °C to reduce possible remnants of solvent in the remaining matter. Vials were then cooled to room temperature in a desiccator and the fat content was determined gravimetrically. For reference (*i.e.*, 100% fat extraction) the fat content was determined using a classical organic extraction procedure.[33]

Evaluating pesticide recovery through a reduced factorial experiment

The dependence of fat and pesticide recovery on four factors was investigated through a reduced factorial design. Two levels were used for each factor and at least two replicate measurements were made for each experimental condition. Factors were supercritical fluid temperature (40/95 °C), density (0.40/0.75 kg l^{-1}), flow rate (0.5/1.5 ml min^{-1}) and dynamic extraction time (40/120 min).

Statistical modelling

Fractional recovery of fat and pesticide was established at fifteen temperature/density conditions (see Table 1). For each condition two to five extractions were performed and from recovery data a recovery surface was modelled for fat

Table 1 Combinations of temperature and density used for modelling recovery of fat and pesticides and subsequent calculation of optimisation response surface

Temperature/°C	25	40	40	40	40	40	60	60	60	80	80	80	100	100	100
Density/kg l^{-1}	0.99	0.45	0.60	0.75	0.90	0.95	0.45	0.60	0.75	0.45	0.60	0.75	0.45	0.60	0.75

($Recovery_{fat}$) and each pesticide investigated ($Recovery_{OPP}$). For optimisation a response was arbitrarily defined as $Recovery_{OPP} - Recovery_{fat}$. Thus, for each pesticide investigated the response was calculated by subtracting the fractional fat recovery from fractional pesticide recovery. For each pesticide this set of fifteen response values was used for modelling a response surface. When plotted against density and temperature the maximum of the response plane thus corresponds to the optimised condition for the pesticide in question. Recovery and response surfaces were calculated using statistical software (SAS 6.11 procedure g3grid/spline).

Results and discussion

Factors affecting extractions

In developing a unified screening and quantification method priority must be given to both speed and good pesticide recovery without matrix contamination. To fulfil these requirements SFE optimisation must encompass extraction as well as trapping and elution of the compounds of interest.

Considering fat contamination two lipid fractions can be distinguished. One fraction of lipid is readily extracted using either organic solvent or SFE, whereas total extraction including phosphorylated and more polar lipids requires a more harsh treatment, such as boiling in hydrochloric acid prior to extraction.[15] Thus the latter lipid fraction is less likely to contaminate residue extracts wherefore optimising SFE of OPPs from fatty matrices implies extraction of pesticides separately from the more accessible lipids.

Addressing selectivity the extraction characteristics of both pesticide and fat needs to be established. As for the processes underlying SFE several authors have provided theoretical descriptions.[34–37] From such studies, it has been found that several extraction parameters such as temperature, density, extraction time, and flow rate exert a strong influence on extractable analyte recoveries. In the present study a reduced factorial design was employed to select the major factors with respect to extraction efficiency. The four factors evaluated were supercritical fluid temperature, density, flow rate, and dynamic extraction time. It was observed that all factors had significant effect upon fat extraction, flow rate being the least significant factor. In contrast, only density and temperature had significant effects on recovery for the pesticides investigated (confidence level 95%). In the present study only moderate flow rates were investigated. A more significant effect on residue recovery could be anticipated when using high flow rates due to low trapping efficiency associated with a high gas flow through the trap. However, Huser and Kleibohmer have reported that high flow rates did not cause low SFE trapping efficiencies of PAHs using solid phase traps.[38]

Having established the principal components for pesticide recovery further method development focused on temperature and density. As the supercritical fluid density is defined by temperature and pressure optimisation strategies may involve only one of these parameters. However, it has been observed that in optimising SFE methods it is of importance to vary both temperature and density, possibly in combination.[1,39] This is based upon the observation that the solubility of an analyte in a supercritical fluid is influenced by both the volatility of the analyte and the solvating effect of the supercritical fluid.[1,35,40]. The present study confirms the need for optimising temperature and density in combination and the experimental approach was to characterise the combined effect of temperature and density through a series of recovery experiments.

SPE of fatty matrix substrances

Throughout method optimisation the amount of coextracted matrix components was determined gravimetrically. Whereas analysis of methyl esters (FAMEs) is preferable when characterising a fat extraction,[41,42] the simpler gravimetric approach was justified considering coextracted fat merely as an impurity. Using an organic solvent based extraction a reference value for 100% fat recovery was established at 0.12 g fat g^{-1} meat. The meat used for method development was characteristic for raw beef having a water content of 67 ± 1% (m/m) and a pH of 6.1 to 6.3.

Fat recovery using SFE is shown in Fig. 1(A). To obtain fat recoveries greater than 50% the density of the supercritical fluid should exceed 0.7 g ml^{-1} at temperatures above 40–50 °C.

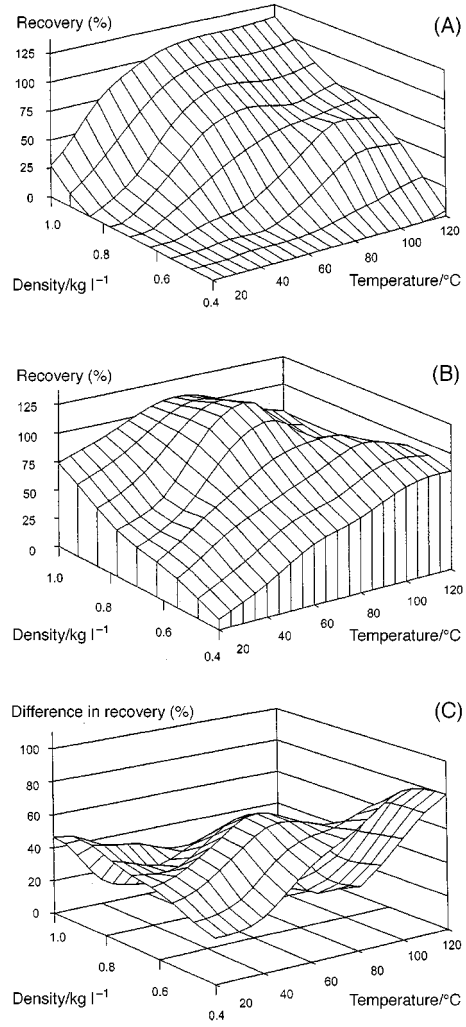

Fig. 1 Recovery planes of fat (A) and chlorpyrifos (B). Planes were modelled from 15 recovery experiments (see text for details). Response plane for the optimisation algorithm $Recovery_{OPP} - Recovery_{fat}$ is shown in (C). Except for density and temperature being optimised the SFE conditions were as described in the Experimental section.

SFE of organophosphorus pesticides

From an analytical point of view pesticides of medium to low polarity are of primary interest, as these are most likely to accumulate in fatty matrices. It has been found[43,44] that fat solubility can be described by the logarithmic value of the partition coefficient between octanol and water ($\log P_{ow}$). At values below 3 compounds have no or very low fat solubility whereas fat solubility can be anticipated for pesticides having values above 4. Pesticides included in this optimisation study have been selected to cover this polarity range of interest: chlorpyrifos ($\log P_{ow}$ 5.1), chlorpyrifos-methyl ($\log P_{ow}$ 4.3), malathion ($\log P_{ow}$ 2.9), pirimifos-methyl ($\log P_{ow}$ 4.2) and prothiofos ($\log P_{ow}$ 5.7). Pesticides ranging from the apolar prothiofos to the more polar malathion showed comparable extraction characteristics with good recovery. A representative example is given by chlorpyrifos extraction in Fig. 1(B). In general, good recoveries are obtained at high temperatures even at rather low densities.

Optimising the response ($Recovery_{OPP} - Recovery_{fat}$)

Having characterised the extraction profiles of fat and each individual pesticide, the data was used to establish the optimal combination of temperature and density. This condition, *i.e.*, the condition leading to high pesticide recovery and low fat contamination, was empirically described as differences in relative recovery: $Recovery_{OPP} - Recovery_{fat}$, where $Recovery_{OPP}$ and $Recovery_{fat}$ are the fractions recovered of pesticide and fat, respectively. This expression was central to the further optimisation. The algorithm may be used with optimisation strategies such as simplex and factorial designs[36,45] and multivariate designs.[46] However, in the present study a simple approach modelling a response surface was used. For this purpose a set of fifteen density/temperature conditions was used for recovery studies.

As can be seen from Fig. 1(C), the largest difference in the recoveries of chlorpyrifos and fat was found at densities less than 0.6 kg l^{-1} and a temperature range of 80–120 °C. This was the case for all OPPs investigated as can be seen from Table 2. Thus good pesticide recovery with minimal fat contamination can be obtained at these conditions. It is, however, crucial that pesticides are not decomposed by elevated temperatures. Coulibaly and Smith[32] have shown that a number of OPPs are rather stable at elevated temperatures up to 80 °C, and their findings are confirmed in the present work, as good recoveries were obtained even at temperatures above 100 °C. To reduce the risk of thermal decomposition of pesticides and wear on equipment the temperature was maintained at 95 °C.

Optimised method

The optimised method (as described in the Experimental section 'SFE extraction of meat samples') allows quantitative extraction of medium to highly apolar pesticides, *i.e.*, the pesticides of primary interest considering residues in fatty matrices. From the time study (Fig. 2), it is evident that an extraction time of 2 h is necessary for full recovery of all pesticides investigated even at a flow rate of 2 ml min^{-1}. However, compared with the total analysis time of traditional solvent-based extraction procedures[39,47] this is rather fast. Addition of modifier may reduce the time necessary for extraction. However, loss of selectivity is a possible drawback of modifier addition. Hence, this approach was not further pursued.

The elution of the trap was optimised through evaluation of six organic solvents (see Table 3). SFE conditions were as described in the Experimental section 'SFE extraction of meat samples', the only parameter varied being the solvent used for the first elution of the trap. All six solvents gave satisfactory recoveries even if values obtained using ethyl acetate and acetonitrile were at the lower end. As solvent evaporation and drying was necessary for fat determination, tetrahydrofuran was less suitable for the present optimisation experiment due to the tendency of peroxide formation. Of the solvents evaluated, heptane provided cleaner GC-chromatograms with less endogenous compounds using nitrogen–phosphorus detection wherefore this solvent was chosen. Using calibration graphs with parathion acting as internal standard the square of the correlation coefficient of the linear regression (r^2) was higher than 0.99 for all pesticides included in the study.

Considering trap packing material, Florisil has been used for trapping of several lipophilic compound after SFE. Examples are chlorinated and brominated pollutants extracted from environmental samples[48] and OPPs, organochlorine, and organonitrogen pesticides from grain matrices.[49] However, using traditional column chromatography[50,51] and SFE,[52] low recovery of OPPs has been reported. Solid phase trapping is a result of both cryogenic trapping and adsorption on active sites on the trapping material,[1] and low OPP recovery is a result of irreversibly binding to the trap material.[52] Thus, the good recoveries obtained in the present study may, in part, result from a decompression of OPPs onto the Florisil phase rather than a chromatographic retention. As good recoveries were obtained, no further optimisation of packing material was done.

EC MRLs for residues in meat[53] are typically set at the limit of determination generally considered to be at 0.01–0.1 mg kg^{-1}. However, at present only a few OPPs have EC MRLs in meat products hence the recoveries of carbofuran, phorate, procymidone and vinclozolin were used to evaluate the SFE method. As can be seen from Table 4 the optimised SFE method meets the quantification requirements imposed by EC MRLs. This is further demonstrated in Fig. 3 showing a typical chromatogram at level 0.05 mg kg^{-1}. Fig. 3 also indicates that by far the most of the pesticide is contained in Fraction 1 [(Fig. 3(A)]. Considering the low limits of detection in relation

Table 2 The optimised conditions of temperature and density for extraction of each OPP included in the study. Optimised conditions correspond to the maximum of the response surface as calculated from $Recovery_{OPP} - Recovery_{fat}$. Except for density and temperature being optimised SFE conditions were as described in the Experimental section

Pesticide	Temperature/°C	Density/kg l^{-1}
Chlorpyrifos	105	0.4
Chlorpyrifos-methyl	95	0.4
Malathion	80	0.4
Pirimifos-methyl	90	0.4
Prothiofos	105	0.4

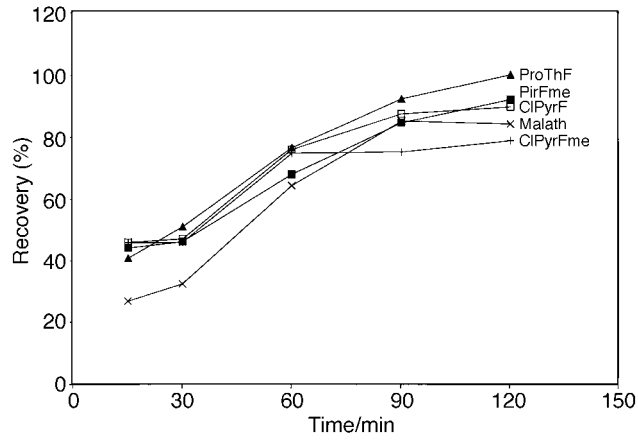

Fig. 2 Time dependence of pesticide recovery when extracted from meat. Chlorpyrifos (ClPyrF, □); chlorpyrifos-methyl (ClPyrFme, +); malathion (Malath, X); pirimifos-methyl (PirFme, ■); prothiofos (ProThF, ▲). Except for the extraction time being studied the SFE conditions were as described in the Experimental section.

to MRLs, GC analysis can be limited to fraction 1 for screening. If the heptane trap rinse used for screening is positive, Fraction 2 must also be assayed to enable final quantification of the pesticides.

As incurred samples could not be obtained, spiked samples were used for the method development. Using spiked samples, residue recovery may be higher than that obtained using incurred samples.[1,54] However, in a study by King *et al.*[55] a satisfactory extraction of incurred organochlorine residues from chicken fat, beef and lard could be obtained using a short 18 min SFE at 3 ml min^{-1}, 50 °C and 204 atm (corresponding to a density of 0.8 g ml^{-1}). Comparing the optimised method of the present study to the method of King *et al.*, it is assumed that a prolonged extraction (120 min *vs.* 18 min) at a higher temperature (95 °C *vs.* 50 °C) is matching a lower density (0.4 g ml^{-1} *vs.* 0.8 g ml^{-1}) and flow rate (2 ml min^{-1} *vs.* 3 ml min^{-1}) in the present extraction procedure. Using the present method for incurred samples, the sample size must also be considered carefully. Thus, using a sample size of 1.0 g the preparation and homogenisation of the sample material must be effective to ensure a representative subsample for SFE analysis. Also, if a larger sample size is to be used, it may be necessary to mix the meat with a sorbent to retain water in the extraction.[1,52]

Methods for extraction of lipophilic compounds such as OPPs and organochlorine compounds have existed for several years, classic methods being established by Stalling *et al.*[56] and Specht and Tindle.[57] Some important methodologies have been reviewed,[22,50,58,59] and methods issued by the European Committee for Standardization[47] (CEN) and the Food and Drug Administration[60] (FDA) are examples of authoritative methods. Also, in a previous study[61] an organic solvent extraction procedure was developed for determination of OPPs in meat, and recoveries obtained are comparable to those obtained using the present SFE method. In traditional methods clean-up procedures employed are primarily solvent partitioning, gel permeation chromatography (GPC), and solid phase extraction

Table 3 Recovery of pesticides (% total, residue level 100% = 0.5 mg kg^{-1} meat, n = 3) using various solvents for the first elution of the trap. Recoveries represent the sum of values obtained for Fraction 1 and 2. See text for details on extraction conditions

Solvent	Pesticide	Mean recovery (%)
Acetonitrile	Chlorpyrifos	85 ± 1
	Chlorpyrifos-methyl	83 ± 9
	Malathion	84 ± 3
	Pirimifos-methyl	79 ± 3
	Prothiofos	87 ± 3
Ethyl acetate	Chlorpyrifos	82 ± 10
	Chlorpyrifos-methyl	85 ± 9
	Malathion	83 ± 7
	Pirimifos-methyl	90 ± 6
	Prothiofos	88 ± 9
Ethylacetate–cyclohexane 1 + 1	Chlopyrifos	90 ± 5
	Chlorpyrifols-methyl	95 ± 8
	Malathion	94 ± 5
	Pirimifos-methyl	93 ± 6
	Prothiofos	87 ± 10
Heptane	Chlorpyrifos	110 ± 12
	Chlorpyrifos-methyl	109 ± 12
	Malathion	84 ± 10
	Pirimifos-methyl	93 ± 14
	Prothiofos	100 ± 2
Methanol	Chlorpyrifos	90 ± 3
	Chlorpyrifos-methyl	119 ± 1
	Malathion	103 ± 3
	Pirimifos-methyl	105 ± 1
	Prothiofos	98 ± 8
Propan-2-ol	Chlorpyrifos	96 ± 7
	Chlorpyrifos-methyl	104 ± 10
	Malathion	91 ± 9
	Pirimifos-methyl	97 ± 9
	Prothiofos	94 ± 10
Tetrahydrofuran	Chlorpyrifos	92 ± 8
	Chlorpyrifos-methyl	106 ± 3
	Malathion	101 ± 3
	Pirimifos-methyl	98 ± 1
	Prothiofos	89 ± 5

Table 4 Recovery data, detection limit (LD) and limit of determination (LOD). Recovery data are based upon two series of each of three samples (total n = 6). LDs and LODs were established using meat extracts containing no residues (n = 6). LD was determined as the mean value of the matrix blank readings plus 3 standard deviations of the mean. LOD was determined as the mean value of the matrix blank readings plus 6 standard deviations of the mean. Where established, EC MRL for meat (^{m}) or fat (^{f}) is given and (*) indicates MRL considered identical to LOD by EC.[53]

Pesticide	LD in meat/ mg kg^{-1}	LOD in meat/ mg kg^{-1}	EC MRL/ mg kg^{-1}	Recovery (%) (residue level/ mg kg^{-1} meat)
Organophosphorus pesticides—				
Chlorpyrifos	0.01	0.03	$0.05^{f,*}$	95 ± 14 (0.10)
Chlorpyrifos-methyl	0.01	0.02	$0.05^{f,*}$	78 ± 3 (0.10)
Malathion	0.01	0.02	—	83 ± 10 (0.10)
Pirimifos-methyl	0.01	0.02	$0.05^{f,*}$	87 ± 1 (0.10)
Prothiofos	0.01	0.02	—	95 ± 6 (0.10)
Other pesticides—				
Carbofuran	0.02	0.03	$0.1^{m,*}$	89 ± 8 (0.20)
Phorate	0.01	0.01	$0.5^{m,*}$	78 ± 9 (0.05)
Procymidone	0.03	0.05	$0.5^{m,*}$	88 ± 9 (0.20)
Vinclozolin	0.02	0.03	$0.5^{m,*}$	87 ± 11 (0.20)

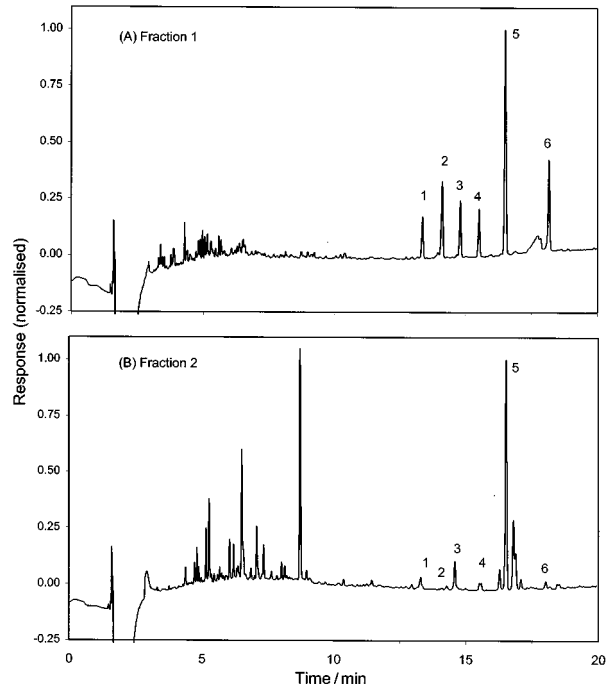

Fig. 3 Representative gas chromatograms using nitrogen–phosphorus detection. Chromatograms are normalised to the standard (5 std, parathion). Meat was spiked at level 0.05 mg kg^{-1}, and chromatograms of heptane trap rinse (A, Fraction 1) and acetone trap rinse (B, Fraction 2) are shown. 1 = chlorpyrifos-methyl, 2 = pirimifos-methyl, 3 = chlorpyrifos, 4 = malathion, 5 = parathion standard, 6 = prothiofos.

(SPE). Recently accelerated solvent extraction (ASE) has been used for extraction of OPPs from meat.[62] Even if ASE is fast, there is a need for clean-up through phase separation and GPC as in other organic solvent based methods. Overall the organic solvent based methods have limit of determinations (LODs) in the range 0.01–0.05 mg kg^{-1}, which is also reflected in the EC MRLs[53] set at the LOD, a level that is obtainable with the SFE method. Also, SFE recoveries are at least as good as those obtainable using organic solvent based methods, the acceptable range being considered as 80–110%. Thus the performance and operating range of the SFE method fully meets the organic based methodologies. Extending the comparison towards solvent based methodologies, low requirement for handling is evident for the SFE methodology, *i.e.*, homogenisation and thimble packing are the primary manual operations involved. Even if some organic solvent methods have succeeded in reducing solvent quantities[60,62] the SFE method presented is substantially more environmentally favourable due to the very low solvent requirement, *i.e.*, less than 20 ml per sample including system cleaning. Additionally, total residue analysis ranging from raw meat sample to fully quantified results can be obtained within hours. In contrast, when analysing an identical sample using an organic solvent extraction method the results will not be available until next laboratory day due to comprised labour intensive procedures. Thus the SFE method presented constitutes a promising alternative for fast routine residue analysis in fatty matrices such as meat.

The author acknowledges the skilled technical assistance of I. Schröder.

References

1 Bøwadt, S., and Hawthorne, S. B., *J. Chromatogr. A.*, 1995, **703**, 549.
2 Chester, T. L., Pinkston, J. D., and Raynie, D. E., *Anal. Chem.*, 1996, **68**, 487R.
3 Mitra, S., and Wilson, N. K., *J. Chromatogr. Sci.*, 1991, **29**, 305.
4 Veress, T., *J. Chromatogr. A*, 1994, **668**, 285.
5 Lou, X., Janssen H.-G., and Cramers, C. A., *J. High Resolut. Chromatogr.*, 1995, **18**, 483.
6 King, J. W., *J. Chromatogr. Sci.*, 1989, **27**, 355.
7 Bartle, K. D., Clifford, A. A., Hawthorne, S. B., Langenfeld, J. J., Miller, D. J., and Robinson, R., *J. Supercrit. Fluids*, 1990, **3**, 143.
8 Hierro, M. T. G., and Santa-Maria, G., *Food Chem.*, 1992, **45**, 189.
9 Levy, J. M., Dolata, L., Ravey, R. M., Storozynsky, E., and Holowczak, K. A., *J. High Resolut. Chromatogr*., 1993, **16**, 368.
10 Langenfeld, J. J., Hawthorne, S. B., Miller, D. J., and Pawliszyn, J., *Anal. Chem.*, 1994, **66**, 909
11 Fahmy, T. M., Paulaitis, M. E., Johnson, D. M., and McNally, M. E. P., *Anal. Chem*., 1993, **56**, 1462
12 Eckert, C. A., Knutson, B. L., and Debenedetti, P. G., *Nature* (*London*), 1996, **383**, 313.
13 Battersby, P., Dean, J. R., Hitchen, S. M., Tomlinson, W. R., and Myers, P., *Anal. Proc.*, 1993, **30**, 473.
14 Arul, J., Boudreau, A., Makhlouf, J., Tardif, R., and Sahasrabudhe, M. R., *J. Food Sci.*, 1987, **52**, 1231.
15 Lembke, P., and Engelhardt, H., *Chromatographia*, 1993, **35**, 509.
16 Hopper, M. L., King, J. W., Johnson, J. H., Serino, A. A., and Butler, R. J., *J. AOAC Int.*, 1995, **78**, 1072.
17 Palmer, M. V., and Ting, S. S. T., *Food Chem.*, 1995, **52**, 345.
18 Valcárcel, M., and Tena, M. T., *Fresenius' J. Anal. Chem.*, 1997, **358**, 561.
19 Ho J. S., and Tang P. H., *J. Chromatogr. Sci.*, 1992, **30**, 344.
20 Gere D. H., Knipe C. R., Castelli P., Hedrick J., Randal Frank L. G., Schulenberg-Schell H., Schuster R., Doherty L., Orolin J., and Lee, H. B., *J. Chromatogr. Sci.*, 1993, **31**, 246.
21 Hopper, M., *J. AOAC Int.*, 1997, **80**, 639.
22 Walters, S. M., *Anal. Chim. Acta*, 1990, **236**, 77.
23 Parks O. W., and Maxwell, R. J., *J. Chromatogr. Sci.*, 1994, **32**, 290.
24 Frances, J. E., King, J. W., and Snyder, J. M., *J. Agric. Food Chem.*, 1991, **39**, 1871.
25 Nam, K.-S., and King, J. W., *J. High Resolut. Chromatogr*., 1994, **17**, 577.
26 Murugaverl B., Gharaibeh A., and Voorhees K. J., *J. Chromatogr. A*, 1993, **657**, 223.
27 France, J. E., and King, J. W., *J. AOAC Int.*, 1991, **74**, 1013.
28 Nam, K.-S., and King, J. W., *J. Agric. Food Chem.*, 1994, **42**, 1469.
29 Lee, S. M., and Richmann, S., *J. Assoc. Off. Anal. Chem.*, 1991, **74**, 893.
30 Kaufmann, B. M., and Clower, M. Jr., *J. AOAC Int.*, 1994, **78**, 1079.
31 *Official Methods of Analysis*, ed. Helrich K, Association of Official Analytical Chemists Inc, Virginia, 15th edn., 1990, vol. 2, section 950.46.
32 Coulibaly K., and Smith, J. S., *Agric. Food Chem.*, 1993, **41**, 1719.
33 Stijve, T., and Cardinale, E., *Mitt. Geb. Lebensmittelunters. Hyg.*, 1974, **65**, 131.
34 King, J. W., and France J. E., in *Analysis with Supercritical Fluids: Extraction and Chromatography*, ed. Wenclawiak B., Springer, Berlin, 1992, ch. 3.6.
35 Clifford A. A., in *Supercritical Fluid Extraction and its Use in Chromatographic Sample Preparation*, ed. Westwood S. A., Blackie, Glasgow, 1993, ch. 1.
36 Kane M., Dean J. R., Hitchen S. M., Dowle C. J., and Tranter R. L., *Anal. Chim. Acta*, 1993, **271**, 83.
37 Young, T. M., and Weber, W. J. Jr., *Anal. Chem*., 1997, **69**, 1612.
38 Husers N., and Kleibohmer W., *J. Chromatogr. A.*, 1995, **697**, 107.
39 Furton, K. G., Jolly, E., and Rein, J., *J. Chromatogr*., 1993, **629**, 3.
40 Janda V., Bartle K. D., and Clifford, A. A., *J. Chromatogr*., 1993, **642**, 283.
41 House, S. D., Larson, P. A., Johnson, R. R., DeWries, J., and Martin, D. L., *J. AOAC Int.*, 1994, **77**, 960.
42 Ali, L. H., Angyal, G., Weaver, C. M., and Rader, J. I., *Food Chem.*, 1997, **58**, 149.
43 Ahlborg, U. G., Black, A. L., Borzelleca, J. F., Clegg, D. J., Hamilton, M. J., Ives, F., Lundehn, J. R., Voldum-Clausen, K., and Zhuang, W. J., *Pesticides in Food—Plant Production and Protection Paper*, FAO, Rome, 1991, **111**, 15.
44 Noble, A., *J. Chromatogr.*, 1993, **642**, 3.
45 Lanças, F. M., Queiroz, M. E. C., and Silva, I. C. E., *Chromatographia*, 1995, **40**, 421.
46 Li, K., Ong, C. P., and Li, S. F. Y., *J. Chromatog. Sci.*, 1994, **32**, 5653.
47 European Committee for Standardization (CEN), Fatty Food—*Determination of Pesticides and Polychlorinated Biphenyls (PCBs)*, EN 1528, part 1–4, Brussels, 1996.
48 Hartonen, K., Bøwadt, S., Hawthorne, S. B., and Riekkola, M. L., *J. Chromatogr. A*, 1997, **774**, 229.
49 King, J. W., Hopper, M. L., Luchtefeld, R. G., Taylor, S. L., and Orton, W. L., *J. AOAC Int.* 1993, **76**, 857.
50 Leoni, V., Caricchia, A. M., and Chiavarini, S., *J. AOAC Int.*, 1992, **75**, 511.
51 Gillespie, A. M., Daly, S. L., Gilvydis, D. M., Schneider, F., and Walters, S. M., *J. AOAC Int.*, 1995, **70**, 109.
52 Hopper, M. L., and King, J. W., *J. Assoc. Off. Anal. Chem.*, 1991, **74**, 661.
53 Council Directive 96/33/EEC, *Off. J. Eur. Commun*., 1996, **L144**, 35.
54 Burford, M. D., Hawthorne, S. B., and Miller, D. J., *Anal. Chem.*, 1993, **65**, 1497.
55 King, J. W., Snyder, J. M., Taylor, S. L., Johnson, J. H., and Rowe, L. D., *J. Chromatogr. Sci.*, 1993, **31**, 1.
56 Stalling, D. L., Tindle, R. C., and Johnson, J. L., *J. AOAC Int.*, 1972, **55**, 32.
57 Specht, W., and Tindle, R. C., *Fresenius' Z. Anal. Chem.*, 1985, **322**, 443.
58 Armishaw, P., and Millar, R. G., *J. AOAC Int.*, 1993, **76**, 1917.
59 Hernández, F., Serrano, R., Beltran, J., and López, F. J., *J. AOAC Int.*, 1996, **79**, 123.
60 *Pesticide Analytical Manual*, ed. McMahon, B. M., and Hardin, N. F., US Food and Drug Administration, Washington, DC, 3rd edn., 1994, vol. 1, section 2, p. 5.
61 Juhler, R. K., *J. Chromatogr*., 1997, **789**, 145.
62 Obana, H., Kikuchi, K., Okihashi, M., and Hori, S., *Analyst*, 1997, **122**, 217.

Paper 8/02323I
Received March 24, 1998
Accepted May 26, 1998