Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1048 (2004) 153–159
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Supercritical fluid extraction for pesticide multiresidue analysis in honey: determination by gas chromatography with electron-capture and mass spectrometry detection

Sandra R. Rissato[a,*], Mário S. Galhiane[a], Fátima R.N. Knoll[b], Bernhard M. Apon[c]

[a] *Department of Chemistry, Faculty of Sciences, Paulista State University (UNESP), P.O. Box 473, 17033-360 Bauru (SP), Brazil*
[b] *Department of Biology, Paulista State University (UNESP), P.O. Box 473, 17033-360 Bauru (SP), Brazil*
[c] *Chromapon Inc., 9815 Carmenite Road, Suite J., Whittier, CA 90605, USA*

Received 23 February 2004; received in revised form 23 July 2004; accepted 26 July 2004

Abstract

An analytical procedure using supercritical fluid extraction (SFE) and capillary gas chromatography with electron-capture detection was developed to determine simultaneously residues of different pesticides (organochlorine, organophosphorus, organonitrogen and pyrethroid) in honey samples. Fortification experiments were conducted to test conventional extraction (liquid–liquid) and optimize the extraction procedure in SFE by varying the CO_2-modifier, temperature, extraction time and pressure. Best efficiency was achieved at 400 bar using acetonitrile as modifier at 90 °C. For the clean-up step, Florisil cartridges were used for both methods LLE and SFE. Recoveries for majority of pesticides from fortified samples of honey at fortification level of 0.01–0.10 mg/kg ranged 75–94% from both methods. Limits of detection found were less than 0.01 mg/kg for ECD and confirmation of pesticide identity was performed by gas chromatography–mass spectrometry in selected-ion monitoring mode. The multiresidue methods in real honey samples were applied and the results of developed methods were compared.

Keywords: Pesticides; Multiresidue analysis; Honey

1. Introduction

The extensive use of pesticides plays an important role in the increase of world food production. Pesticides are applied worldwide to a broad variety of crops for both field and post-harvest protection. The increasingly public concern, in recent years, about health risks from pesticide residues in the diet, has deeply modified the strategy for crop protection, with emphasis on food quality and safety [1].

Unfortunately, honey bees are insects that are greatly affected by insecticides as well as pesticides in general. There are several ways by which honey bees can be killed and contaminated by pesticides. One is the direct contact of the pesticide on the bee while it is foraging in the field. The bee immediately dies and does not return to the hive. In this case, the queen, brood and nurse bees are not contaminated and the colony survives. The second more deadly way, is when the bee comes in contact with a pesticide and transports it back to the colony, either as a contaminated pollen or nectar or on its body. As a consequence, residues of certain pesticides could appear in apiarian products, thus it is convenient to evaluate them in order to maintain the characteristics that a natural product, such as honey should bear [2].

Honey is a very complex matrix according to the botanical origin and the pesticide residues determination often

* Corresponding author. Tel.: +55 14 3103 6135; fax: +55 14 3203 2856.
E-mail address: srissato@fc.unesp.br (S.R. Rissato).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.07.053

Table 1
Retention times, recoveries (R.S.D.%, n = 5) and detection limits of the selected pesticides obtained by liquid–liquid extraction and supercritical fluid extraction

Pesticides	t_R (min)	Recovery (R.S.D.)		LOD (mg/kg)
		LLE	SFE	
Organohalogen				
Aldrin	27.99	83 (5.9)	98 (4.5)	0.008
Bromopropylate	39.71	85 (7.1)	89 (5.3)	0.007
Chlorothalonil	26.47	86 (6.4)	90 (5.6)	0.005
Diclofop-methyl	38.65	89 (6.6)	92 (5.8)	0.006
Dicofol	30.03	83 (6.7)	89 (6.0)	0.007
Endosulfan alfa	32.50	85 (7.2	94 (5.4)	0.005
Endosulfan beta	36.41	85 (6.5)	95 (5.8)	0.005
Hexachlorobenzene	21.11	87 (6.9)	88 (5.5)	0.006
Metoxychlor	41.29	88 (7.1.)	91 (4.6)	0.007
Tetradifon	42.20	77 (6.8)	96 (5.5)	0.005
Organonitrogen				
Buprofezin	34.96	86 (5.9)	88 (5.3)	0.010
Dicloran	23.50	88 (6.6)	90 (5.6)	0.008
Etaconazole	36.55	84 (7.0)	97 (5.3)	0.008
Hexaconazole	33.27	83 (6.5)	91 (4.2)	0.007
Imazalil	33.90	88 (7.1)	93 (4.5)	0.006
Linuron	8.84	90 (6.4)	92 (5.9)	0.008
Metolachlor	28.87	87 (6.8)	97 (4.8)	0.010
Prochloraz	45.64	89 (7.3)	95 (5.6)	0.010
Propiconazole	37.73	85 (6.7)	98 (5.7)	0.008
Quizalofop-ethyl	49.40	89 (6.2)	95 (5.3)	0.010
Tebuconazole	38.41	83 (6.8)	94 (5.9)	0.008
Triadimefon	29.17	84 (6.6)	96 (6.3)	0.005
Triadimenol	31.28	85 (6.4)	94 (4.8)	0.010
Trifluralin	17.30	86 (5.9)	97 (4.6)	0.007
Vinclozolin	26.57	83 (6.2)	98 (4.6)	0.007
Organophosphorus				
Chlorpyrifos	29.53	75 (7.3)	98 (4.9)	0.002
Diazinon	24.08	78 (6.5)	94 (5.6)	0.005
Dichlorvos	7.27	77 (6.2)	97 (5.7)	0.006
Dimethoate	25.16	76 (6.4)	95 (4.8)	0.007
Pyrethroid				
Cyfluthrin[a]	46.08, 46.34, 46.68	84 (7.1)	92 (4.9)	0.009
Cypermethrin[a]	47.67, 48.20, 48.36	87 (7.0)	93 (5.4)	0.008
Fenvalerate[a]	52.29, 53.51	85 (6.8)	95 (5.5)	0.005

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

includes isolation-concentration steps. Sample preparation is a key element in the pesticide residues analysis in honey. It is performed to produce clean samples, sufficiently free from impurities, for the chromatographic analysis in order to achieve a good separation and high selectivity. Since sample pre-treatment is most essential, but at the same time the most time consuming step in chromatographic analysis, there is a reason to carefully investigate possible alternatives to shorten the time consumed [3].

Pesticide residues in honey are usually extracted by treating the sample with an organic solvent [4–6], or in the solid-phase, by passage through octadecylsilane cartridges [7,8], after dilution of the honey sample with water.

The sample clean-up is crucial for honey analysis, since high molecular weight compounds can contaminate the chromatographic system and specially when using electron-capture detectors (ECD), the interfering compounds make it difficult to interpret the chromatograms due to overlapping peaks. The honey extract has been subjected to a clean-up step by liquid–liquid partitioning, an octadecylsilane or Florisil column or gel permeation chromatography [4,8–10]. For residues, the extract is commonly analyzed by gas chromatography (GC) [7,11–13] or high performance liquid chromatography (HPLC) [8,14,15].

The general drawbacks, such as the use of large amounts of solvents, time-consumption, labor-intensity and considerable waste production, associated with these classical extraction techniques could be reduced by using supercritical fluid extraction (SFE). SFE has shown to be an efficient and rapid method for the isolation of pesticides from complex matrices such as honey [16,17].

SFE has gained increased attention as a potential replacement for conventional liquid solvent extraction due to its properties of supercritical fluids such as higher diffusivity and low viscosity which allow selective extractions of different chemicals without additional clean-up as well as the use of little sample amounts [18,19].

The goal of the present work is to develop a rapid and accurate multiresidue method to determine organochlorine, organophosphorus, organonitrogen and pyrethroid pesticides in routine testing of honey samples based on supercritical fluid extraction (SFE) and to compare it with liquid–liquid extraction. The development of SFE was performed by the optimization of several parameters; mainly the pressure, temperature and the addition of an organic modifier to the fluid (acetone and acetonitrile). Clean-up was based on Florisil, 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 liquid–liquid extraction.

The applicability of the newly developed procedure for the multiresidue analysis of honey is also presented.

2. Experimental

2.1. Chemicals

2.1.1. Pesticide standards

Pesticide reference standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany) with purity range of 96–100%. Pesticides investigated are listed in Table 1.

2.1.2. Pesticide solutions

Pesticide stock solutions (approximately 500 mg/L) of individual pesticide standards were prepared by dissolving

approximately 0.050 g of the pesticide in 100 mL of acetone: *n*-hexane (1:1,v/v) and stored in a freezer at −18 °C in glass bottles with PTFE-faced screw caps. Pesticide working solutions were prepared for recovery tests of liquid–liquid extraction and SFE methods by appropriate dilution with acetone: *n*-hexane (50:50, v/v).

2.1.3. Organic solvents and reagents

Acetone, *n*-hexane, methylene chloride, ethyl acetate and methanol, all of special grade for pesticide residue analysis were purchased from Mallinkrodt, Merck. Sep-Pak® Vac Florisil cartridges (3 mL, 500 mg) were bought from Waters (Milford, MA). A special syphonated CO_2 from White Martins was also used in SFE.

2.2. Liquid–liquid extraction (conventional method)

The pesticide extraction method used in the determination of pesticides in honey was based on the literature with a few modifications [8].

A 10 g portion of honey sample was weighed in an Erlenmeyer flask and fortified when required with the pesticide standard solution. The sample was mixed with 5 mL water and homogenized by shaking to reduce its viscosity and facilitate its handling. After that, the sample was mixed with a 50 mL solution of *n*-hexane/acetone (60:40, v/v) submitting it to extraction by shaking for 20 min. Then, the organic phase was separated by centrifugation at 2500 × *g* for 10 min and then collected. The sample was once again extracted with 40 mL of solvent and the above-described procedure was repeated. The two portions collected were combined and the solvent was evaporated in a rotary evaporator under reduced pressure at 65 °C and the sample was dried under a gentle stream of pure nitrogen. Finally, the residue was dissolved in 5 mL of acetone and passed through a 0.50 μm sized pore PTFE filter.

For honey fortification, 10 g of the sample was heated in a water bath at 40 °C for 20 min, being 5 min allowed. The fortified samples were prepared by adding an appropriate volume of the standard working solution to the honey samples. The mixture was mechanically stirred in a blender, so as to ensure homogenization and then submitted to the extraction step.

2.3. Supercritical fluid extraction (SFE)

SFE was carried out by using the SFX-220 extraction system (ISCO, Lincoln, NE, USA) that consists of an SFX-220 extractor, an SFX-200 controller, 100 DX syringe pump, and a syphonated carbon dioxide (CO_2) cylinder that was pressurized up to work pressure.

A 5 g of honey sample was mixed with water ca. 3 mL and heated in a water bath at 40 °C to improve and facilitate the handling of the mixture. The honey samples were then fortified by adding an appropriate volume of standard working solution, after which the mixture was treated with cellulose powder (2 g) to facilitate the lyophilization process, homogenized by shaking, and frozen at −18 °C prior to lyophilization. The honey samples were poured into a stainless steel extraction cell (5.6 cm × 1.6 mm i.d.) in a sandwich mode, using a silanized glass wool at both the bottom and the top of the cell to protect cell sealing. Before extraction, whenever necessary, a modifier (acetone and acetonitrile) was added to the pre-mixture chamber by pipeting a calculated volume in relation to the total volume of SFE cell 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 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 fortified honey samples were: 10% of acetone modifier, extraction pressure, 200 bar; extraction temperature, 60 °C; extraction time, 20 min. The extraction conditions were varied from 40, 60 and 90 °C and 200, 400 and 600 bar, using flow rate of expanded gas, 1.5 mL/min. CO_2 or CO_2 modified with 10% of acetone and acetonitrile. The extraction time was tested at 10, 20 and 30 min, so as to optimize the pesticide recovery in honey samples.

A fused-silica capillary tube (30 cm × 100 μ i.d.) was attached to the outlet of the extractor as a restrictor and the pesticides were collected on-line in a Florisil cartridge at 10 °C (the procedure is described in Section 2.4).

2.4. Clean-up SPE

The clean-up of samples was performed by means of a Supelco VISIPREP-12 manifold using Florisil cartridges which were conditioned with approximately 5 mL of 1:1 ethyl acetate/*n*-hexane. When 0.5–1 mL of the ethyl acetate/*n*-hexane remained in the cartridge, the valve of the manifold was closed to prevent cartridges drying. The extract was added to the column and eluted under gravity with two portions of 5 mL each, methylene chloride/*n*-hexane (80:20,v/v) and *n*-hexane/acetone (60:40, 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.

2.5. 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 increased 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) operated

in the splitless mode; purge off time, 1 min; injection volume, 1 μL; detector temperature, 300 °C; make-up gas, N_2.

2.6. GC/MS

Confirmatory run analysis was done on a Hewlett-Packard Model 5890 Series II gas chromatograph with a 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 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 final temperature being held for 30 min; injector temperature, 250 °C; carrier gas He operated in the splitless mode; purge off time, 1 min; injection size, 1 μL; GC–MS transfer line, 280 °C; MS conditions: solvent delay, 2.9 min; electron impact ionization voltage, 70 eV; scan rate, 1.5 scan/s; scanned-mass range, 40–600 *m*/*z*.

3. Results and discussion

3.1. Liquid–liquid extraction

The recoveries were determined by adding the pesticides to honey samples at a final concentration of 0.01 or 0.10 mg/kg. Table 1 shows the recovery and precision achieved by applying the solvent extraction procedure to fortified honey samples at the 0.01 mg/kg level. For the high fortification concentration (0.10 mg/kg), recoveries greater than 74% were found for 33 total pesticides from the extracted honey samples. These numbers were similarly reflected for the honey fortified at the low concentration (0.01 mg/kg), showing that most pesticides were extracted using acetone/*n*-hexane.

The clean-up using Florisil was tested in order to reduce matrix effects. Fig. 1 presents an amplified view of a blank extract of honey, both (A) without any clean-up and (B) following Florisil clean-up. As it can be seen, the clean-up procedure decreased the number and height of the chromatographic peaks of the blank extract of honey, which could result in interferences of the target pesticides in the GC/ECD analysis. Furthermore, studies with all pesticide standards in a blank, using Florisil for the clean-up test showed recoveries above 92%.

Regarding precision, the relative standard deviation (R.S.D., $n = 5$) was below 8% and the limits of detection (LOD) were less than 0.01 mg/kg for ECD, the extraction and the clean-up procedure was considered reliable enough for routine multiresidue screening in honey samples.

3.2. Supercritical fluid extraction (SFE)

The selection of operating conditions in SFE is still a difficult task and an area of active research [20]. In order to obtain optimum conditions for multiresidue pesticide in SFE, experimental variables were continuously varied during an extraction to maximize selectivity, as well as overall recoveries through fortified honey samples.

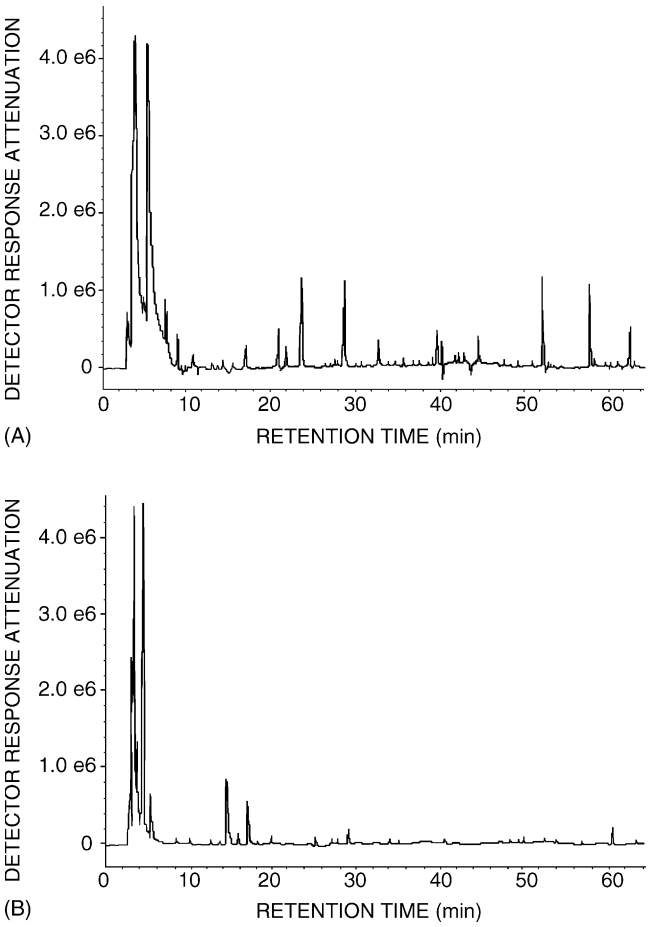

Fig. 1. GC-ECD chromatograms of blank extract of honey obtained by the liquid–liquid extraction method, (A) without clean-up and (B) after Florisil clean-up. GC conditions are described in Section 2.5.

3.2.1. Modifier effects

Depending on the type of sample matrix and the analyte's retaining nature on the matrix, the modifier may influence the extraction in three different ways: (1) increasing the analyte's solubility in the supercritical fluid, as a result of analyte–modifier interactions in the fluid phase; (2) facilitating the analyte desorption—the molecules of polar modifiers are able to interact with the matrix and compete efficiently with the analytes for the active sites in the matrix; (3) distorting the matrix–analyte diffusion and penetration of the supercritical fluid inside the matrix are favored when the modifier swells the matrix.

The development and the widespread application of carbon dioxide (CO_2) based on SFE has been mentioned in several reviews [21,22].

Nevertheless, the use of pure CO_2 in multiresidue pesticide analysis is limited because CO_2 is considered a nonpolar

solvent with a liquid solubility equal to that of hexane. However, for quantitative extraction of moderately polar and polar pesticides, a modifier such as methanol has been applied in order to obtain satisfactory results [23].

Aiming at improving pesticide recovery, the effect of the modifier on multiresidue extraction efficiency was investigated at 40 °C and 200 bar during a period of 10 min. The test showed that the average recovery of pesticides from honey matrices with acetonitrile as a modifier greatly improved compared with CO_2 modified with acetone for some pesticides investigated: tetradifon, etaconazole, hexaconazole, imazalil, metolachlor, prochloraz, propiconazole, triadimenol, chlorpyrifos, diazinon, dichlorvos and dimethoate, increasing from 32% to 61%. However, for other compounds, the increased pesticide recovery results were lower or no effect was observed (e.g. organochlorine pesticides). The increase in average recovery indicated that acetonitrile increased the solvating power of CO_2 sufficiently for the extraction of several classes of pesticides. Furthermore, since analytes with different polarity show a better recovery in the fluid added with acetonitrile, the effect of the modifier might be related not only to the change in polarity of the extraction fluid, but also to its interaction with the matrix. Based on these results, CO_2 modified by acetonitrile was applied in further experiments.

3.2.2. Effect of extraction time

The length of extraction time influenced the extraction efficiency and selectivity of the fluid. In the first phase of this study, the effects of extraction periods (10, 20 and 30 min) on pesticide SFE efficiency were demonstrated. The pressure and temperature were fixed at 200 bar and 40 °C using CO_2 modified with 10% acetonitrile. The results showed that by increasing the period from 10 to 20 min, improved the extraction efficiency of the studied pesticides in more than 25%. However, the increase in the extraction time from 20 to 30 min had minimal effects on the extraction efficiencies of pesticides.

3.2.3. Effects of extraction pressure

Fluid pressure is the main parameter that influences the recovery of organic compounds. Studies revealed that a maximum fluid density could be obtained at high pressures at a given temperature, which can enhance the strength of the solvent [24]. In this work, when the pressure was increased from 200 to 400 bar, the recoveries of pesticides studied increased around 20% for all the compounds. By increasing the density, the solvating power of the extraction solvent, which is responsible for the higher recoveries, is increased, but at higher densities, the diffusion coefficients decrease. The decrease in the diffusion coefficients can cause lower recoveries at higher pressures owing to the kinetics of the extraction process [24]. Therefore, 400 bar was considered as the optimal fluid pressure for the extraction and under these conditions, the relative standard deviation (R.S.D.) was lower than 6%.

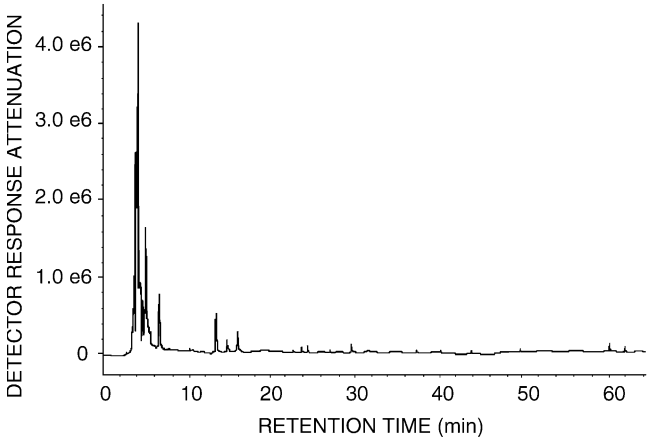

Fig. 2. GC-ECD chromatogram of blank extract of honey obtained by the SFE method. GC conditions are described in Section 2.5.

3.2.4. Effects of extraction temperature

Temperature is an essential experimental variable for SFE as it affects three extraction steps: desorption, diffusion and dissolution. While the CO_2 density may decrease with the increase of temperature at constant pressure, the solubility of many organic compounds can dramatically increase because of an increase in the solute's vapor pressure. However, very little solubility data are available in the literature to assess the effects of elevated temperature [22]. Consequently, isolating the effects of temperature on analyte–matrix interactions is extremely difficult. In this work, three temperatures (40, 60 and 90 °C) were evaluated to optimize the extraction process. The recovery results showed that by increasing he temperature from 40 to 90 °C, one enhances the extraction efficiency mainly for some pesticides: aldrin, tetradifon, etaconazole, metolachlor, prochloraz, propiconazole, triadimenol, chlorpyrifos, diazinon, dichlorvos and dimethoate. It is probably due to the increase in the solvating power of the solvent, at higher temperatures, that the analyte molecules are provided with more energy to overcome the barrier of interaction forces. Therefore, temperature increase not only enhances the analyte's solubilities, as proposed by several researchers [21,25], but also provides more energy to improve pesticide recovery. It is evident from the above results that good recovery results (above 88%) and high precision (R.S.D. below 6%) were obtained using SFE (Table 1) and hence SFE can be efficiently applied for the determination of multiresidue pesticides in honey samples. Figs. 2 and 3 show the chromatograms of blank extract of honey and fortified honey extract with the 33 pesticides, respectively in the better conditions of SFE.

3.3. Analysis of real honey samples

In order to compare SFE and liquid extraction performances, the data obtained by performing SFE in the experimental conditions, providing the highest extraction yield inside the experimental domain, were compared with those

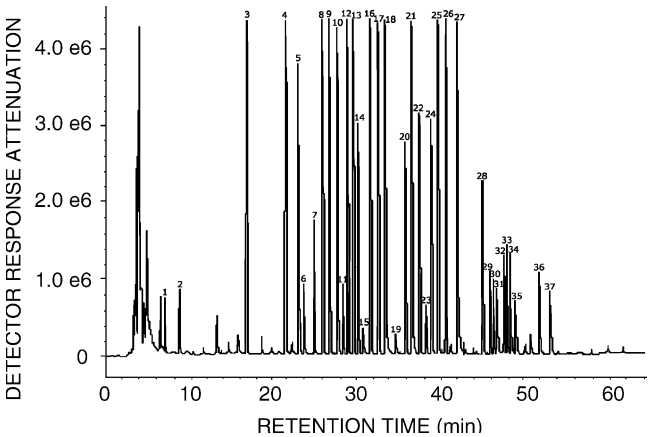

Fig. 3. GC-ECD chromatogram of fortified honey extract with the 33 pesticides obtained by the SFE method. GC conditions are described in Section 2.5. 1: Dichlorvos (0.20 mg/kg); 2: linuron (0.18 mg/kg); 3: trifluralin (0.30 mg/kg); 4: hexachlorobenzene (0.23 mg/kg); 5: dicloran (0.14 mg/kg); 6: diazinon (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 alfa (0.27 mg/kg); 17: hexaconazole (0.26 mg/kg); 18: imazalil (0.28 mg/kg); 19: buprofezin (0.22 mg/kg); 20: endosulfan beta (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); 29–31: cyfluthrin (I, II, III sum 0.38 mg/kg); 32–34: 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).

obtained by performing a traditional solvent extraction on the same sample (Table 1).

Firstly, 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 made by GC–MS. The spectra obtained were studied and three minimum selected ions for quantification were used, which are summarized in Table 2. The interference by matrix effects was studied through fortified sample by selecting the base peak of their mass spectra, after the acquisition of the samples' total ion chromatogram. The absence of co-extracted interferences at the pesticides retention times was then confirmed.

Table 2
Main ions and relative abundance of selected pesticides detected by GC/MS

Pesticides	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–IV)	163 (100); 206 (80); 226 (51)
Cypermethrin (I–IV)	163 (100), 181 (86); 209 (27)
Fenvalerate (I, II)	125 (100), 167 (84), 419 (19)

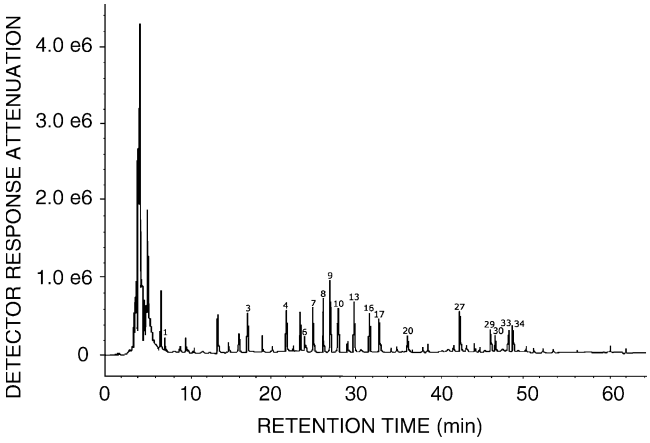

Fig. 4. GC-ECD chromatogram of real honey sample obtained by the SFE method. GC conditions are described in Section 2.5.

Fig. 4 presents a chromatogram of a real honey sample obtained by SFE. Compounds such as chlorothalonil, chlorpyrifos, endosulfan alfa, trifluralin and vinclozolin were determined by the two methods, however, higher quantity of residues was found by SFE (Table 3). In addition, endosulfan beta, hexachlorobenzene, tetradifon, and cypermethrin, were not found in real honey samples by the liquid–liquid extraction method. These differences can be explained by the properties of the supercritical fluid resulting in higher efficiency of extraction than the liquid–liquid extraction or losses during sample preparation.

SFE applied to honey analyses presents advantages as compared to conventional methods regarding the organic solvent saving and time consumption (60% less, after

Table 3
Residue (mg/kg) (R.S.D.%, $n = 5$) of the selected pesticides determined in real honey sample by liquid–liquid extraction and supercritical fluid extraction methods

Pesticides	Residue (mg/kg)	
	Liquid–liquid extraction	Supercritical fluid extraction
Organohalogen		
Aldrin	nd	nd
Bromopropylate	0.012 (4.4)	0.015 (4.4)
Chlorothalonil	0.008 (5.1)	0.010 (5.8)
Diclofop-methyl	nd	nd
Dicofol	nd	nd
Endosulfan alfa	0.007 (5.9)	0.013 (4.7)
Endosulfan beta	nd	0.009 (5.6)
Hexachlorobenzene	nd	0.003 (5.5)
Metoxychlor	nd	nd
Tetradifon	0.012 (4.5)	0.019 (4.4)
Organonitrogen		
Buprofezin	nd	nd
Dicloran	nd	nd
Etaconazole	nd	nd
Hexaconazole	nd	nd
Imazalil	nd	nd
Linuron	nd	nd
Metolachlor	nd	nd
Prochloraz	nd	nd
Propiconazole	nd	nd
Quizalofop-ethyl	nd	nd
Tebuconazole	nd	nd
Triadimefon	nd	nd
Triadimenol	nd	nd
Trifluralin	nd	0.009 (4.8)
Vinclozolin	nd	0.008 (5.3)
Organophosphorus		
Chlorpyrifos	nd	0.0031 (5.2)
Diazinon	0.017 (5.3)	0.019 (4.7)
Dichlorvos	0.007 (6.1)	0.008 (5.3)
Malathion	0.092 (4.9)	0.148 (4.3)
Pyrethroid		
Cyfluthrin[a]	0.007 (5.3)	0.009 (3.8)
Cypermethrin[a]	0.006 (4.4)	0.014 (4.6)
Fenvalerate[a]	nd	nd

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

optimization of extraction conditions). Concerning the quantitative results, the detection limits reached using SFE were basically of the same magnitude as those achieved with the liquid–liquid extraction, while the reproducibility obtained clearly presented R.S.D. lower than 6%, due to the SFE's better precision, which reduces the number and magnitude of mistakes.

In addition, the SFE proved to be easier and faster than the solvent extraction and thus, more effective and advantageous.

4. Conclusions

An SFE method using modified supercritical CO_2 has been developed and optimized for simultaneous extractions of organochlorine, organophosphorus, organonitrogen and pyrethroid pesticides from honey samples. The study of the influence of the parameters affecting SFE, allowed a better understanding of the mechanisms that take place in the supercritical extraction process, providing a larger base to improve the analytical results. The result of this study demonstrated that the use of SFE is fast, accurate and specific for multiresidue analyses in honey samples.

Compared with the conventional methodology, the main advantages of SFE are that the chances of sample contamination are greatly diminished as sample handling is minimized and the use of organic solvents is reduced. A much lower solvent evaporation, a simplified clean-up step, higher power diffusion and solubility are the other advantages of SFE.

References

[1] Analytical Methods for Pesticide Residues in Foodstuffs, sixth ed., General Inspectorate for Health Protection, The Netherlands, 1996.
[2] M.C. Rodríguez, M. Hidalgo, A. Belleza, F. Capón, Contaminantes en Alimentos y Toxicología 4 (1989) 23.
[3] C. Tomlin, The Pesticide Manual, 12th ed., The British Crop Protection Council, Surrey, UK, 2000.
[4] M. Szerletics-Turi, E.S. Matray, Apidologie 26 (1999) 321.
[5] A. Tsigouri, U. Menkissoglu-Spiroudi, A.T. Thrasyvoulou, G.C. Diamantes, J. Assoc. Off. Anal. Chem. Int. 83 (2000) 1225.
[6] M. Fernández, Y. Picó, S. Girotti, J. Mañes, J. Agric. Food Chem. 49 (2001) 3540.
[7] S. Bogdanov, V. Kilchenmann, A. Imdorf, J. Apic. Res. 37 (1998) 57.
[8] A.C. Martel, S. Zeggane, J. Chromatogr. A 954 (2002) 173.
[9] J.J. Jiménez, J.L. Bernal, Mª.J. del Nozal, L. Toribio, Mª.T. Martín, J. Chromatogr. A 823 (1998) 381.
[10] A.P. Dalpero, S. Rossi, S. Ghini, R. Colombo, A.G. Sabattini, S. Girotti, J. Chromatogr. A 905 (2001) 223.
[11] U. Menkissoglu-Spiroudi, G.C. Diamantidis, V.E. Georgiou, A.T. Thrasyvoulou, J. AOAC Int. 83 (2000) 178.
[12] J. Yu, C. Wu, J. Xing, J. Chromatogr. A 1036 (2004) 101.
[13] U. Menkissoglu-Spiroudi, G.C. Diamantidis, V.E. Georgiou, A.T.T. Thrasyvoulou, J. AOAC Int. 83 (2000) 178.
[14] D.B. Gomis, J.J. Mangas, A. Castano, M.D. Gutierrez, Anal. Chem. 68 (1996) 3867.
[15] A-C. Martel, S. Zeggane, J. Chromatogr. A 954 (2002) 173.
[16] M. Fernández, Y. Picó, J. Mañes, J. Food Prot. 65 (2002) 1502.
[17] J.J. Jiménez, J. Atienza, J.L. Bernal, J. High Resol. Chromatogr. 18 (1995) 367.
[18] P. Karasek, J. Planeta, E. Varad'ova Ostra, M. Mikesova, J. Golias, M. Roth, J. Vejrosta, J. Chromatogr. A 1002 (2003) 13.
[19] S.B. Hawthorne, C.B. Grabanski, E. Martin, D.J. Miller, J. Chromatogr. A 892 (2000) 421.
[20] G.R. van der Hoff, P. van Zoonen, J. Chromatogr. A 843 (1999) 301.
[21] S.J. Lehotay, J. Chromatogr. A 785 (1997) 289.
[22] W.H. Hauthal, Chemosphere 43 (2001) 123.
[23] F.M. Lanças, S.R. Rissato, M.S. Galhiane, Supercritical Fluid Methods and Protocols, The Humana Press Inc., UK, 2000.
[24] S. Reindl, F. Höfler, Anal. Chem. 66 (1994) 1808.
[25] D.J. Miller, S.B. Hawthorne, Anal. Chem. 67 (1995) 273.