
Supercritical Fluid Extraction of Organophosphorus Pesticides from Orange Samples: Effect of Solid Additives on Recovery

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Key Words

Supercritical fluid extraction (SFE)

Oranges

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Dichloran

Summary

A method based on extraction with supercritical carbon dioxide has been developed for the analysis of pesticides in fruit. The method was tested on ten organophosphorus pesticides and dicloran in orange samples. Various solid additives including anhydrous sodium sulfate, Extrelut and diatomaceous earth were examined as extraction enhancers. Also tested were magnesium silicate as interference adsorbent, to increase selectivity, and sodium carbonate as alkaline salt, to prevent acidification of the sample by CO₂. The recoveries obtained by SFE (92–104 %; mean relative standard deviation 10 %) were higher than those of conventional methods. The polar pesticide methamidophos, however, was not recovered.

Introduction

Supercritical fluid extraction (SFE) has, for various reasons, been widely used as an alternative to solvent-based extraction methods [1–3] in routine analysis of pesticides in food [4–8]. Thus, SFE facilitates automation and miniaturization – amounts of sample can be reduced considerably (from typically 35 g in traditional liquid solvent methods to 1 g in SFE-based methods). Because SFE avoids several steps of conventional methods (e.g. partitioning, clean-up, evaporation) not only does it reduce the uncertainty in the results, it also reduces analysis times and is less labor-intensive. Use of chlorinated solvents is completely eliminated and that of non-

chlorinated organic solvents is drastically reduced, in conformity with EPA recommendations. The high selectivity of SFE enables the supercritical CO₂ extract to be injected directly with no previous clean-up.

Despite the suitability of SFE for multiresidue analysis of pesticides in food [8–10], most reported applications involve a single compound and soil samples. The problems posed by sample moisture initially restricted the application of SFE to dry samples such as grain and seeds [11–13].

Hydrophobic matrices facilitate penetration of supercritical CO₂ and too high a moisture content can render analytes inaccessible to the supercritical fluid because it is water-immiscible. In addition, the rate of extraction can be significantly increased if the sample is previously dried [14]. Matrix water can result in undesirable partitioning of the more polar analytes and extracted water can hinder retention in traps or analysis of the extracts. Real samples with high moisture contents result in plugging of restrictors if the water they contain freezes at restrictor tips. This can be overcome by increasing the restrictor temperature, but at the expense of losses of the most volatile analytes. Although freeze-dried samples are used in many SFE-based methods for analysis of pesticide residues in vegetables [15, 16], the use of solid additives [9, 10, 17] seems a more promising alternative to freeze-drying as it is simpler to implement and avoids losses of volatile compounds. Solids such as desiccating salts, diatomaceous earth (Celite or Hydromatrix), sand and glass wool can be added to liquid or semi-solid samples with a high water content, either to absorb the water or to disperse it and thereby increase the surface exposed to the supercritical fluid. In this way, the extraction efficiency is improved and potential problems arising from water circulating through the SFE system are avoided.

Johansson et al. [18] have proposed the use of fat-selective adsorbents, e.g. basic alumina, at the outlet of the extraction cell to improve SFE selectivity and obtain cleaner extracts. This additive is recommended if the analytes are stable in an alkaline medium. The aim of

this research was to study the effects of various solid additives on the supercritical fluid extraction recovery of organophosphorus pesticides and dicloran from fruit samples. The organophosphorus pesticides selected included effective insecticides and acaricides widely used on a number of fruit crops including citrus trees; the organophosphorus pesticide fenamiphos has systemic nematocidal activity and dicloran is a protective fungicide.

Experimental

All extractions were performed with SFC-grade CO₂ from Air Liquide (Paris, France).

Pesticide standards including *S*-2-ethylthioethyl-*O,O*-dimethylphosphorodithioate (Thiometon), 2,6-dichloro-4-nitroaniline (Dicloran), *O,O*-dimethyl-*O*-3,5,6-trichloro-2-pyridylphosphorothioate (Chlorpyrifos-methyl), *O*-2-diethylamino-6-methylpyrimidin-4-yl-*O,O*-dimethyl phosphorothioate (Pirimiphos-methyl), *O,O*-diethyl-*O*-3,5,6-trichloro-2-pyridylphosphorothioate (Chlorpyrifos-ethyl), 2-chloro-1-(2,4-dichlorophenyl)vinyl-diethyl phosphate (Chlorfenvinphos), ethyl 4-methylthio-*m*-tolyl-*iso*-propylphosphoramidate (Fenamiphos), *S*-4-chlorophenylthiomethyl-*O,O*-diethylphosphorodithioate (Carbophenothion), *S*-3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl-*O,O*-dimethylphosphorodithioate (Azinphos-methyl) and *S*-3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl-*O,O*-diethylphosphorodithioate (Azinphos-ethyl) were purchased from Dr Ehrenstorfer and used as received to prepare individual stock solutions in acetone. A mixed working standard of concentrations ranging from 11 to 45 µg mL⁻¹ was used as spiking solution and as stock solution to prepare calibration standards (the latter by dilution with isooctane). All solvents used were pesticide residue grade. Acid-washed diatomaceous earth (95 % SiO₂, Sigma), Florisil (60–100 mesh, Merck), Extrelut (packing material for high-volume extraction columns, Merck) and anhydrous sodium sulfate (analysis grade, Merck) were used as additives before SFE.

Apparatus

Extraction was performed with a Hewlett-Packard 7680T supercritical fluid extractor equipped with a Hewlett-Packard 1050 quaternary modifier pump, an automated variable restrictor and an ODS analyte trap. Table I shows the extraction and collection conditions used in the most of the experiments. Carbon dioxide modified with 5 % methanol was used as extraction fluid. Trap and nozzle temperatures were increased to 70°C during extraction with modified CO₂ to prevent condensation of methanol in the trap. The efficiency of the rinse step was checked; 100 % of each analyte retained in the trap was collected in the first vial (1.3 mL – no pesticides were detected in the second vial (1.3 mL additional rinse volume)).

Table I. SFE conditions used for the analysis of organophosphorus pesticides in orange.

Pressure (bar)	299
Temperature (°C)	50
Fluid flow-rate (liquid; mL min ⁻¹)	2
Extraction fluid	CO ₂
Extraction vessel size (mL)	7
Static extraction period (min)	1
Dynamic extraction period (min)	20
Trap temperature during collection (°C)	20
Trap temperature during rinse (°C)	30
Rinse solvent	<i>n</i> -hexane
Trap adsorbent	ODS
Rinse volume (mL)	1.3

Gas chromatography of the extracted analytes was performed with a Varian 3400CX chromatograph equipped with a Varian 8200CX automatic sample injector, an alkaline flame thermionic specific detector (TSD) and a Supelco 30 m × 0.25 mm × 0.25 µm film thickness SPB-20 fused silica capillary column. The injector and detector temperatures were 240 and 275 °C, respectively, and the bead intensity was 3.0 A. Split injection was performed at a split ratio of 1:60. The oven temperature was held at 80 °C for 1 min after injection, then programmed initially at 30 °C min⁻¹ to 180 °C, which was held for 15 min, and then at 5 °C min⁻¹ to 250 °C. Nitrogen was used as carrier gas at a flow-rate of 1.2 mL min⁻¹; the detector air and hydrogen flow-rates were 125 and 4 mL min⁻¹.

Sample Preparation

Oranges (853 g) were cut into pieces and homogenized in a Polytron blender for 5 min. The working standard mixture of pesticides (20 mL) was then added to sample while blending. The spiked sample was divided among small containers and stored at –40 °C until analysis. Aliquots of approximately 1 g were mixed with different amounts and types of solids before introduction into the supercritical fluid extractor. SFE extracts were injected directly into the GC with no additional clean-up. A typical chromatogram is shown in Figure 1.

Solvent Extraction and Clean-Up Procedures

Spiked orange samples (35 g) were extracted with ethyl acetate [3] or acetone [2]. The acetone extracts were partitioned with *n*-hexane and dichloromethane or with dichloromethane alone. Before GC analysis the sample extracts obtained with both solvents were subjected to GPC clean-up on a 60 cm × 2.5 cm i.d. column slurry-packed with Bio-Beads SX-3 resin. The eluent was a 1:1 mixture of ethyl acetate and cyclohexane pumped at a flow-rate of 5 mL min⁻¹; the loop volume was 5 mL. The GPC system was set at a 14 min dump and 8 min collect cycle.

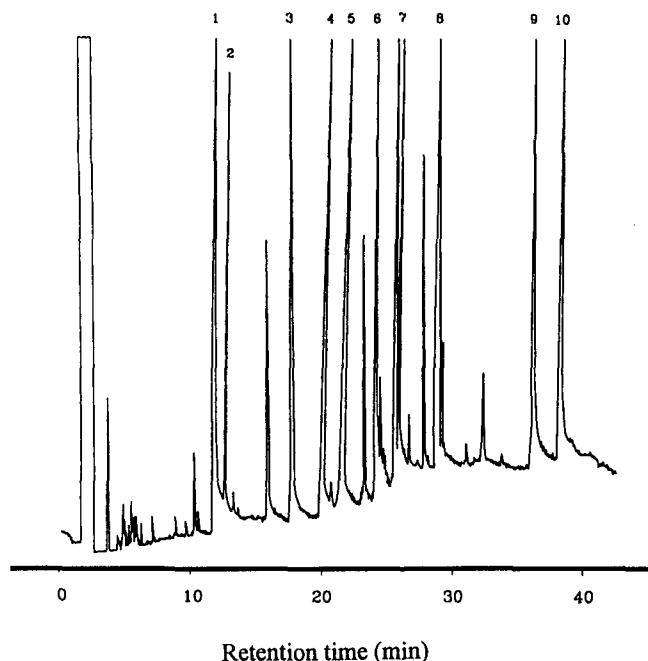


Figure 1

GC-TSD chromatogram obtained from an SFE extract of spiked orange subjected to no clean-up. Addition of diatomaceous earth (1:1 ratio). For GC and SFE conditions, see text and Table I, respectively. Peak identification: **1** = thiometon; **2** = dicloran; **3** = chlorpyrifos-methyl; **4** = pirimiphos-methyl; **5** = chlorpyrifos-ethyl; **6** = chlorfenvinphos; **7** = fenamiphos; **8** = carbophenothion; **9** = azinphos-methyl; **10** = azinphos-ethyl.

Table II. Pesticide recovery (% \pm s.d.; $n = 2$) with various water-absorbing solids.

Compound	Anhyd. Na_2SO_4	Extrelut	Diatomaceous earth
Thiometon	18 \pm 6	64 \pm 2	72 \pm 1
Dicloran	34 \pm 14	91.4 \pm 0.8	79 \pm 2
Chlorpyrifos-methyl	23 \pm 9	45 \pm 3	76 \pm 2
Pirimiphos-methyl	22 \pm 8	45 \pm 3	69 \pm 4
Chlorpyrifos-ethyl	26 \pm 9	31 \pm 3	71 \pm 3
Chlorfenvinphos	19 \pm 8	54 \pm 2	69 \pm 2
Fenamiphos	8 \pm 7	44 \pm 2	47 \pm 3
Carbophenothion	27 \pm 11	31 \pm 2	69 \pm 4
Azinphos-methyl	11 \pm 7	65 \pm 2	58 \pm 2
Azinphos-ethyl	11 \pm 6	62 \pm 1	60 \pm 1

Results and Discussion

Pesticide Recovery with Different Water-Absorbing Solids

Samples were mixed with different solids before SFE to evaluate the ability of the solid to enhance recovery by water absorption and sample dispersion; to obtain a loose mixture with 1.2 g sample, 3.5 g anhydrous Na_2SO_4 or 0.6 g Extrelut or diatomaceous earth was needed. After addition of the drying agent some mois-

ture remains in the sample and can increase the polarity of supercritical CO_2 by acting as modifier (the solubility of water in supercritical CO_2 is approximately 0.3 %). Table II lists the extraction recoveries obtained for ten of the eleven organophosphorus pesticides studied using the additives. The addition of Extrelut or diatomaceous earth improved both extraction recovery and reproducibility. Methamidophos was not recovered. For most of the pesticides the best recovery results were achieved with diatomaceous earth.

Influence of the Sample/Absorbent Ratio on Recovery

The significant role of diatomaceous earth as a water absorbing and dispersing agent is apparent from Figure 2, which shows the effect of varying the diatomaceous earth/sample ratio on the percentage recoveries of pesticides. Increasing the amount of absorbent used resulted in markedly increased recoveries of some pesticides. For instance, the recovery of fenamiphos increased from 33 % to 97 % on tripling the amount of diatomaceous earth used. These results are consistent with those reported by Hopper et al. [9]. The effect of addition of an alkaline salt to neutralize the acidity introduced in the aqueous phase of the sample by the CO_2 [19] was also investigated. An acidic medium can hinder the extraction of ionizable compounds containing an NH_2 group (e.g. methamidophos). The sample was mixed with approximately the same amount of sodium carbonate and the diatomaceous earth was added. As is apparent from Figure 2, the recoveries obtained with and without addition of alkali were nearly identical for most of the analytes. Again no methamidophos was recovered.

Addition of Florisil to the Sample

Florisil (magnesium silicate) is used as a selective adsorbent for removal of interferents from lipid extracts. Its effect as a sample additive on SFE recovery was studied in the presence and absence of diatomaceous earth. As is apparent from Figure 3, the addition of Florisil to the sample did not increase recovery to the same extent as did diatomaceous earth; worse, it significantly reduced the efficiency of extraction of some pesticides (e.g. pirimiphos-methyl and fenamiphos). The improved extract cleanness provided by Florisil was not apparent owing to the high selectivity of SFE and the GC detector, and the type of sample used,

Comparison of SFE and Regulatory Solvent-Based Extraction Methods

Table III lists results obtained from the analysis of the eleven pesticides studied by SFE and two conventional liquid-solvent extraction methods. Supercritical CO_2 extractions were performed in triplicate on different days. SFE furnished significantly higher recoveries than solvent extraction with acetone and ethyl acetate for all the

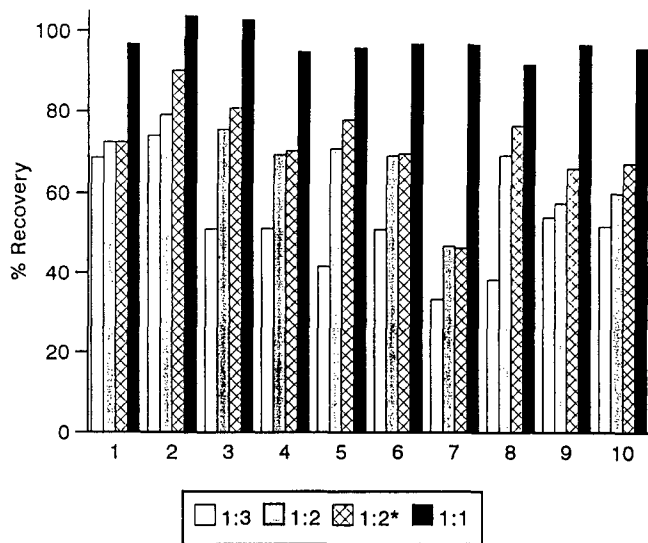


Figure 2
Recovery of organophosphorus pesticides from orange with supercritical CO₂ using different diatomaceous earth/sample ratios. *With addition of sodium carbonate. Compound numbering as for Figure 1.

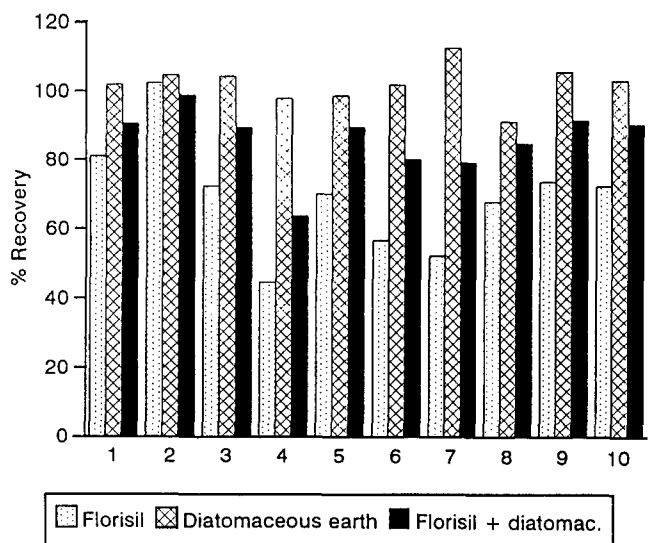


Figure 3
Influence of Florisil on the SFE recovery of organophosphorus pesticides from orange. Compound numbering as for Figure 1. The sample/Florisil and sample/diatomaceous earth ratios were 1:1; the sample/Florisil/diatomaceous earth ratio was 1:1:1.

organophosphorus pesticides studied except the extremely polar methamidophos, which is more than 100 times more soluble in water than in organic solvents [20] and partitioned into the aqueous phase remaining in the sample. There is some controversy about this compound. Whereas as the CO₂ extraction of methamidophos has proved unsuccessful for some authors [8, 9], Valverde-Garcia et al. [21] reported SFE recoveries above 70 % using CO₂ with anhydrous magnesium sulfate as a solid additive and methanol as a static modifier.

Table III. Recovery of organophosphorus pesticides from spiked orange samples using SFE and regulatory solvent-based extraction methods.

Compound	Conc. added (µg g ⁻¹)	Acetone Conc. ± S.d. (µg g ⁻¹)	Recovery ± s.d. (%)	Ethyl acetate Conc. ± S.d. (µg g ⁻¹)	Recovery ± s.d. (%)	SFE, pure CO ₂ Conc. ± S.d. (µg g ⁻¹)	Recovery ± s.d. (%)	SFE, 5 % MeOH - CO ₂ Conc. ± s.d. (µg g ⁻¹)	Recovery ± s.d. (%)
(%)Methamidophos	0.74	-	0	0.485 ± 0.007	66 ± 1	-	0	-	0
Thiometon	1.05	0.71 ± 0.04	96 ± 5	0.53 ± 0.03	71 ± 3	1.02 ± 0.08	97 ± 7	0.94 ± 0.07	90 ± 7
Dicloran	1.73	1.3 ± 0.2	73 ± 9	0.66 ± 0.03	38 ± 2	1.79 ± 0.08	104 ± 5	1.5 ± 0.2	88 ± 11
Chlorpyrifos-methyl	0.47	0.43 ± 0.04	91 ± 8	0.22 ± 0.02	47 ± 5	0.48 ± 0.03	103 ± 7	0.38 ± 0.08	81 ± 17
Pririmphos-methyl	0.47	0.40 ± 0.03	84 ± 7	0.20 ± 0.03	43 ± 5	0.45 ± 0.02	95 ± 4	0.31 ± 0.07	65 ± 15
Chlorpyrifos-ethyl	0.77	0.78 ± 0.06	101 ± 8	0.43 ± 0.05	56 ± 6	0.74 ± 0.04	96 ± 6	0.6 ± 0.1	81 ± 19
Chlorfenvinphos	1.02	0.8 ± 0.1	75 ± 10	0.37 ± 0.04	36 ± 4	1.0 ± 0.1	97 ± 10	0.8 ± 0.2	77 ± 19
Fenamiphos	0.78	0.28 ± 0.06	36 ± 8	0.24 ± 0.05	30 ± 6	0.8 ± 0.2	97 ± 28	0.5 ± 0.1	69 ± 18
Carbophenothion	0.47	0.47 ± 0.04	100 ± 8	0.27 ± 0.02	58 ± 5	0.43 ± 0.02	92 ± 4	0.39 ± 0.08	83 ± 17
Azinphos-methyl	1.00	0.44 ± 0.09	44 ± 8	0.17 ± 0.04	17 ± 4	1.0 ± 0.2	97 ± 15	0.89 ± 0.07	89 ± 7
Azinphos-ethyl	1.10	0.9 ± 0.1	82 ± 12	0.32 ± 0.06	29 ± 6	1.1 ± 0.2	96 ± 14	0.9 ± 0.1	81 ± 12

Diatomaceous earth/sample ratio, 1:1; n = 3.

As can be seen in Table III the use of 5 % methanol-modified CO₂ did not improve recovery and reproducibility was worse. In addition, Lehotay et al. [8] reported that the use of a modifier does not improve recoveries but increases matrix effects.

Conclusions

The proposed SFE method surpasses conventional solvent-based extraction for preparing fruit samples for organophosphorus pesticide analysis. It uses smaller samples, simplifies and automates preliminary operations, expedites analyses, and dramatically reduces organic solvent consumption. Extraction with supercritical CO₂ gives higher recoveries than extraction with acetone or ethyl acetate for all the pesticides except methamidophos, which is only partially recovered with ethyl acetate.

As shown in this work, diatomaceous earth is essential for quantitative recovery of pesticides from semi-solid samples. None the solid additives tested enhanced the efficiency of SFE by an amount comparable with that obtained using diatomaceous earth.

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