

Supercritical Fluid Extraction of Pesticides from Vegetables Using Anhydrous Magnesium Sulfate for Sample Preparation[†]

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Supercritical fluid extraction (SFE) was evaluated for the extraction of imidacloprid, methiocarb, chlorpyrifos, chlorothalonil, endosulfan I, endosulfan II, and endosulfan sulfate from pepper and tomato, using vegetable sample:anhydrous magnesium sulfate (5:7) mixtures to carry out the extractions and HPLC/DAD, GC/ECD, and GC/FPD for analysis. Preliminary experiments were performed to study the extractability of these pesticides from glass wool and different water absorbent materials. Spiked tomato and pepper samples were extracted with supercritical carbon dioxide assessing different extraction conditions. The chosen SFE conditions were 300 atm, 50 °C, 200 μ L of methanol static modifier, 1 min static time, dynamic extraction with 15 mL of CO₂, and collection in 3 mL of ethyl acetate. Except for imidacloprid, which was not recovered under any of the assessed conditions, pesticide recoveries were greater than 80%. Incurred residues of chlorpyrifos, endosulfan, and methiocarb in a number of pepper and tomato samples were as efficiently extracted with SFE as with conventional extraction methods.

Keywords: SFE; pesticides; vegetables

INTRODUCTION

Analytical methods for pesticide residues have their main application in the control of foods for human consumption, especially in the control of fruits and vegetables since they are generally produced using direct applications of pesticides (FAO, 1993). These controls are usually carried out by official agencies of developed countries, this being the reason that the analytical manuals published by these agencies (FDA, 1994; McLeod and Graham, 1986; Thier and Zeumer, 1987) are an excellent information source to know the most used methodologies in pesticide residue analysis. Such manuals and reviews published on multiresidue analysis of pesticides in fruits and vegetables (Ambrus and Thier, 1986; Valverde-García and Gonzalez, 1989; Holland and Malcolm, 1992) show that in the last decades few changes have been introduced in the basic scheme of the extraction step of these analyses, which is still being performed with large sample size and large volumes of organic solvents.

The recent development of analytical scale supercritical fluid extraction (SFE) has opened new perspectives to improve the sample preparation step in any analytical process (Hawthorne, 1990; King and France, 1992). In fact, the unique properties of supercritical fluids, especially supercritical carbon dioxide, have already been exploited to extract different pesticides from soils and other solid matrices with low water content (Camel et al., 1993; Richter, 1992; Dean, 1993), making these analyses simpler, faster, less expensive, and with smaller environmental impact and greater chances of miniaturization and automatization. However, these inherent advantages in SFE have scarcely been ex-

ploited in the analysis of pesticide residues in fruits and vegetables since this technique presents some practical limitations to be applied to high-water content samples (Burford et al., 1993).

Until now, two different approaches have been proposed to solve the problems caused by the water content of vegetable samples when pesticide residues want to be analyzed by SFE. One is to carry out the extraction on lyophilized samples (Jimenez et al., 1994), but this method is time consuming and can lead to the loss of volatile analytes. The second and more promising approach is to mix samples, prior to SFE, with an appropriate material to absorb water and disperse the sample (Burford et al., 1993). This approach was first used by Hopper and King (1991), who obtained good recoveries for some organochlorine and organophosphorus pesticides from spiked potato, incurred lettuce, and carrot samples, using pelletized diatomaceous earth (Hydromatrix) as water absorbent material. More recently, Lehotay and Eller (1995), Lehotay et al. (1995), and Aharonson et al. (1994), using this same material and commercial SFE equipment, have obtained excellent results in the extraction of close to 50 pesticides from spiked and incurred fruits and vegetable samples. However, the use of Hydromatrix led to obtain, in all cases, null recoveries for methamidophos, a very polar pesticide with a *n*-octanol/water partition coefficient (K_{ow}) of 0.16 (Tomlin, 1994).

Valverde et al. (1995) previously described an approach for sampling in SFE using anhydrous magnesium sulfate as the drying agent and showed that methamidophos can be efficiently extracted by SFE from different vegetables. In this work, the sample preparation method proposed in the previous paper on methamidophos has been assessed to extract by SFE different classes of nonpolar and intermediate polar pesticides from pepper and tomato samples, after carrying out a preliminary study on their extractability from glass wool and some water absorbent materials. Common name,

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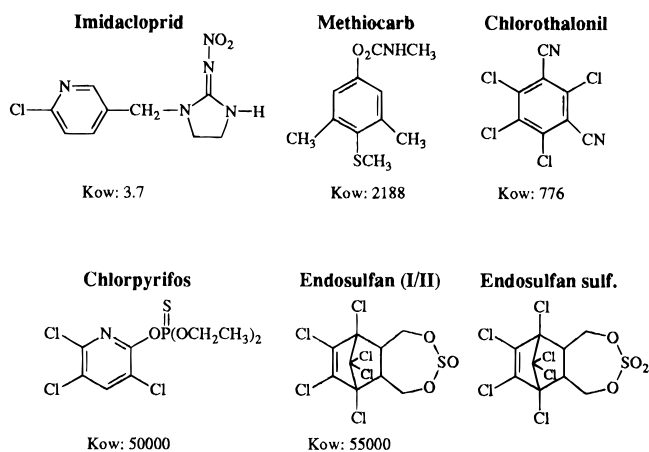


Figure 1. Common name, formula, and K_{ow} value of the studied pesticides.

formula, and *n*-octanol/water partition coefficient of the studied pesticides are indicated in Figure 1 (Tomlin, 1994).

EXPERIMENTAL PROCEDURES

Reagents and Apparatus. (a) Pesticide standards (purity > 98%) were supplied by Riedel de Haën (Seelze, Germany) except imidacloprid which was obtained from Bayer AG (Leverkusen, Germany). For each pesticide, a stock standard solution (about 500 mg/L) was prepared in acetone. Spiking standard solution, containing 50 mg/L imidacloprid, methiocarb, chlorothalonil, chlorpyrifos, endosulfan I, endosulfan II, and endosulfan sulfate, was prepared in acetone from the stock standard solutions.

(b) All the solvents used were Panreac (Barcelona, Spain), pesticide residue grade. Carbon dioxide, 99.995% purity, was supplied by SEO (Madrid, Spain). Anhydrous magnesium sulfate (>99% purity; Fluka, Buchs, Switzerland), Florisil (60–100 mesh; Baker, Deventer, Holland), wet sample support (diatomaceous earth; Isco, Lincoln, NE), and glass wool (pure and washed; Panreac) were used.

(c) The HPLC/DAD system was a HP-1040 M Series II/HP-1050 pump (Hewlett-Packard, Palo Alto, CA), equipped with a Lichrosphere ODS column, 5 μ m particle size, 4.6 mm i.d. \times 125 mm (Merck, Darmstadt, Germany). The following acetonitrile/water mobile phase program was used 25/75 (0–2 min), 90/10 (12 min), and 100/0 (15 min). Flow rate was 1 mL/min, and injection volume was 20 μ L.

(d) The gas chromatograph was a Hewlett-Packard 5890 with electron capture detection (ECD), equipped with a HP-5 fused silica gel capillary column, 30 m length \times 0.25 mm i.d., 0.25 μ m film thickness. The operating conditions were injector temperature, 280 $^{\circ}$ C; detector temperature, 300 $^{\circ}$ C; oven temperature program, 140 $^{\circ}$ C (1 min) and 5 $^{\circ}$ C/min to 280 $^{\circ}$ C (5 min); helium flow rates, 1 mL/min (carrier), 10 mL/min (split), and 30 mL/min (make up); splitless time, 0.75 min.

(e) A Perkin-Elmer 8600 gas chromatograph (Norwalk, CT) with flame photometric detection (FPD) in phosphorus mode, equipped with a BP-10 wide bore fused silica gel capillary column, 25 m length \times 0.53 mm i.d., 1 μ m film thickness, was also used. The operating conditions were injector and detector temperature, 300 $^{\circ}$ C; oven temperature program, 180 $^{\circ}$ C (1 min), 30 $^{\circ}$ C/min to 190 $^{\circ}$ C (7 min), and 30 $^{\circ}$ C/min to 270 $^{\circ}$ C (7 min); carrier (nitrogen) flow rate, 10 mL/min.

(f) An Isco SFE system, consisting of one Model 260D syringe pump and controller, a SFX 2-10 extractor with restrictor heater set at 70 $^{\circ}$ C, and 10 mL stainless steel extraction cartridges with removable 2 μ m frits, was used in this study. Uncoated and deactivated fused silica gel capillary column, 30 cm length \times 50 μ m i.d., was used as restrictor, and 10 mL graduated test tubes, immersed in a 15–20 $^{\circ}$ C water bath, containing 3 or 5 mL of ethyl acetate were used as the collection system.

Recovery Tests with Glass Wool and Drying Agents. Some preliminary experiments were performed to evaluate the

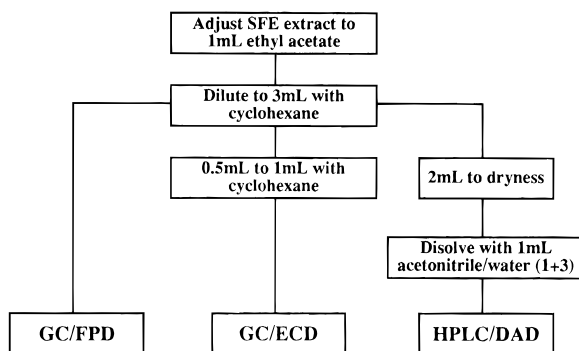


Figure 2. Preparation process of SFE extracts for GC and HPLC analysis.

extractability of the pesticides from glass wool (as inert material) and different water absorbent materials such as Florisil, Isco wet sample support, and anhydrous magnesium sulfate. Extractions were carried out in 10 mL extraction cartridges packed with 2.4 g of glass wool, 4.8 g of Florisil, 4.1 g of wet sample support, and 11.2 g of anhydrous magnesium sulfate or 10.2 g of anhydrous magnesium sulfate:water mixture (2 + 1) with a layer of 1 g of anhydrous magnesium sulfate at the bottom. Materials were spiked with 1.5 μ g of each pesticide by addition to the packed cartridge of 30 μ L of spiking standard solution and extracted with supercritical carbon dioxide in dynamic mode (after 1 min static), assessing the extraction conditions specified under Results and Discussion.

Preparation and SFE of Vegetable Samples. Pepper and tomato samples used in this study were provided by the Association of Producers and Exporters of Fruits and Vegetables of Almería (COEXPHAL). All vegetable samples were previously analyzed in the Residue Control Laboratory of COEXPHAL, using a conventional ethyl acetate–sodium sulfate-based extraction method and GC/FPD–ECD and HPLC/DAD (Agüera et al., 1993; Fernandez-Alba et al., 1994, 1995). Samples that were demonstrated not to contain any detectable pesticide residue were selected to perform the SFE experiments with spiked samples. Five vegetable samples determined to contain some pesticide residue were used as incurred samples. The sample preparation method was that proposed in the previous paper on SFE of methamidophos from vegetables (Valverde-García et al., 1995). Specifically, 20 g of blended fresh vegetable sample was thoroughly mixed with 28 g of anhydrous magnesium sulfate in a glass beaker immersed in an ice/water bath (the ice/water bath was used because anhydrous magnesium sulfate mixed with water generates heat). After 5 min, the mixture was thoroughly pounded in a porcelain mortar until obtaining a dry and homogeneous powdered mixture. This mixture was named SFE sample.

Extractions were done in 10 mL extraction cartridges packed with 8 g of SFE sample, placing first 1 g of anhydrous magnesium sulfate at the bottom of the cartridge to bind any water that migrated during the extraction. All the extractions were performed with supercritical carbon dioxide, in dynamic mode after 1 min static equilibrium period. The assessed extraction conditions (CO₂ volume, pressure, temperature and static modifier) are specified under Results and Discussion.

Chromatographic Analysis. Evaporation of the collection solvent during SFE led to an ethyl acetate volume of 0.8–2 mL after extraction, depending on the extraction conditions. In all cases, the extract volume was adjusted to 1 mL by evaporation with a nitrogen stream or addition of ethyl acetate. Different portions of this extract were then prepared for GC and HPLC analysis following the scheme indicated in Figure 2. In the experiments carried out with vegetable samples, the GC/FPD, GC/ECD, and HPLC/DAD analytical extracts contained 1.11, 0.55, and 2.22 g of vegetable sample/mL, respectively.

Imidacloprid and methiocarb were determined by HPLC/DAD at 210 and 270 nm, respectively. Chlorpyrifos was determined by GC/FPD, whereas chlorothalonil and endosul-

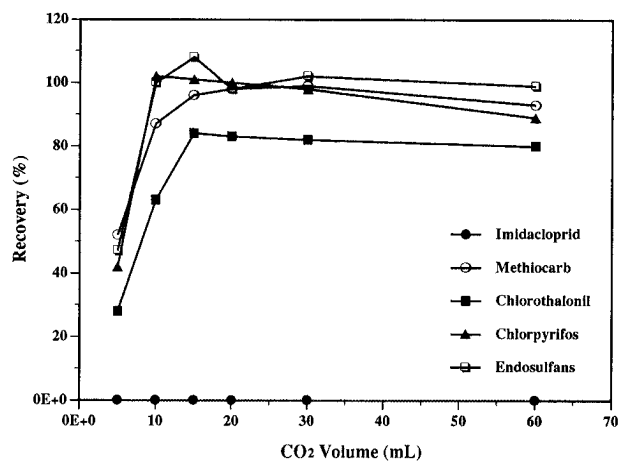


Figure 3. Effect of CO₂ volume on pesticide recoveries from glass wool spiked with 1.5 µg of each pesticide (SFE conditions: dynamic mode after a 1 min static equilibrium, 50 °C, 300 atm).

fans were determined by GC/ECD. Pesticide standard solutions to make external calibrations were prepared by suitable dilution of the spiking standard solution with cyclohexane/ethyl acetate (2 + 1) for GC/FPD analysis, cyclohexane/ethyl acetate (5 + 1) for GC/ECD analysis, and acetonitrile/water (1 + 3) for HPLC/DAD analysis.

RESULTS AND DISCUSSION

Glass Wool. Figure 3 shows the effect of CO₂ volume on pesticide recoveries from spiked glass wool when extractions were carried out at 300 atm pressure and 50 °C temperature. Recoveries obtained for the three endosulfans were very similar, and values indicated in this figure are the mean recoveries obtained for endosulfan I, endosulfan II, and endosulfan sulfate. Recoveries greater than 80% were obtained for methiocarb, chlorothalonil, chlorpyrifos, and endosulfans with only 10 or 15 mL of CO₂, whereas imidacloprid was not recovered at all, including when extractions were done with 60 mL of CO₂. Previous CO₂-bubbling experiments, conducted with empty extraction cartridges and collecting CO₂ in 3 or 5 mL of ethyl acetate solution containing 1.5 µg of each pesticide, demonstrated that none of these pesticides is practically removed during the bubbling and the analytical extracts preparation processes.

The effects of pressure and temperature on the extractability of the pesticides from spiked glass wool were also studied, performing extractions with 15 mL of CO₂ at different pressures (200, 400, and 500 atm) and 50 °C temperature and at different temperatures (40, 70, and 90 °C) and 300 atm pressure. In all cases, recoveries obtained in these experiments were close to those given in Figure 3 for 15 mL of CO₂. Recovery data obtained from glass wool indicate that, such as was expected, the solubility of these pesticides in pure CO₂ is higher when their polarity is lower. However, polarity issues alone cannot explain why imidacloprid was not recovered at all. Hawthorne (1990) noted that labile compounds, such as imidacloprid, may not be extracted by supercritical CO₂.

Drying Agents. Table 1 shows the results obtained in the extractions carried out on different water absorbent materials, spiked with 1.5 µg of each pesticide, using 15 mL of CO₂ at 50 °C at 300 or 500 atm. In this table we can point out the following results: (i) imidacloprid was not recovered at all from these materials;

Table 1. Pesticide Recoveries Obtained by SFE^a of Florisil, Isco Wet Sample Support (Isco Wss), Anhydrous Magnesium Sulfate, and Anhydrous Magnesium Sulfate:Water (2 + 1) Mixture, Spiked with 1.5 µg of Each Pesticide

pesticide	recovery, %							
	florisil		Isco WSS		MgSO ₄		MgSO ₄ :H ₂ O (2 + 1)	
	A	B	A	B	A	B	A	B
imidacloprid	0	0	0	0	0	0	0	0
methiocarb	0	0	79	90	0	0	73	92
chlorothalonil	0	0	70	76	85	90	84	108
chlorpyrifos	85	104	83	85	79	75	88	107
endosulfan I	92	92	100	94	85	89	80	88
endosulfan II	0	0	101	96	82	78	88	91
endosulfan sulfate	0	0	98	94	91	79	90	90

^a Dynamic extraction (1 min static equilibrium time) with 15 mL of compressed CO₂, at 50 °C temperature and two different pressures: (A) 300 atm, and (B) 500 atm.

(ii) only chlorpyrifos and endosulfan I were recovered from Florisil; (iii) recoveries obtained from Isco wet sample support and anhydrous magnesium sulfate were similar to those obtained from glass wool, except the zero values obtained for methiocarb from anhydrous magnesium sulfate; and (iv) such as was observed for methamidophos (Valverde-García et al., 1995), the water contained in the magnesium sulfate:water (2 + 1) mixture seems to be an efficient modifier for the extraction of methiocarb from magnesium sulfate.

It is interesting to note that, under the extraction conditions indicated in Table 1, endosulfan I is efficiently extracted from Florisil whereas endosulfan II and endosulfan sulfate are not recovered at all. These results are in agreement with those previously published by Valverde-García et al. (1992) on the elution patterns of endosulfans in short Florisil columns using different petroleum ether:ethyl ether mixtures as elution solvent. Likewise, previous studies carried out by ISCO (1991) have shown that endosulfan II and endosulfan sulfate are more difficult to extract than endosulfan I from spiked soils with supercritical CO₂.

Vegetable Samples. First recovery tests were conducted on SFE tomato samples spiked into the extraction cartridge with 1.5 µg of each pesticide (30 µL of spiking standard solution), assessing different pressures (300 and 500 atm), temperatures (50, 70, and 90 °C), CO₂ volumes (15 and 30 mL), and modifier (no modifier, 200 µL of methanol static modifier) conditions. With our sample preparation method, the potential difficulties (Burford et al., 1993) caused by the water content of vegetables were avoided. Extractions were performed without plugging the capillary restrictor or significantly affecting flow rate. Additionally, extracts did not contain water, and cleanup was not needed before analysis. Figure 4 shows the HPLC chromatograms obtained at 210 and 270 nm for a pesticide standard solution containing 1 mg/L of each pesticide and the extract corresponding to a spiked SFE tomato sample, when it was extracted using the extraction conditions indicated in the figure caption.

Pesticide recoveries obtained in the experiments carried out on spiked SFE tomato samples ranged from 77% to 116%, except for imidacloprid which was not recovered at all, in all cases. Figure 5 shows the results obtained when extractions were carried out with 15 mL of CO₂ at 50 °C and 300 atm/no modifier, 500 atm/no modifier, or 300 atm/200 µL of methanol static modifier. Additional extractions on SFE tomato samples spiked with only 1.5 µg of imidacloprid were performed with 30 or 60 mL of CO₂ at 500 atm and 50 °C and using

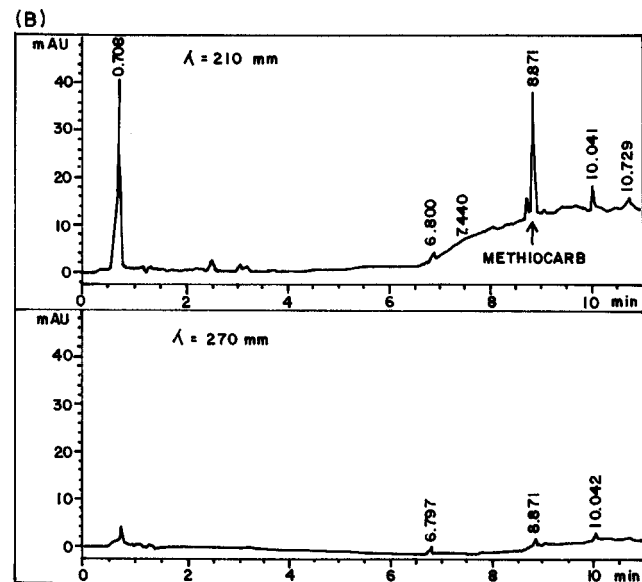
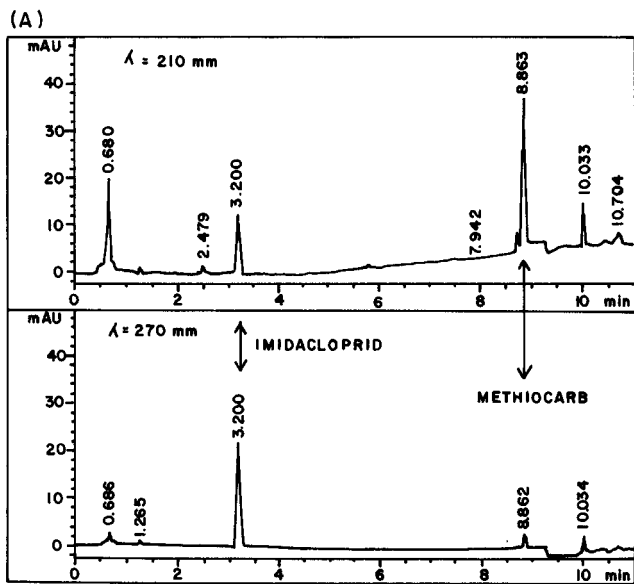


Figure 4. HPLC chromatograms at 210 and 270 nm corresponding to (A) pesticide standard solution containing 1 mg/L of each pesticide and (B) extract corresponding to a SFE tomato sample spiked with 1.5 μ g of each pesticide (SFE conditions: dynamic extraction after 1 min static equilibrium, 50 °C, 300 atm, with 15 mL of CO₂ and 200 μ L of methanol static modifier).

200 μ L of methanol, acetone, or 2-propanol as static modifier, obtaining again, in all cases, null recoveries for this pesticide.

The extraction conditions selected to evaluate the proposed method on spiked and incurred pepper and tomato samples were 300 atm, 50 °C, 15 mL of CO₂, and 200 μ L of methanol as modifier. Under these conditions, it was already demonstrated that methamidophos is efficiently extracted from different vegetables (Valverde et al., 1995). Table 2 shows the results obtained for pepper and tomato samples spiked with 0.5 and 0.1 mg/kg of each pesticide (these samples were spiked by adding 1 mL or 200 μ L of the spiking standard solution to 100 g aliquots of blended fresh tomato or pepper samples). In this table, mean recovery data correspond to single extraction of three different SFE vegetable samples prepared from each spiked fresh vegetable sample. In all cases, mean recoveries were greater than 80%, except, as was expected, for imidacloprid which was not recovered at all.

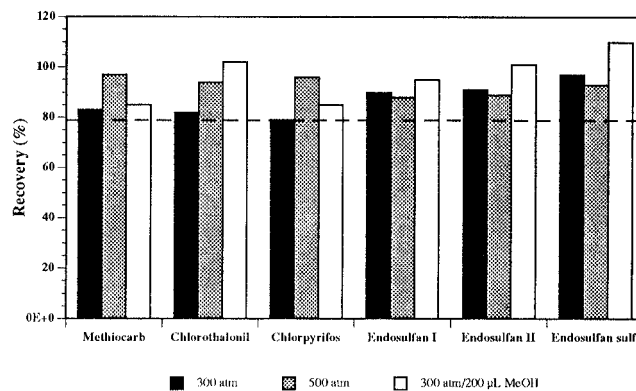


Figure 5. Pesticide recoveries from SFE tomato samples spiked with 1.5 μ g of each pesticide (SFE conditions: dynamic extraction after 1 min static equilibrium, with 15 mL of CO₂ at 50 °C and 300 atm/no modifier, 500 atm/no modifier, and 300 atm/200 μ L of methanol static modifier).

Table 2. Pesticide Recoveries Obtained by SFE^a of Pepper and Tomato Samples Spiked with 0.5 and 0.1 mg/kg of Each Pesticide

pesticide	recovery, ^b % (CV, %)			
	pepper		tomato	
	0.5 ppm	0.1 ppm	0.5 ppm	0.1 ppm
imidacloprid	0 (0)	0 (0)	0 (0)	0 (0)
methiocarb	92 (8)	81 (24)	103 (12)	83 (17)
chlorothalonil	86 (7)	90 (10)	81 (10)	87 (8)
chlorpyrifos	96 (4)	96 (6)	90 (5)	85 (9)
endosulfan I	80 (8)	95 (9)	91 (6)	93 (11)
endosulfan II	89 (8)	104 (11)	102 (7)	100 (13)
endosulfan sulfate	92 (11)	98 (14)	99 (4)	108 (16)

^a Dynamic extraction (1 min equilibrium static period) at 50 °C and 300 atm, with 15 mL of compressed CO₂ and 200 μ L of methanol as static modifier. ^b Mean value of single extraction of three replicate SFE samples.

Table 3. Pesticide Levels Determined by the Proposed SFE Method in Incurred Vegetable Samples Previously Analyzed by Conventional Analysis

sample	pesticide	residue level, mg/kg	
		conventional anal.	SFE anal. ^a
tomato	endosulfan ^b	0.37	0.81 (7) ^d
pepper	endosulfan ^b	0.16	0.19 (13)
	methiocarb	0.55	0.38 (17)
	methiocarb sulfoxide	na ^c	0.47 (16)
tomato	endosulfan ^b	0.05	0.08 (11)
pepper	methiocarb	0.16	0.12 (28)
	methiocarb sulfoxide	na ^c	0.08 (18)
pepper	endosulfan ^b	0.14	0.09 (16)
	chlorpyrifos	1.11	0.90 (9)
	methamidophos	0.35	0.42 (11)

^a Mean value of three replicate extractions (CV in parentheses). ^b Sum of endosulfan I, endosulfan II, and endosulfan sulfate. ^c Not analyzed. ^d Mean value of three replicate extractions of two replicate SFE samples.

Pesticide levels determined by SFE and conventional analysis in five incurred pepper and tomato samples are given in Table 3. Results obtained for methiocarb, chlorpyrifos, and endosulfan indicate that these pesticides are as efficiently extracted with SFE as with the conventional extraction method. Table 3 also shows that residues of methiocarb sulfoxide can be extracted from incurred vegetable samples by using the proposed SFE method. Finally, Figure 6 shows the GC/FPD and GC/ECD chromatograms obtained for a SFE extract corresponding to the pepper sample containing incurred residues of endosulfan, chlorpyrifos, and methamidophos. The residue levels determined for methamidophos in this sample confirm the results previously

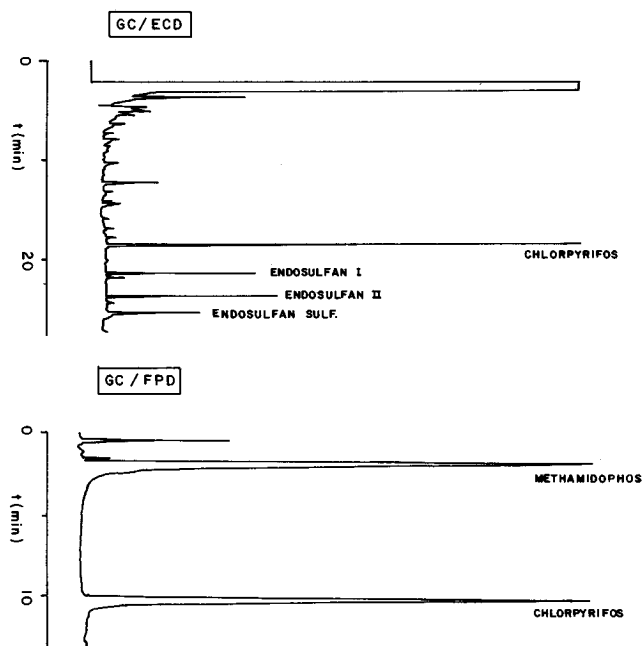


Figure 6. GC/ECD and GC/FPD chromatograms corresponding to an extract obtained by the SFE proposed method for the pepper sample containing incurred residues of endosulfan, chlorpyrifos, and methamidophos.

published (Valverde-García et al., 1995) on SFE of this pesticide from vegetables.

In conclusion, supercritical fluid extraction has been demonstrated to be a suitable alternative to conventional solvent extraction methods to extract some polar and nonpolar pesticides from vegetables, when samples are mixed with anhydrous magnesium sulfate. Compared with the standard methodology, the proposed method is faster, is less expensive, is environmentally safer, and requires less glassware and laboratory space. However, future works on the proposed method must be made to succeed in extracting imidacloprid residues, to test other pesticides and matrices, and to validate our results by suitable interlaboratory tests.

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