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Multiresidue Analysis of Pesticides in Soil by Supercritical Fluid Extraction/Gas Chromatography with Electron-Capture Detection and Confirmation by Gas Chromatography−Mass Spectrometry

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The applicability of supercritical fluid extraction (SFE) in pesticide multiresidue analysis (organohalogen, organonitrogen, organophosphorus, and pyrethroid) in soil samples was investigated. Fortification experiments were conducted to test the conventional extraction (solid−liquid) and to optimize the extraction procedure in SFE by varying the CO_2 modifier, temperature, extraction time, and pressure. The best efficiency was achieved at 400 bar using methanol as modifier at 60 °C. For the SFE method, C-18 cartridges were used for the cleanup. The analytical screening was performed by gas chromatography equipped with electron-capture detection (ECD). Recoveries for the majority of pesticides from spiked samples of soil at different residence times were 1, 20, and 40 days at the fortification level of 0.04−0.10 mg/kg ranging from 70 to 97% for both methods. The detection limits found were <0.01 mg/kg for ECD, and the confirmation of pesticide identity was performed by gas chromatography−mass spectrometry in a selected-ion monitoring mode. Multiresidue methods were applied in real soil samples, and the results of the methods developed were compared.

KEYWORDS: Pesticide multiresidue analysis; supercritical fluid extraction; capillary gas chromatography−mass spectrometric detection with selective ion monitoring; soil

1. INTRODUCTION

Pesticides are generally recognized as significantly benefiting our ability to meet the world's need for abundant, safe, and affordable food and fiber. Pesticides reach the soil environment by direct or indirect application from aerial and ground applications. The main processes potentially affecting the ultimate fate of pesticides in soil are retention by soil materials (involving adsorption/desorption processes), transformation processes (biological and chemical degradation), and transport (throughout soil, atmosphere, surface water, or groundwater) (*1*, *2*).

Contrary to food, the extraction of soil samples is an issue for which new approaches are published more often. The interaction between the matrix and the analytes is stronger than that in food; thus, bound residues can be formed with an extraction behavior different from that of a nonbound fraction. The occurrence and significance of bound pesticide residues in soil have become critical in dealing with the persistence, degradation, and biological availability of pesticide residues (*3*). To obtain comparable results, an extraction procedure capable of liberating the bound residues of these analytes is required (*2*).

The Soxhlet extraction has been applied for 30 years, and although it is time-consuming, it is regarded as the most exhaustive procedure. Due to high recovery rates, it is seen as a reference method for soil extraction (*2*). Conventional methods have been applied in the multiresidue determination of pesticides in soil using sonication with ethyl acetate (*4*) and methanol (*5*), as well as liquid−solid extraction. Because conventional liquid−solid extraction techniques, such as Soxhlet extraction, sonication, and mechanical shaking are laborious and time-consuming and need large volumes of toxic organic solvents, closer attention is being paid to the development of more efficient environmentally friendly techniques for the rapid analytical-scale extraction of solid matrices, such as the supercritical fluid extraction (SFE) (*6*).

The key factors in the extraction process related to pesticide solubility in the supercritical fluid are pesticide desorption from the matrix surface and diffusion of the desorbed pesticide into the bulk solvent (*7*). Upon the application of SFE, extraction is performed by supercritical CO_2, with solubility of the analytes

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in supercritical CO_2 tuned by changing the density of the fluid. This is generally obtained by the optimization of CO_2 pressure and temperature of the extraction cell (*8*−*10*). However, for quantitative extraction of moderately polar pesticide residues in soil, a modifier such as methanol has to be applied to obtain satisfactory results (*11*−*13*). SFE has shown to be an extraction technique with which a few groups of compounds can be isolated from soil, focusing on each application (*6*).

In this study, one rapid analytical multiresidue method for the determination in soil of organochlorine, organophosphorus, organonitrogen, and pyrethroid pesticides, based on SFE, was developed and compared with the conventional extraction. The development of SFE included variations of instrumental parameters such as modifier, temperature, pressure, and time. The cleanup was based on C-18 followed by GC-ECD for simultaneous determination, and confirmatory analysis was carried out by GC-MS in the selected-ion monitoring (SIM) mode. The extraction efficiencies were directly compared to those achieved using solid−liquid extraction. After this point, the methods were applied to the analysis of real samples and the results were discussed.

2. EXPERIMENTAL PROCEDURES

2.1. Soil Samples. Soil samples (∼1 kg) were collected from agricultural fields in the fall of 2003 in Bauru (São Paulo state), Brazil. The bulk soil samples were ground and passed through a 2 mm sieve to remove stones and plant material, after which the samples were homogenized and placed in brown glass bottles.

Blank soils, collected from preserved local sites, were air-dried, ground, and sieved through a 2 mm sieve. To prepare pesticide-free samples, the soil samples were sequentially immersed in methanol, acetone, methylene chloride, and *n*-hexane for at least 24 h, for each solvent. Finally, it was determined that there were no detectable levels of target analytes in the soil samples before spiking, by using both conventional and supercritical methods.

2.2. Soil Sample Fortification. Freshly fortified samples were prepared by adding an appropriate volume of a standard working solution to 25 g of dried homogenized soil sample. Additional acetone was added until the solvent completely covered the soil particles. The bulk of the solvent was slowly evaporated to an air-dried level. The mixture was then thoroughly mixed for 50 min in a mechanical shaker. Samples with aged residues were prepared by spiking soil samples with an appropriate volume of the standard working solution. After overnight air-drying, the sample was stored at room temperature for different times (1, 20, and 40 days) and thereafter stored at 4 °C until the extraction moment. Fortification levels for each pesticide, ranging from 0.04 to 0.10 mg/kg, are reported in **Table 1**.

It was assumed that the contaminants were uniformly distributed in the sample and that, because the soil retained residual moisture throughout the storage period, any analyte−matrix interactions would have occurred, over the weathering period, to a similar extent as in real contaminated soil with similar properties.

2.3. Chemicals. *2.3.1. Pesticide Standards.* Reference pesticide standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany) with purity ranging from 95 to 100%. The pesticides investigated are listed in **Table 1**.

2.3.2. Pesticide Solutions. Pesticide stock solutions (∼500 mg/L) of individual pesticide standards were prepared by dissolving ∼0.050 g of the pesticide in 100 mL of acetone/*n*-hexane (50:50, v/v) and storing in a freezer at −18 °C in glass bottles with PTFE-faced screw caps. Pesticide working solutions were prepared to test recovery of conventional and SFE methods by an appropriate dilution of acetone/*n*-hexane (50:50, v/v).

2.3.3. Organic Solvents and Reagents. Acetone, *n*-hexane, methylene chloride, ethyl acetate, and methanol, of special grading for the pesticide residue analysis, were purchased from Mallinckrodt, Merck. Sep-PakVac C-18 cartridges (3 mL, 500 mg) were purchased from Waters (Milford, MA). A special siphonated CO_2 from White Martins was also used in SFE.

Table 1. Retention Times, Recoveries of Spiked Soil Samples Obtained by Solid–Liquid Extraction in Different Aging Days, and Detection Limits of Pesticides Studied

pesticide	t_R (min)	spiking level (mg/kg)	recovery (RSD, %) 1 day	20 days	40 days	LOD (mg/kg)
organohalogen						
aldrin	27.99	0.10	87 (6.1)	81 (6.4)	79 (6.2)	0.003
bromopropylate	39.71	0.10	85 (6.3)	85 (6.9)	78 (5.8)	0.004
chlorothalonil	26.47	0.05	83 (6.9)	80 (5.3)	81 (5.4)	0.002
diclofop-methyl	38.65	0.08	82 (5.7)	81 (6.8)	79 (6.1)	0.006
dicofol	30.03	0.05	81 (5.8)	83 (6.6)	82 (5.3)	0.005
endosulfan α	32.50	0.05	84 (5.5)	86 (7.9)	81 (5.9)	0.003
endosulfan β	36.41	0.06	88 (5.1)	81 (5.9)	80 (6.6)	0.003
hexachlorobenzene	21.11	0.07	89 (5.8)	83 (5.8)	81 (7.9)	0.004
metoxychlor	41.29	0.06	83 (5.2)	84 (6.3)	82 (8.0)	0.005
tetradifon	42.20	0.05	81 (6.7)	82 (7.1)	78 (7.2)	0.002
organonitrogen						
buprofezin	34.96	0.05	88 (8.5)	89 (7.2)	80 (7.3)	0.005
dicloran	23.50	0.05	82 (5.7)	90 (6.6)	81 (5.5)	0.003
etaconazole	36.55	0.06	91 (5.9)	86 (7.4)	76 (6.8)	0.003
hexaconazole	33.27	0.05	83 (6.5)	85 (6.8)	79 (5.7)	0.005
imazalil	33.90	0.05	86 (6.1)	88 (5.9)	83 (5.4)	0.005
linuron	8.84	0.07	82 (7.7)	84 (6.2)	80 (7.5)	0.006
metolachlor	28.87	0.05	92 (5.5)	89 (6.8)	82 (7.6)	0.007
prochloraz	45.64	0.06	86 (5.8)	84 (5.1)	81 (5.1)	0.006
propiconazole	37.73	0.05	78 (7.3)	83 (7.5)	82 (6.5)	0.004
quizalofop-ethyl	49.40	0.08	80 (5.3)	85 (6.8)	77 (6.8)	0.007
tebuconazole	38.41	0.05	83 (8.2)	90 (7.4)	79 (6.7)	0.006
triadimefon	29.17	0.06	84 (5.8)	91 (6.3)	81 (6.3)	0.003
triadimenol	31.28	0.06	87 (6.3)	88 (6.8)	75 (5.9)	0.009
trifluralin	17.30	0.05	84 (6.0)	86 (7.9)	72 (7.3)	0.004
vinclozolin	26.57	0.07	89 (6.2)	87 (5.3)	73 (6.8)	0.003
organophosphorus						
chlorpyrifos	29.53	0.06	93 (6.2)	91 (6.6)	73 (5.8)	0.002
diazinon	24.08	0.07	85 (7.3)	82 (5.7)	74 (6.2)	0.005
dichlorvos	7.27	0.05	89 (5.4)	84 (6.5)	71 (6.7)	0.006
dimethoate	25.16	0.05	86 (5.3)	85 (7.0)	73 (7.5)	0.007
pyrethroid						
cyfluthrin	46.08	0.06	88 (5.5)	89 (6.8)	77 (6.1)	0.006
	46.34					
	46.68					
cypermethrin	47.67	0.05	86 (6.9)	90 (7.2)	76 (5.9)	0.005
	48.20					
	48.36					
fenvalerate	52.29	0.06	89 (7.8)	91 (6.8)	80 (7.3)	0.005
	53.51	0.06	83 (6.7)	85 (5.8)	76 (5.8)	0.005

2.4. Solid−Liquid Extraction (Conventional Method). The multiresidue extraction method used in the determination of pesticides in soil was based on the literature with a few modifications (*14*).

A 25 g portion of the homogenized soil sample was weighed in an Erlenmeyer flask and fortified when required with the pesticide standard solution (described under section 2.2). The sample was extracted with 25 mL of acetone containing 1 mL of 2 N ammonium acetate. Sufficient water was added with continuous stirring so as to disintegrate the soil into small particles, and the flask was shaken for 30 min under constant stirring. After resting for some time, the extracts were decanted and the extraction was repeated once again with 15 mL of acetone. The extracts were vacuum-filtered by means of a Büchner funnel fitted with a no. 1 Whatman filter paper, and the residue was washed with two portions of 10 mL of acetone. The extracts were transferred to a 1 L separatory funnel, 250 mL of 2% NaCl was added, and the extracts were partitioned with 50 mL of methylene chloride in two steps. Both phases were combined, dehydrated by their passing through a filter containing a bed of anhydrous Na_2SO_4, and concentrated in a rotary evaporator under reduced pressure at 65 °C; the sample was dried under a gentle stream of pure nitrogen. The residue was dissolved in 1 mL of acetone and submitted to the GC-ECD analysis.

2.5. Supercritical Fluid Extraction. SFE was carried out by using an SFX-220 extraction system (ISCO, Lincoln, NE), which consists of an SFX-220 extractor, an SFX-200 controller, a 100 DX syringe pump, and a siphonated carbon dioxide, which was pressurized until working pressure.

Six grams of soil sample was spiked in a 50 mL beaker with the pesticide standard solution as described under section 2.2. According to Lopez-Avila et al. (*15*) and Lehotay and Eller (*16*) the mixture was distributed on the sample surface and homogenized, by mixing 1.0 g of hydromatrix, improving the homogeneous CO_2 flow through the soil sample with different water contents. The soil samples were poured into the stainless steel extraction cell (5.6 cm × 1.6 mm i.d.) in a sandwich mode, using silanized glass wool at both the bottom and the top of the cell to protect cell sealing. Prior to extraction, whenever necessary, a modifier (acetone and methanol) was added to the samples by pipetting a calculated volume in relation to the total volume of the SFE cell so as to obtain a 10% v/v supercritical fluid volume.

Optimized extraction conditions were obtained by sequentially varying one experimental parameter while all other parameters remained fixed. The parameters were varied in the order of modifier species, temperature, pressure, and extraction time. The results of the current test were used to determine the next extraction parameter change for optimization. The optimized extraction conditions obtained using the spiked blank soil were 10% of methanol modifier, extraction pressure of 400 bar, extraction temperature of 60 °C, and extraction time of 20 min.

The varied extraction conditions were 40, 60, and 90 °C and 200, 400, and 600 bar, using a flow rate of expanded gas of 1.5 mL/min of CO_2 or CO_2 modified with 10% of acetone or methanol. The extraction time was tested at 20, 40, and 60 min to optimize pesticide recoveries in soil samples.

A fused-silica capillary tube (30 cm × 100 μm i.d.) was attached to the outlet of the extractor as a restrictor, and the pesticides were collected on-line in a C-18 cartridge at 10 °C (the procedure is described under section 2.6).

2.6. SPE Cleanup. The extracts obtained as described under section 2.5 were submitted to an SPE column.

A Supelco Visiprep-12 manifold was used for the cleanup of the samples. The cleanup is performed in C-18 cartridges attached to the vacuum manifold and conditioned with ~5 mL of ethyl acetate/*n*-hexane (50:50, v/v). When only 1 mL of ethyl acetate/*n*-hexane remained in the cartridge volume, the cartridge valve of the manifold was closed to prevent the drying of the cartridges. The extract was added to the column and eluted under gravity with two portions of 5 mL of methylene chloride/*n*-hexane (50:50, v/v), *n*-hexane/acetone (80:20, v/v, and 20:80, v/v). Once elution was completed, the collected extracts were concentrated under a gentle N_2 stream.

The residue was quantitatively dissolved in 1 mL of acetone and submitted to analysis by GC-ECD and GC-MS.

2.7. GC-ECD. A Hewlett-Packard model 5890 series II gas chromatograph equipped with a ^{63}Ni electron-capture detector and a fused-silica capillary column HP-608 (30 m × 0.25 μm i.d.; film thickness = 0.25 mm) was used. The operating conditions were as follows: initial temperature, 45 °C (1 min), increased at 20 °C/min to 150 °C, kept for 5 min, then ncreased at 4 °C/min to 280 °C for 20 min; injector temperature, 250 °C; H_2 carrier gas; column linear velocity (μ = 45 cm/s); detector temperature, 300 °C; makeup gas N_2; operated in the splitless mode; purge off time, 1 min; injection volume, 1 μL.

2.8. GC-MS. Confirmatory run analysis was performed on a Hewlett-Packard model 5890 series II gas chromatograph with an HP 5972 mass selective ion detector (quadrupole) and a fused-silica capillary column LM-5−5% phenyl 95% dimethylpolysiloxane (35 m × 0.25 mm i.d., film thickness = 0.25 μm). GC was operated under the following conditions: initial temperature, 45 °C (1 min), increased at 21 °C/min to 150 °C, kept for 5 min, then increased at 4 °C/min to 280 °C, and the final temperature being held for 30 min; injector temperature, 250 °C; He carrier gas; GC-MS transfer line, 280 °C; operated in the splitless mode; purge off time, 1 min; injection size, 1 μL. MS conditions were as follows: solvent delay, 2.9 min; electron impact ionization voltage, 70 eV; scan rate, 1.5 scan/s; scanned-mass range, *m*/*z* 40−600.

3. RESULTS AND DISCUSSION

3.1. Solid−Liquid Extraction. To determine the influence of soil matrix on the determination of pesticides, it is necessary to obtain data relating to their sorption. The sorption of pesticides by soil is governed by various intermolecular interactions including van der Waals, hydrogen bonding, charge transfer, ligand exchange, direct and induced ion−dipole, dipole−dipole interactions, and chemisorption (*17*). Among the known processes, sorption−desorption behavior is considered to be the most important process affecting organic contaminants (*18*). The specific portion of the soil with which a pesticide interacts will depend on both the compound and the soil, as

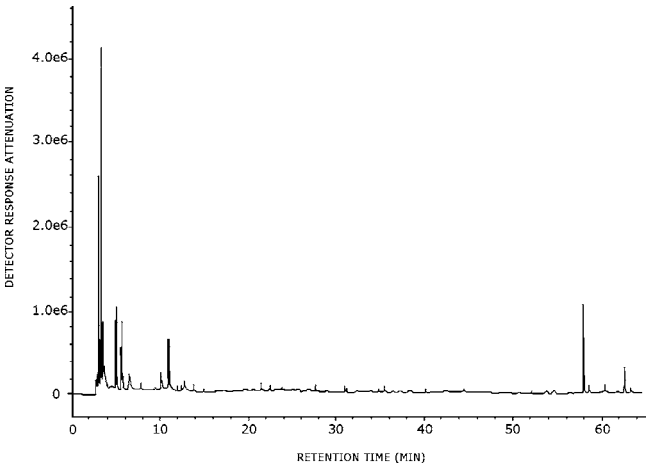

Figure 1. GC-ECD chromatogram of soil extract blank obtained by solid–liquid extraction method (see conditions under section 2.4).

Table 2. Recoveries of Pesticides in Spiked Soil Samples by SFE Using CO_2 Modified with 10% Acetone and 10% Methanol

	recovery (%)	
pesticide	CO_2 10% acetone	CO_2 10% methanol
organohalogen		
aldrin	54 (6.3)	56 (3.9)
bromopropylate	55 (5.2)	59 (3.7)
chlorothalonil	58 (4.2)	61 (6.1)
diclofop-methyl	61 (5.1)	63 (5.8)
dicofol	63 (6.2)	67 (5.4)
endosulfan α	62 (5.3)	65 (5.9)
endosulfan β	64 (6.8)	69 (3.8)
hexachlorobenzene	61 (5.2)	66 (7.4)
metoxychlor	56 (4.9)	68 (4.8)
tetradifon	24 (5.3)	51 (6.1)
organonitrogen		
buprofezin	54 (5.6)	59 (6.6)
dicloran	46 (5.3)	44 (5.9)
etaconazole	28 (4.7)	56 (5.2)
hexaconazole	31 (5.2)	57 (4.8)
imazalil	22 (3.9)	53 (5.3)
linuron	51 (4.9)	55 (6.4)
metolachlor	27 (5.6)	56 (4.9)
prochloraz	29 (5.5)	54 (6.2)
propiconazole	32 (6.3)	56 (5.1)
quizalofop-ethyl	61 (6.2)	67 (5.5)
tebuconazole	63 (5.7)	68 (5.6)
triadimefon	63 (5.9)	67 (7.1)
triadimenol	31 (4.8)	55 (6.8)
trifluralin	64 (4.5)	67 (5.2)
vinclozolin	61 (3.9)	69 (3.9)
organophosphorus		
chlorpyrifos	25 (3.9)	53 (5.7)
diazinon	31 (5.4)	52 (4.8)
dichlorvos	30 (5.9)	54 (4.4)
dimethoate	29 (4.6)	51 (5.3)
pyrethroid		
cyfluthrin[a]	59 (4.5)	62 (4.8)
cypermethrin[a]	59 (6.1)	61 (5.9)
fenvalerate[a]	63 (5.7)	60 (6.1)

[a] Quantification done by the sum of the peak areas of isomer forms.

Table 3. Recoveries of Pesticides in Spiked Soil Samples by SFE Using CO_2 Modified with 10% Methanol in Different Temperatures

	recovery (%)		
pesticide	40 °C	60 °C	90 °C
organohalogen			
aldrin	66 (3.9)	68 (5.1)	69 (4.9)
bromopropylate	69 (3.7)	70 (4.6)	72 (5.2)
chlorothalonil	61 (6.1)	62 (3.7)	68 (5.6)
diclofop-methyl	63 (5.8)	64 (5.3)	63 (5.5)
dicofol	67 (5.4)	69 (5.5)	75 (4.6)
endosulfan α	65 (5.9)	66 (5.5)	68 (4.1)
endosulfan β	69 (3.8)	68 (4.4)	70 (5.3)
hexachlorobenzene	66 (4.4)	69 (5.1)	72 (4.6)
metoxychlor	68 (4.8)	70 (5.2)	68 (4.8)
tetradifon	51 (5.1)	70 (6.5)	71 (4.4)
organonitrogen			
buprofezin	59 (5.6)	63 (4.7)	69 (5.3)
dicloran	44 (5.9)	68 (7.2)	69 (4.7)
etaconazole	56 (5.2)	61 (3.8)	68 (4.8)
hexaconazole	57 (4.8)	64 (5.3)	69 (4.6)
imazalil	53 (5.3)	71 (6.2)	72 (5.5)
linuron	55 (6.4)	69 (4.4)	70 (4.2)
metolachlor	56 (4.9)	73 (5.1)	71 (5.5)
prochloraz	54 (6.2)	72 (6.5)	73 (4.7)
propiconazole	56 (5.1)	76 (4.4)	77 (4.9)
quizalofop-ethyl	67 (5.5)	69 (6.2)	71 (3.7)
tebuconazole	68 (5.6)	71 (6.3)	70 (7.4)
triadimefon	67 (7.1)	69 (6.6)	72 (5.6)
triadimenol	55 (6.8)	74 (5.7)	75 (4.9)
trifluralin	67 (5.2)	71 (5.8)	71 (4.9)
vinclozolin	69 (3.9)	72 (4.6)	73 (4.5)
organophosphorus			
chlorpyrifos	53 (5.7)	75 (6.1)	76 (4.8)
diazinon	52 (4.8)	70 (4.7)	73 (5.1)
dichlorvos	54 (4.4)	71 (5.6)	70 (5.3)
dimethoate	51 (5.3)	73 (6.2)	75 (4.4)
pyrethroid			
cyfluthrin[a]	62 (4.8)	64 (4.5)	70 (5.0)
cypermethrin[a]	61 (5.9)	63 (5.8)	67 (5.2)
fenvalerate[a]	60 (6.1)	62 (5.5)	66 (5.5)

[a] Quantification done by the sum of the peak areas of isomer forms.

Table 4. Recoveries of Pesticides in Spiked Soil Samples by SFE Using CO_2 Modified with 10% Methanol at 60 °C, in Different Pressures

	recovery (%)		
pesticide	200 bar	400 bar	600 bar
organohalogen			
aldrin	68 (5.1)	89 (3.9)	90 (5.9)
bromopropylate	70 (4.6)	91 (5.3)	91 (5.5)
chlorothalonil	62 (3.7)	93 (5.6)	268 (3.9)
diclofop-methy	64 (5.3)	88 (5.7)	87 (4.8)
dicofol	69 (5.5)	90 (5.4)	175 (11.2)
endosulfan α	66 (5.5)	89 (4.9)	192 (9.1)
endosulfan β	68 (4.4)	91 (5.5)	89 (6.3)
hexachlorobenzene	69 (5.1)	94 (4.9)	95 (6.6)
metoxychlor	70 (5.2)	88 (5.3)	268 (5.9)
tetradifon	70 (4.4)	92 (5.8)	90 (3.9)
organonitrogen			
buprofezin	63 (4.7)	87 (4.7)	88 (6.3)
dicloran	68 (5.2)	95 (5.2)	155 (9.3)
etaconazole	61 (3.8)	92 (4.1)	92 (6.8)
hexaconazole	64 (5.3)	93 (4.5)	169 (7.2)
imazalil	71 (4.8)	90 (5.5)	89 (5.3)
linuron	69 (4.4)	89 (4.9)	90 (5.4)
metolachlor	73 (5.1)	94 (4.3)	231 (12.2)
prochloraz	72 (4.5)	91 (5.2)	182 (9.7)
propiconazole	76 (4.4)	96 (5.4)	95 (5.8)
quizalofop-ethyl	69 (5.2)	91 (5.8)	90 (3.9)
tebuconazole	71 (5.3)	93 (3.9)	289 (10.3)
triadimefon	69 (5.6)	89 (4.8)	88 (7.2)
triadimenol	74 (5.7)	92 (5.3)	85 (6.1)
trifluralin	71 (5.8)	92 (4.4)	93 (6.6)
vinclozolin	72 (4.6)	88 (6.1)	63 (5.6)
organophosphorus			
chlorpyrifos	75 (5.1)	95 (5.6)	167 (5.1)
diazinon	70 (4.7)		77 (6.3)
dichlorvos	71 (5.6)	89 (5.3)	91 (4.9)
dimethoate	73 (5.2)	90 (5.8)	88 (5.3)
pyrethroid			
cyfluthrin[a]	64 (4.5)	93 (4.4)	94 (5.7)
cypermethrin[a]	63 (4.8)	89 (4.9)	267 (8.2)
fenvalerate[a]	62 (5.5)	93 (4.7)	92 (5.5)

[a] Quantification done by the sum of the peak areas of isomer forms.

well as the time of contact between them. The most realistic situation for the evaluation of an extraction method is the use of a native uncontaminated soil. However, because it is practically impossible to find a natural uncontaminated matrix with studied analytes, the use of an aged spiked soil appears to be a good model for the evaluation of the tested methods. The residence times in spiked soil samples were carried out at 1, 20, and 40 days following fortification. **Figure 1** presents the chromatogram of the blank soil sample where some matrix peaks can be observed when the extract is analyzed by ECD: however, these peaks do not interfere with the selected pesticide analysis, showing to be satisfactory for the samples studied. **Table 1** lists the percent recovery and relative standard deviation for the pesticides studied from spiked soil at different residence times. The average recoveries for the pesticides at 1 and 20 days are very similar and >80%. However, at 40 days, a pronounced difference in recoveries was observed for most classes of pesticides studied, mainly for organophosphorus pesticides, the recovery results of which were ~70%. The strong analyte−matrix interactions are therefore responsible for low recoveries. The results indicated that the residence time influenced the extraction efficiency by higher interactions of the pesticides with the soil, decreasing the recoveries of the pesticides studied.

The limits of detection (LOD) were <0.01 mg/kg for ECD, and the relative standard deviation (RSD) ranged from 4 to 8%. Although the recovery results (70−to 83%) for the 40-day-aged spiked samples, the extraction and the cleanup procedure could be considered to be reliable enough for routine multiresidue screening in soil samples.

3.2. Supercritical Fluid Extraction. Sample preparation methods generally used by analytical chemists are both time- and solvent-consuming. According to a recent survey, two-thirds of the analysis time is devoted to sample preparation, and this step accounts for at least one-third of the errors generated during the performance of an analytical method (*19*). Several concerns about the hazards associated with most of the solvents used, and the costs and environmental dangers of waste solvent disposal, have led to the application of alternative sample extraction methods such as solid-phase extraction (SPE) and SFE (*20*).

SFE has gained increased attention as a potential replacement for conventional liquid solvent extraction (sonication or Soxhlet) owing to its properties of supercritical fluids such as higher diffusivity and low viscosity (*20*).

In this context, SFE appears to be one of the most appropriate techniques for multiresidue pesticide analyses in soil samples. It allows the performance of selective extractions of different chemicals without the additional cleanup, as well as the use of a smaller sample size (*21*).

Various analyte−matrix interaction forces ranging from van der Waals, to water bridging, to H-bonding, to covalent bonding are involved in the sorption of organic chemicals by soils (*17*, *18*). To obtain optimum conditions for multiresidue pesticides

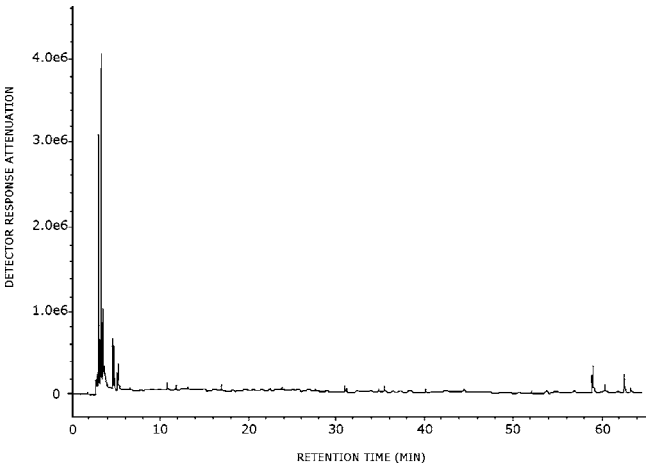

Figure 2. GC-ECD chromatogram of soil extract blank obtained by SFE method (see conditions under section 2.5).

in SFE, experimental variables were continuously varied during an extraction to maximize selectivity, as well as overall recoveries. The spiked soil samples used for the optimization step in SFE were done through blank samples, as stated under section 2.2 for the 1-day period, which was assessed in the optimal experimental conditions after the optimization study.

Excess water in the soil samples causes the restrictor to be plugged by ice, and, as a result, water flows into the collection trap. In this work, whenever necessary, a hydromatrix was used as a drying agent to control excess water.

3.2.1. Modifier Test. The modifier test showed that the average recovery of pesticides from soil matrix with methanol as a modifier has been greatly improved as compared with CO_2 modified with acetone for some pesticides investigated: tetradifon, etaconazole, hexaconazole, imazalil, metolachlor, prochloraz, propiconazole, triadimenol, chlorpyrifos, diazinon, dichlorvos, and dimethoate. As one observes in **Table 2**, the response of these pesticides increased from 22 to 57%. However, for other compounds, the increase in recovery results was lower, or no effect was observed. The increase in average recovery indicated that methanol sufficiently increased the solvating power of CO_2 for the extraction of several classes in spiked soil samples. It might increase the polarity of the supercritical fluid and enhance the partitioning of polar analytes into fluid. In addition, it might compete with polar analytes for the active sites in the matrix and displace them into the fluid. Finally, it might swell the soil matrix and expose the small internal cavities, allowing a better access of the supercritical fluid to the absorbed analytes (*22*). On the basis of these results, methanol-modified CO_2 was applied in further experiments.

3.2.2. Temperature and Pressure Optimization. Supercritical fluids have densities (and solvating powers) comparable to those of liquids, which can be continuously varied by as much as an order of magnitude by varying the temperature and pressure of the extraction vessel. The choice of pressure and temperature in SFE to affect selectivity is a main advantage over mixtures of liquid solvents, which cannot achieve such control (*21*).

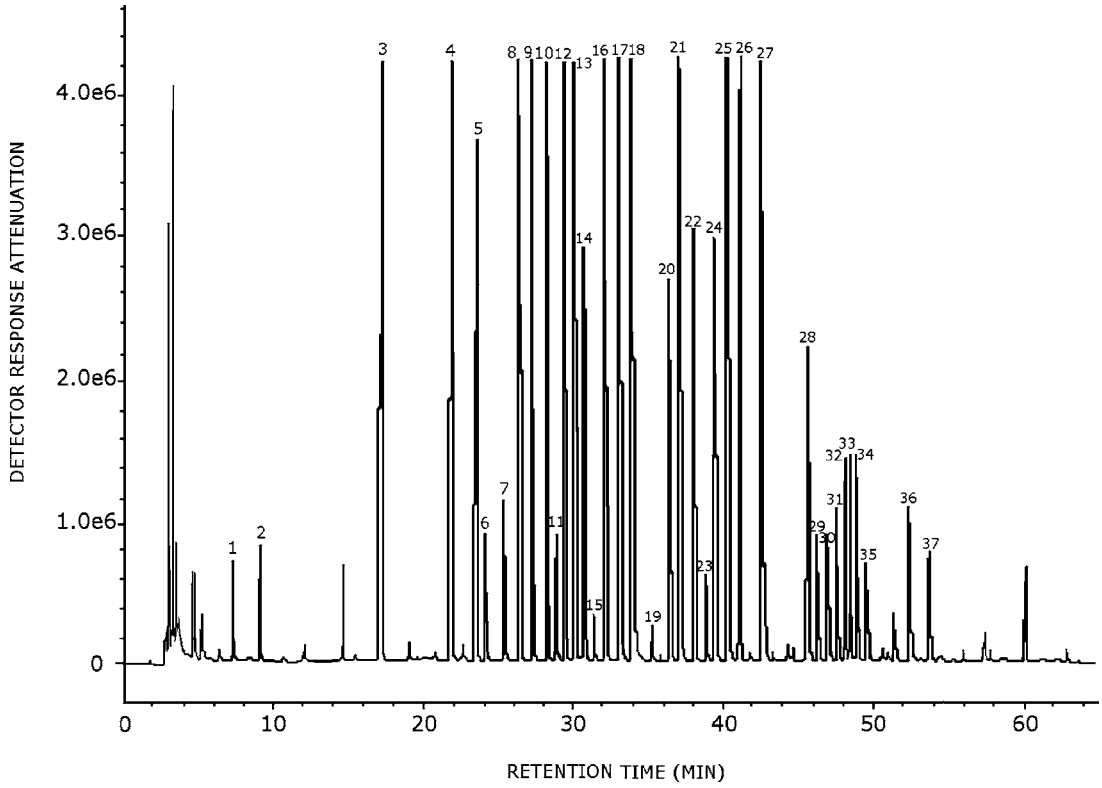

Figure 3. GC-ECD chromatogram of spiked soil extract obtained by SFE method (see conditions under Section 2.5). Peaks: 1, dichlorvos (0.20 mg/kg); 2, linuron (0.18 mg/kg); 3, trifluralin (0.30 mg/kg); 4, hexachlorobenzene (0.23 mg/kg); 5, dicloran (0.14 mg/kg); 6, diazinon (0.19 mg/kg); 7, dimethoate (0.17 mg/kg); 8, chlorothalonil (0.23 mg/kg); 9, vinclozolin (0.21 mg/kg); 10, aldrin (0.28 mg/kg); 11, metolachlor (0.20 mg/kg); 12, triadimefon (0.25 mg/kg); 13, chlorpyrifos (0.27 mg/kg); 14, dicofol (0.25 mg/kg); 15, triadimenol (0.18 mg/kg); 16, endosulfan α (0.27 mg/kg); 17, hexaconazole (0.26 mg/kg); 18, imazalil (0.28 mg/kg); 19, buprofezin (0.22 mg/kg); 20, endosulfan β (0.22 mg/kg); 21, etaconazole (0.29 mg/kg); 22, propiconazole (0.29 mg/kg); 23, tebuconazole (0.21 mg/kg); 24, diclofop-methyl (0.23 mg/kg); 25, bromopropylate (0.38 mg/kg); 26, metoxychlor (0.32 mg/kg); 27, tetradifon (0.28 mg/kg); 28, prochloraz (0.25 mg/kg); 29, 30, 31, cyfluthrin (I, II, III; sum = 0.38 mg/kg); 32, 33, 34, cypermethrin (I, II, III; sum = 0.35 mg/kg); 35, quizalofop-ethyl (0.26 mg/kg); 36, 37, fenvalerate (I, II; sum = 0.37 mg/kg).

Table 5. Residues of Pesticides Determined in Real Soil Sample by Solid–Liquid and SFE Methods

pesticide	residue (mg/kg) (RSD %) solid–liquid extraction	supercritical fluid extraction
organohalogen		
aldrin	0.022 (6.3)	0.038 (4.5)
bromopropylate	nd	nd
chlorothalonil	0.021 (7.1)	0.047 (5.8)
diclofop-methyl	nd	nd
dicofol	0.188 (6.2)	0.250 (5.3)
endosulfan alfa	nd	0.189 (4.6)
endosulfan beta	nd	0.116 (5.5)
hexachlorobenzene	nd	0.104 (4.8)
metoxychlor	nd	nd
tetradifon	nd	0.227 (5.3)
organonitrogen		
buprofezin	nd	nd
dicloran	nd	nd
etaconazole	nd	nd
hexaconazole	nd	nd
imazalil	0.114 (6.9)	0.249 (5.5)
linuron	nd	nd
metolachlor	0.206 (6.5)	0.291 (5.3)
prochloraz	nd	0.168 (5.7)
propiconazole	nd	nd
quizalofop-ethyl	nd	nd
tebuconazole	0.388 (6.3)	0.512 (4.9)
triadimefon	nd	nd
triadimenol	nd	nd
trifluralin	nd	0.225 (4.4)
vinclozolin	nd	nd
organophosphorus		
chlorpyrifos	nd	0.119 (5.6)
diazinon	nd	nd
dichlorvos	nd	nd
dimethoate	nd	nd
pyrethroid		
cyfluthrin[a]	nd	nd
cypermethrin[a]	nd	0.218 (3.7)
fenvalerate[a]	nd	0.158 (5.9)

[a] Quantification done by the sum of the peak areas of isomer forms.

Table 6. Main Ions and Relative Abundance of Pesticides Detected by GC-MS

pesticide	main ions, *m*/*z* (relative abundance %)
organohalogen	
aldrin	263 (71), 293 (25), 329 (9)
bromopropylate	149 (100), 167 (25), 279 (18)
chlorothalonil	263 (70), 293 (28), 329 (9)
diclofop-methyl	253 (100), 281 (44), 340 (80)
dicofol	111 (41), 139 (12), 251 (72)
endosulfan	237 (100), 265 (63), 339 (28)
hexachlorobenzene	214 (22), 249 (24), 284 (100)
metoxychlor	227 (100), 274 (8), 374 (3)
tetradifon	159 (100), 229 (55), 356 (38)
organonitrogen	
buprofezin	105 (100), 172 (35), 305 (18)
dicloran	124 (100), 176 (90), 206 (80)
etaconazole	173 (100), 191 (35), 245 (63)
folpet	104 (100), 260 (82), 295 (21)
hexaconazole	83 (100), 214 (45), 231 (20)
imazalil	173 (96), 215 (100), 296 (10)
linuron	61(100), 160 (18), 248 (15)
metolachlor	162 (100), 211 (12), 238 (52)
prochloraz	180 (100), 266 (26), 308 (91)
propiconazole	173 (100), 221 (58), 259 (58)
quizalofop-ethyl	243 (39), 299 (100), 372 (96)
tebuconazole	125 (84), 250 (100), 307 (10)
triadimefon	57 (100), 208 (44), 293 (5)
triadimenol	112 (100), 128 (45), 168 (59)
trifluralin	263 (74), 306 (100), 335 (10)
vinclozolin	187 (100), 212 (99), 285 (75)
organophosphorus	
chlorpyrifos	97 (100), 197 (78), 314 (46)
diazinon	88 (100), 179 (71), 304 (38)
dichlorvos	109 (100), 185 (35), 220 (9)
dimethoate	87 (100), 125 (55), 229 (12)
pyrethroid	
cyfluthrin (I, II, III, IV)	163 (100), 206 (80), 226 (51)
cypermethrin (I, II, III,IV)	163 (100), 181 (86), 209 (27)
fenvalerate (I, II)	125 (100), 167 (84), 419 (19)

The rate of pesticide extraction from the soil matrix will be partially controlled by the unaltered supercritical fluid diffusing through the matrix.

One way to improve this diffusion is to control the temperature and pressure or density. The analytes must be desorbed from the soil surface in a first step, followed by diffusion through the matrix, and then partitioned into the supercritical fluid before being extracted.

The effect of temperature on pesticide extraction was checked at 40, 60, and 90 °C. As shown in **Table 3** for the compounds studied, the best efficiency was found at 60 °C. Pesticides such as chlorothalonil, dicofol, buprofezin, etaconazole, hexaconazole, cyfluthrin, cypermethrin, and fenvalerate showed a slight increase in the recovery at 90 °C, but this increase did not greatly affect the extraction efficiency. It can be observed that tetradifon, etaconazole, hexaconazole, imazalil, metolachlor, prochloraz, propiconazole, triadimenol, chlorpyrifos, diazinon, dichlorvos, and dimethoate presented a low recovery at 40 °C, but at 60 °C, the recovery increased. These results correspond to the experiments reported by Nemoto et al. (30−70 °C) (*23*) and Rice et al. (*24*) (40−100 °C). The behavior observed for each pesticide depends on its polarity. As can be seen in **Table 3**, the solubility of the pesticides increased with the temperature, with maximum recovery at 60 °C, which was selected as the optimum temperature for the SFE method.

The influence of pressure was significant for the optimization of SFE conditions. Control of density in SFE has enabled unique applications to separate classes of pesticides from common matrix interference that can plague traditional methods. In this work, the experiments were carried out to check the pressure behavior. The combination of three different pressures (200, 400, and 600 bar) using 10% of methanol as a modifier was evaluated. The recovery results are summarized in **Table 4**. These results show that the increase in extraction pressure (200−400 bar) resulted in an improvement of the recoveries of all compounds studied, demonstrating that the increase in pressure influenced the SFE process and the solubility of analytes in the fluid. However, the use of a 600 bar pressure did compromise the analytical results, influencing recoveries of >170% for many of the pesticides.

The effect of time on the extraction efficiency appears to be negligible. Some tests were done to check this effect at 20, 40, and 60 min; however, no clear evidence showed the importance of these variable in the range studied.

This study is only a model system, when SFE recoveries were obtained from spiked samples, and it was used to determine the best conditions to extract target analyte from real samples. The GC-ECD chromatogram presented in **Figure 2** shows analytes extracted from a spiked blank soil sample under optimized SFE conditions of modifier, temperature, pressure, and time in the multiresidue pesticide. Accuracy was determined by analyzing five replicate samples consecutively. The standard deviation ranged from 4 to 8%, and the recovery results suggest

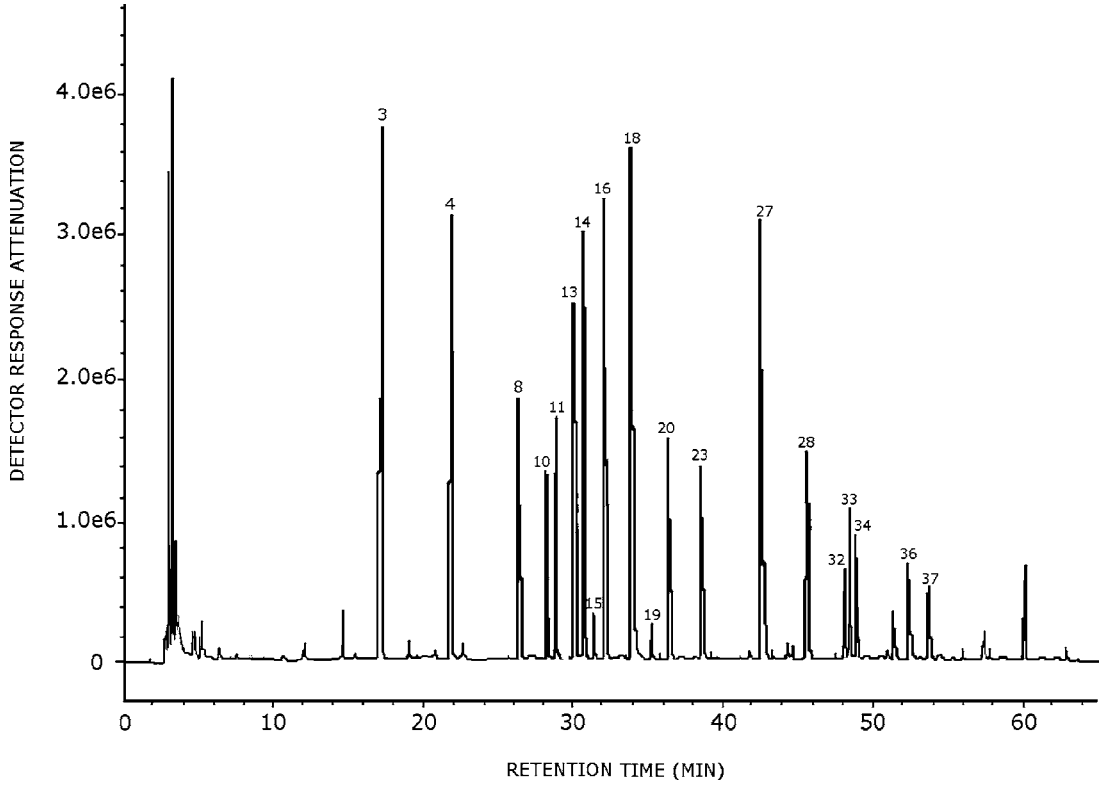

Figure 4. GC-ECD chromatogram of soil real sample obtained by SFE method (see conditions under section 2.5).

that the SFE procedure could be considered to be suitable for the multiresidue screening of 32 pesticides of several classes in soil samples (**Figure 3**).

3.2.3. Residence Fortification Time. Soil capacity to retain or sorb pesticides is a key parameter controlling the extent to which pesticides leach through soil into groundwater or run off into surface water. The strength of pesticide binding depends on the types of bonds between the organic molecule and the surface. Although sorption is affected by the physical and chemical properties of the pesticide and soil, it also appears that sorption can be affected by the residence time in the soil. The aim of this research was to determine the effect of aging on sorption of pesticides, a potential mobile to soil. To judge the efficiency of the analytical procedure, recovery experiments were performed by spiking soil with different quantities of the pesticides studied. Studies indicated that reliable recovery data cannot be obtained from freshly spiked samples alone (*25*). Fortification procedures do not necessarily give a good indication of extraction efficiencies because it is more difficult to remove pesticides from soils following field treatment than from a fortified soil sample. Therefore, SFE procedures evaluated by fortification should be considered critically. In light of the above arguments, we tested the extractability of the pesticides from aged soil samples to simulated recovery from actual field sample. Fortified soil samples were extracted at different aging times (1, 20, and 40 days) using the best experimental conditions of optimized SFE. These studies did not reveal any great differences in the extractability of several pesticides investigated.

3.3. Analysis of Real Soil Samples. Finally, SFE was compared with conventional solid−liquid extraction. **Table 5** shows the results for real soil samples collected in agricultural fields in Bauru (São Paulo state, Brazil) and analyzed by both solid−liquid and SFE. First, the identification of the compounds was performed by ECD comparing the retention times of the standards and the peaks. The confirmation of residue identity of the studied pesticides was performed by GC-MS. The selected ions for quantification are summarized in **Table 6**. The selected ions are in agreement with those reported by other authors for the mass spectra of these compounds (*26*, *27*). The quantification of these pesticides was performed by selecting the base peak of their mass spectra, after the acquisition of the total ion chromatogram of the samples. The absence of coextracted interferences at the pesticides retention times was confirmed by blank extract analysis.

As can be observed, the main difference within the methods was found for the extraction of some pesticides such as endosulfan α, endosulfan β, hexachlorobenzene, tetradifon, trifluralin, clorpirifos, and cypermethrin, which are not found in real soil samples by the solid−liquid extraction. **Figure 4** presents a chromatogram of real soil samples obtained by SFE. Compounds such as aldrin, chlorothalonil, imazalil, metolachlor, and tebuconazole were determined by the two methods; nevertheless, higher quantities of residues were found by SFE. These differences can be explained by the properties of supercritical fluid resulting in higher extraction efficiency of bound residue as compared to the solid−liquid extraction method due to a high power diffusion, which was evidenced by the aged soil samples studied.

Another positive factor of the SFE is its better precision, which reduces the number and magnitude of errors. This is related to the SFE procedure, which is more automatic and involves fewer steps. The RSD values obtained for both conventional and SFE methods (**Tables 1** and **4**) show that, in general, values corresponding to SFE are lower than for the conventional method. The other advantages in the use of SFE versus solvent-based extraction methods are the reduced use of organic solvents, shorter extraction time, smaller sample size, and its properties enabling higher extraction power.

3.4. Conclusions. This study led us to three major findings. First, the strong confirmation that SFE may be used to extract a wide range of pesticide residues in major significant ranges of chemical classes from soil samples. Second, the task of

analyzing these extracts by gas chromatography with an electron capture detector while minimizing labor, time, and environmental concerns prompted us to develop a fast and efficient method to do the separation and quantification, and the results can be obtained in the routine analysis of real soil samples, confirming the reliability and efficacy of this method for the multiresidue analysis of soil samples. Last, but not least, this work proved that SFE may be combined with other techniques such as GC-ECD and GC-MS to analyze and confirm a wide range of pesticides in soil samples with good precision (RSD < 7%) and with average recoveries of >80%.

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