Determination of pesticides in high-water-content samples by off-line supercritical fluid extraction–gas chromatography–electron-capture detection

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Abstract

The optimization of supercritical fluid extraction of several organochlorine and organophosphorus pesticides is presented. The optimized method is compared with the classical sonication coupled to gel permeation chromatography as a clean-up method. The SFE method has been tested for 11 pesticides in spiked strawberries. At a spiking level of 100 ng/g, the pesticide recoveries were higher than 80%. The influence of the water content present in the sample is discussed. Two different approaches to solve this problem, which involve the use of anhydrous sodium sulfate mixed with the sample and alternatively the lyophilization, are presented too. © 1998 Elsevier Science B.V.

Keywords: Extraction methods; Food analysis; Pesticides

1. Introduction

Analytical methods for pesticide residues have their main application in the control of food for human consumption, especially in the control of fruits or vegetables since they are generally produced using direct application of pesticides [1].

Over the last years, several multi-residue methods have been reported that allow the screening for one or more of the classes of pesticides in plant materials such as fruits and vegetables. These methods are usually based on liquid–liquid partition [2–5], Soxhlet extraction or sonication, and most of them use additional clean-up steps like gel permeation chromatography (GPC) [5–7], and/or other materials such as Florisil [8,9], silica gel [10], or charcoal–Celite [11]. Other techniques used are matrix solid-phase dispersion [12,13] or solid-phase extraction [14,15].

Recently, supercritical fluids have been used as an alternative system for the extraction and preparation of samples for residue analysis. In this case, the unique properties exhibited by supercritical fluids, especially supercritical carbon dioxide, have already been applied for the analysis of pesticide residues in food samples [16–21]. However, the advantages of supercritical fluid extraction (SFE) have scarcely been applied to the analysis of pesticide residues in fruits and vegetables, since the technique presents some practical limitations to be applied to high-water-content samples [22,23]. The main problem is the relatively high solubility of water in supercritical carbon dioxide, approximately 0.3% [24]. This can cause restrictor plugging by ice during the supercritical fluid expansion and carry over water into the collection trap and into the collection solvent and ultimately into the chromatographic system.

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There are two different approaches proposed to solve the problems caused by the water content of vegetable samples in SFE. One of them is to lyophilize the sample prior to extraction [25] and the second one is to mix the sample, prior to SFE with an appropriate material to absorb water [20–22,26].

The selection of the operating conditions in SFE is still a difficult task and an area of active research [27]. Some developers optimize extraction methods one parameter at a time [28]. This strategy is time consuming and rarely effective for determining the true optimum. Factor design has been used for the simultaneous determination of various analytical SFE parameters, including temperature, pressure, supercritical fluid density, fluid flow-rate and extraction time [29,30]. Only two or three variables are considered in most cases, so a large number of experiments are necessary in order to achieve reasonably good results. In this paper, a folded Plackett–Burman factorial design was used to optimize the SFE process [31].

In this study, both the lyophilization and the use of a drying material have been studied and SFE has been optimized for the determination of 11 organochlorine pesticides (OCPs) and organophosphorus pesticides (OPPs) in spiked strawberries. Care was taken in the spiking procedure to simulate the real natural situation of pesticides in the whole matrix.

Both SFE methods were compared with the sonication followed by GPC as clean-up procedure. The accuracy and precision of the three methods have been statistically evaluated.

2. Experimental

2.1. Gas chromatographic analysis

A Varian (Harbor City, CA, USA) Star 3400 CX gas chromatograph equipped with a ^{63}Ni electron-capture detection (ECD) system with split/splitless injector and a SGL-5 column (60 m×0.25 mm I.D., film thickness 0.25 μm) supplied by Sugelabor (Madrid, Spain) was used.

The temperatures were as follows: injector temperature, 210°C; detector temperature, 300°C; initial oven temperature, 50°C, hold 1 min, linear temperature gradient 25 C°/min to 215°C, hold 1.5 min,

linear temperature gradient 2 C°/min to 250°C, hold 1.5 min, linear temperature gradient 25 C°/min to 280°C and hold 5 min. Splitless time, 0.6 min.

The carrier gas was hydrogen at a flow-rate of 1.5 ml/min.

2.2. Supercritical fluid extraction

A Prepmaster (Suprex, Pittsburgh, PA, USA) stand-alone SFE system was used. Extractions were accomplished by using a 3-ml extraction vessel (Suprex) for a lyophilized sample or 5-ml extraction vessel (Suprex) for a natural sample. An off-line collection module, the Accutrap (Suprex) was used to perform the cryogenic adsorbent trap collection on a sylanized glass beads cartridge (80/100 mesh, Suprex). This collection module also includes a liquid pump for delivering an appropriate liquid solvent for analyte desorption from the trap. For this work, 1.5 ml of *n*-hexane with a flow-rate of 1.0 ml/min was sufficient to quantitatively carry the pesticides into a GC autosampler vial. The scheme of both apparatus has been described elsewhere [32,33].

2.3. Gel permeation chromatography

The GPC was carried out by using a Bio-Beads S-X3 (200–400 mesh, Bio-Rad Labs., Richmond, CA, USA) column (400 mm×20 mm I.D.) which was connected to a UV-Vis spectrophotometer (Perkin-Elmer, Lambda 3 spectrophotometer) or a fraction collector (Gilson FC203). The flow-rate was held at 2.0 ml/min for the whole GPC work by using a low-pressure pump (Kontron Instruments, LC T-414).

The preparation of the column was as follows: 50 g of Bio-Beads SX-3, which consisted of a spherical porous styrene–divinylbenzene copolymer with 3% crosslinkage, were placed in a 500-ml flask and mixed with 100 ml of the GPC elution solvent (cyclohexane–dichloromethane, 70:30, v/v) and the gel was left to stand for 24 h to 4°C. The fully swollen gas was then de-gassed by applying a vacuum to the flask, before filling the column with the slurry. Elution solvent was pumped through the column at a flow-rate of 2 ml/min for 1.5 h prior to use.

2.4. Reagents

Pesticide standards (purity>98%) chlorpyrifos, procymidone, malathion, endosulfan-beta, vinclozolin, tolclofos-methyl, 4,4'-dichlorobenzophenone, bromopropylate and tetradifon were supplied by Dr. S. Ehrenstorfer, (Ausburg, Germany). *o,p'*-DDE was Certified Reference Material (Terdington, UK) and chlorobenzilate was from Riëdel-de Haen (Seelze, Germany). 2,4,5,2',3',4'-Hexachlorobiphenyl, PCB 138, used as internal standard, was from Chem Service (West Chester, PA, USA).

All the solvents used were from Merck (Darmstad, Germany), Suprapur Quality for gas chromatography. Anhydrous sodium sulfate, ACS reagent grade, supplied by Panreac (Barcelona, Spain), was washed with ethyl acetate and then dried at 300°C for 5 h.

2.5. Procedures

2.5.1. Spiked samples

0.2 g of a methanol standard solution containing 4 μg/g of all the pesticides under study were added to 10 g of natural strawberries previously grinded to obtain an homogeneous slurry. The pesticides were selected according to those detected in plastic used for the strawberry cultivation [34]. The mixture was shaken for 30 min and it was stored in the dark at 4°C for at least 32 h before the analysis.

2.5.2. Ultrasonic extraction coupled to gel permeation chromatography (US–GPC)

2.5.2.1. Pesticide elution profile

Mixtures of the selected pesticides were prepared in dichloromethane. Three independent aliquots of 2 ml of the standard dichloromethane solution were injected into the GPC column which was connected to an UV-Vis spectrophotometer held at 254 nm. The flow of elution solvent was held at 2.0 ml/min for the whole GPC work. The pesticide elution profile was recorded by using a Hewlett–Packard (Palo Alto, CA, USA) 3396A Integrator. This way, the selected fraction was from 20 to 37 min.

2.5.2.2. Extraction of samples

Approximately 10 g of sample were added to a 100 ml round-bottomed flask and mixed with anhydrous sodium sulfate at an optimum ratio of 1:1.6 (w/w). The mixture was mechanically shaken to get an homogeneous and water-free powder. Then, it was covered with 25 ml of acetone for 2 min. This acetone was collected, filtered through silanized glass wool and added to a 500 ml round-bottomed flask.

Then three sequential ultrasonic extractions of 10 min with 60 ml of dichloromethane each were applied to the sample. All the extracts were filtered through silanized glass wool and collected together in the 500-ml flask. The silanized glass wool was rinsed with two 10-ml aliquots of dichloromethane, which were also added to the flask. The large extract was carefully evaporated to dryness using a rotary evaporator (25 rpm, 40°C) followed by nitrogen stream (45°C). The residue was dissolved in 5 ml of the elution solvent and injected on to the GPC column under the experimental conditions described above. The GPC column was washed for a further 10 min before the next sample was introduced. The total GPC run time was 57 min.

The solvent of the fraction was evaporated to dryness by rotary evaporation and nitrogen stream, under the same conditions listed above. The residue was dissolved in *n*-hexane, filtered through a Teflon syringe filter of 0.2-μm pore size and transferred to a 5-ml calibrated flask. Finally, the internal standard, PCB 138, was added and the extract was diluted to the mark with hexane for subsequent GC–ECD analysis. Gravimetric control was used through all the study.

2.5.3. SFE using anhydrous sodium sulfate as drying agent

Before each extraction, ≈0.5 g of spiked grinded strawberry was thoroughly mixed with the appropriate amount of anhydrous sodium sulfate (1:1.4, w/w).

Extractions were done in 5-ml extraction vessels packed with 3 g of the mixture, placing at the bottom of the vessel, sylanized glass wool followed by 0.5 g of anhydrous sodium sulfate to trap any water that could migrate during the extraction. The optimum SFE conditions are specified under Section 3.

2.5.4. SFE of lyophilized sample

The lyophilization process consists of three sequential steps. In the first one, the sample is frozen at −16°C. In the second one, vacuum (<0.1 Torr; 1 Torr=133.322 Pa) is applied to the frozen sample. Lastly, the sample under vacuum is heated at 40°C to eliminate the water by sublimation. A home-made lyophilizator was used.

This way, a final powdered and homogeneous dried sample was obtained. The lyophilized sample is stored in a drier before the analysis.

Extractions were accomplished in a 3-ml extraction vessel packed with 1 g of lyophilized sample, placing at the bottom of the vessel sylanized glass wool and anhydrous sodium sulfate. Anyway, sodium sulfate is necessary to eliminate traces of internal water, but in this case, its amount is reduced to a simple layer of ≈5 mm thickness.

The optimum SFE conditions are also listed in Section 3.

3. Results and discussion

3.1. SFE optimization

Firstly, the criteria for optimization was defined. In this case, the criteria was the overall extraction efficiency for pesticides in spiked strawberries. The extraction efficiency was 100% or the highest achievable within the experimental range.

The number of variables potentially affecting the extraction efficiency and the recovery percentages was very large. Eight factors, including extraction pressure and temperature, CO_2 flow-rate, static extraction time, modifier identity, percentage of modifier, and adsorption and desorption temperatures of the trap were studied. A full, two level factor design (2^8) would involve a total of 256 experiments. Therefore, a folded Plackett–Burman ($2^8 \times 3/32$) type IV resolution design that allowed 15 degrees of freedom and involved 24 randomized runs was applied. The experimental design matrix is already described [31] and it has been previously applied to the SFE of these pesticides in recycled post-consumer plastics used as agricultural soil covers for strawberry cultivation [35]. Table 1 lists the upper

Table 1
Factor levels in the folded Plackett–Burman factorial design

Variable	Levels	
	High	Low
Extraction pressure (atm)[a]	440	200
Extraction temperature (°C)	75	25
CO_2 flow (ml/min)	3.0	1.0
Static extraction time (min)	10	2
Modifier identity	Acetone	Methanol
Modifier percentage (v/v)	10	2
Adsorption temperature (°C)	0	−20
Desorption temperature (°C)	45	10

[a] 1 atm=101 325 Pa.

and lower values given to each factor for both (lyophilized and natural) sample matrix.

The optimum SFE values obtained are listed in Table 2. As can be seen, the only difference between the two sample preparation methods conditions is the supercritical fluid density, which is given by combination of the extraction pressure and temperature. Working with lyophilized sample, lower density is necessary to achieve the best recoveries. This fact has a clear explanation.

The higher the density is, the higher the solubility parameter is, through the well-known Hildebrand equation [36,37]. The pesticide nature is the same in both situations, so their interaction with the matrix should be equal too. However, the penetration ability of supercritical CO_2 into the sample is very different if the sample is mixed with a considerable amount of sodium sulfate, which traps the water. In this case, supercritical CO_2 has to be more dense to penetrate through the whole matrix. The lyophilized sample is also more accesible to the supercritical fluid and the

Table 2
Optimum SFE conditions obtained for lyophilized strawberry (A) and natural strawberry mixed with anhydrous sodium sulphate as drying agent (B). (Spiking level: 100 ng/g)

Variable	Sample preparation	
	A	B
CO_2 density (g/ml)	0.86	1.01
CO_2 flow (ml/min)	3.0	3.0
Static extraction time (min)	10	10
Modifier identity	Acetone	Acetone/methanol
Modifier percentage (v/v)	10	10
Adsorption temperature (°C)	0	0
Desorption temperature (°C)	45	45

extraction is easier than in the sample mixed with the water absorbent solid. Then, to work in more aggressive conditions of pressure and temperature is not necessary to achieve quantitative recoveries with lyophilized samples.

As can be seen in Table 2, the effect of modifier in the natural matrix is not clear. This means that some pesticides such as chlorpyrifos, 4,4′-dichlorobenzophenone, o,p'-DDE, β-endosulfan, bromopropylate and tetradifon gave its maximum recoveries working with acetone, whereas the rest of them, namely vinclozolin, tolclofos-methyl, malathion, procymidone and chlorobenzilate gave its maximum results working with methanol. Moreover, from the literature [38,39] and according to our previous experience [35], modifiers can also be added directly to the sample matrix prior to the extraction (as static modifier) and not only to the CO_2 stream by using a pump (dynamic modifier).

So, a new set of experiments was carried out to check the modifier behaviour. The following possibilities were tested: (a) methanol as static modifier, (b) acetone as static modifier, (c) methanol as both static and dynamic modifier, (d) acetone as both static and dynamic modifier, (e) acetone as static modifier plus methanol as dynamic modifier, (f) methanol as static modifier plus acetone as dynamic modifier. All the experiments were carried out within 400 ml of modifier in static conditions and 10% (v/v) as the dynamic one. The results obtained are shown in Table 3.

As can be seen, the combination of both modifiers, one as static modifier and other as dynamic modifier leads to the best results. The effects of the different modifiers should be different. The efect of the static modifier could be related with the matrix itself, that means, with the change of some physical properties of the matrix. It penetrates into the matrix and facilitates the accesibility of the supercritical fluid to the analytes. The second modifier could change the polarity and consequently, the solubility of the pesticides in the supercritical fluid. This is in good agreement with the experimental results; for the lyophilized sample which has a more homogeneous and accessible matrix, only the dynamic modifier is necessary. Nevertheless, for the sample mixed with the anhydrous sodium sulfate, which is less homogeneous and more difficult to penetrate in, the role of the static modifier is very important in order to obtain good recoveries.

Finally, Table 4 shows the results obtained by both procedures for strawberry spiked sample at two different spiking levels, 100 and 500 ng/g, using the optimum SFE conditions obtained. For comparison purposes, the results obtained by using the sonication/GPC method are also listed.

For SFE, recoveries were higher than 80% except for tolclofos-methyl and o,p'-DDE at both levels

Table 3

Modifiers study in SFE of natural strawberry mixed with anhydrous sodium sulfate as drying agent (Spiking level: 100 ng/g: dynamic modifier: 10%, v/v)

Pesticide	Recovery (%) (n=4)					
	a	b	c	d	e	f
Vinclozolin	75.9	59.1	79.9	85.0	46.0	100.8
Tolclofos-methyl	58.8	50.9	63.1	71.3	41.1	74.8
Malathion	72.7	56.3	69.8	73.1	39.8	102.3
Chlorpyrifos	51.5	53.9	55.5	71.2	36.7	81.8
4,4′-Dichlorobenzophenone	69.0	70.6	64.1	67.5	34.1	95.6
Procymidone	98.1	77.7	96.2	96.4	53.0	101.0
o,p'-DDE	23.5	36.6	25.7	48.0	19.8	70.1
Chlorobenzilate	66.3	64.0	63.3	65.4	37.3	95.5
β-Endosulfan	48.1	50.0	51.6	74.7	33.0	96.3
Bromopropylate	48.2	49.4	50.7	68.9	38.4	84.5
Tetradifon	64.0	65.5	66.2	68.0	42.1	94.3

(a) Methanol as static modifier, (b) acetone as static modifier, (c) methanol as both static and dynamic modifier, (d) acetone as both static and dynamic modifier, (e) acetone as static modifier and methanol as dynamic modifier, (f) methanol as static modifier and acetone as dynamic modifier.

Table 4
Pesticide recoveries obtained by SFE for lyophilized (A) and mixed with anhydrous Na$_2$SO$_4$ (B) samples spiked with 500 and 100 ng/g of each pesticide[a], (C) US–GPC at 100 ng/g spiking level

Pesticide	Recovery (% RSD) ($n=5$)				
	A		B		C
	100 ng/g	500 ng/g	100 ng/g	500 ng/g	100 ng/g
Vinclozolin	100.3 (7)	90.2 (4)	95.5 (4)	92.3 (4)	95.5 (7)
Tolclofos-methyl	75.1 (7)	75.5 (5)	98.9 (4)	94.1 (4)	85.2 (5)
Malathion	103.5 (7)	103.2 (7)	98.5 (2)	93.1 (3)	79.1 (4)
Chlorpyrifos	83.6 (8)	82.4 (6)	88.2 (6)	89.8 (5)	72.1 (8)
4,4′-Dichlorobenzophenone	93.1 (9)	103.5 (7)	93.2 (3)	94.1 (3)	97.1 (4)
Procymidone	99.3 (5)	100.3 (4)	95.2 (4)	92.1 (4)	90.9 (9)
o,p′-DDE	69.2 (10)	74.6 (6)	85.6 (6)	84.3 (5)	87.0 (9)
Chlorobenzilate	95.2 (5)	91.2 (6)	92.3 (2)	91.3 (3)	83.1 (6)
β-Endosulfan	95.3 (6)	92.1 (6)	96.3 (2)	95.6 (3)	89.7 (5)
Bromopropylate	84.9 (7)	81.0 (6)	91.0 (5)	90.6 (4)	93.5 (3)
Tetradifon	88.3 (10)	76.4 (5)	82.1 (8)	83.1 (4)	83.4 (2)
Overall average	89.8 (7.4)	88.2 (5.6)	92.4 (4.2)	90.9 (3.8)	87.0 (5.6)

working with the mixture sample/anhydrous sodium sulfate and for tetradifon at 500 ng/g spiking level, working in the same conditions. As can be seen, except for the pesticides mentioned above, recovery percentages were nearly identical for both SFE sample preparation systems tested. Nevertheless, the use of a lyophilized sample gaves better precision results. This could be attributed to the more homogeneous characteristics of this sample, which results in more reproducible extraction yields.

3.2. Comparison of the extraction methods

Table 4 and Fig. 1 summarize the recovery data obtained for the extraction methods, US–GPC, SFE of lyophilized samples and SFE of natural samples mixed with anhydrous sodium sulfate. As can be seen, the main differences within the methods were found for chlorpyrifos, malathion, tolclofos-methyl and *o,p′*-DDE.

The average recoveries of each compound were statistically evaluated using the *T* test at the 95% confidence level to determine if there was a significant difference within the system used [40]. No significant differences were detected.

The precisions of the methods were also statistically evaluated for each compound using the *F* test

at the 95% confidence level [40]. No differences were observed except for tetradifon when SFE of mixed sample and US–GPC were compared.

4. Conclusions

SFE has been shown to be a successful analytical technique in extracting organochlorine and organophosphorus pesticides from a spiked sample with a high content of water, such as strawberry. Compared with the standard methodology, the SFE method is faster, less expensive and environmentally safer.

Two different approaches to solve this water problem has been tested. Both of them were suitable, but the use of lyophilization as sample preparation was found to have the best overall precision as indicated by the overall average R.S.D. for the 11 pesticides tested, of 4.2%. However, for all the individual pesticides there was no significance in the precisions at the 95% confidence level within the three methods tested.

Nevertheless, the use of lyophilization is time consuming and could lead to the loss of volatile compounds. Therefore, future works to ensure the suitability of the proposed method to another kinds of samples and analytes must be made.
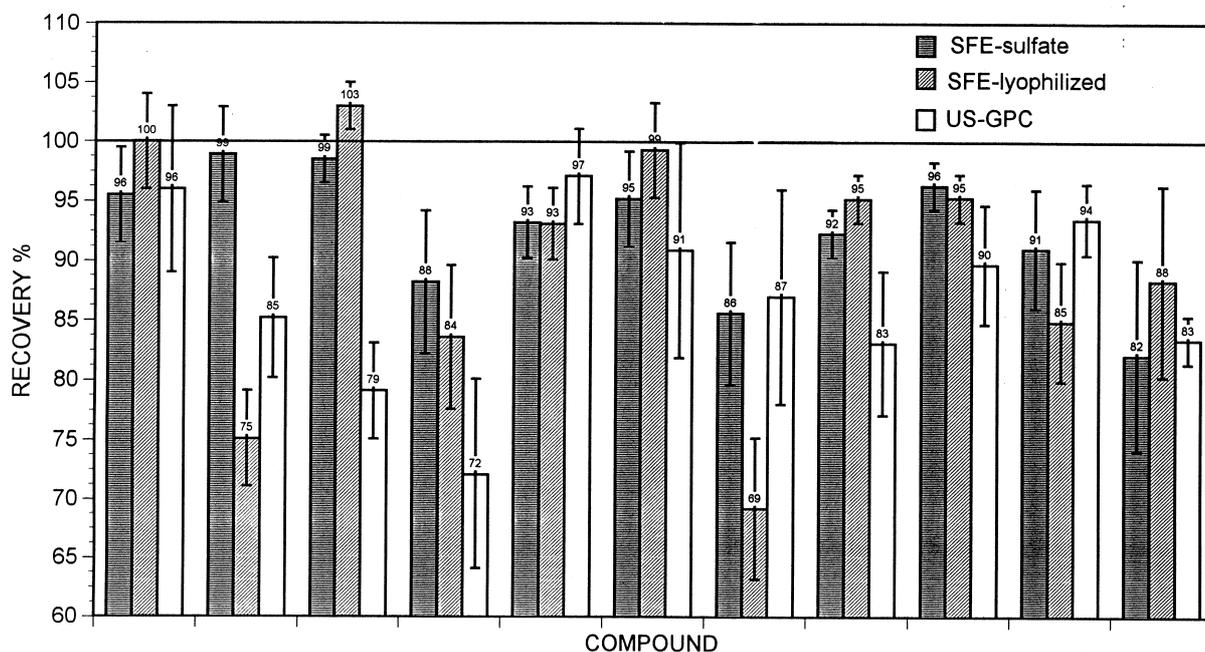
Fig. 1. Comparison of mean recoveries of the three extraction methods at 100 ng/g spiking level: US–GPC; SFE lyophilization and SFE of mixed sample/anhydrous sodium sulfate.

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