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Journal of Chromatography A, 1030 (2004) 77–85

JOURNAL OF CHROMATOGRAPHY A

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Comparison of solid-phase microextraction and stir bar sorptive extraction for determining six organophosphorus insecticides in honey by liquid chromatography–mass spectrometry

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Abstract

Two approaches based on sorptive extraction, solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE), in combination with liquid chromatography (LC)–atmospheric pressure chemical ionization mass spectrometry (MS) have been assayed for analyzing chlorpyriphos methyl, diazinon, fonofos, phenthoate, phosalone, and pirimiphos ethyl in honey. In both, SPME and SBSE, enrichment was performed using a poly(dimethylsiloxane) coating. Significant parameters affecting sorption process such as sample volume, sorption and desorption times, ionic strength, elution solvent, and dilution (water/honey) proportion were optimized and discussed. Performance of both methods has been compared through the determination of linearity, extraction efficiencies, and limits of quantification. Relative standard deviations for the studied compounds were from 3 to 10% by SPME and from 5 to 9% by SBSE. Both methods were linear in a range of at least two orders of magnitude, and the limits of quantification reached ranging from 0.04 to 0.4 mg kg^{-1} by SBSE, and from 0.8 to 2 mg kg^{-1} by SPME. The two procedures were applied for analyzing 15 commercial honeys of different botanical origin. SPME and SBSE in combination with LC–MS enabled a rapid and simple determination of organophosphorus pesticides in honey. SBSE showed higher concentration capability (large quantities of sample can be handled) and greater accuracy (between 5 and 20 times) and sensitivity (between 10 and 50 times) than SPME; thus, under equal conditions, SBSE is the recommended technique for pesticide analysis in honey.
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Keywords: Extraction methods; Honey; Pesticides; Organophosphorus compounds

1. Introduction

Some monitoring programs established to control the quality of commercial honey have revealed low levels of organophosphorus pesticide (OPP) residues [1–3]. The extensive use of OPPs in agricultural practice is the reason of why residues of these pesticides contaminate bees during pollination process and are transferred by them into honey [4]. As OPPs constitute a potential risk to human health, their occurrence in honey is a matter of public concern. However, the European Union (EU) has set maximum residue limits (MRLs) in honey for several acaricides, but neither the *Codex Alimentarius* nor the EU have established MRLs for OPPs [5].

Sample preparation, chromatographic separation systems and detection techniques developed to determine pesticide residues in bee products have been recently reviewed [6] showing that most analytical methods for pesticide determination are based on gas chromatography (GC) or liquid chromatography (LC). Nowadays, LC coupled to mass spectrometry (MS) provides clear advantages in terms of the range of compounds traceable and higher sensitivity/selectivity than conventional LC methods. This review also pointed out that sample preparation is the critical step.

Most common techniques for extracting pesticides from honey have been liquid–liquid extraction (LLE) [7–11] and solid-phase extraction (SPE) [12–18]. However, modern trends in analytical chemistry are towards the simplification and miniaturization of sample preparation, and the minimization of organic solvent used. Solid-phase microextraction (SPME), and stir bar sorptive extraction (SBSE) are easy and fast techniques, which avoid (toxic) solvents, and, in the case of SPME, easily automated.

SPME is performed by immersion of a silica fiber coated with a stationary phase in an aqueous sample, and SBSE by stirring the sample with a stir bar covered with poly(dimethylsiloxane) (PDMS) for a given time. The

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doi:10.1016/j.chroma.2003.11.037

analyte enrichment is by partitioning between the polymer and the aqueous phase according to their distribution constant [19] and its desorption by temperature in the injector (for GC) or by liquid removal (for LC).

Notwithstanding the number of studies published dealing with the use of SPME for different applications and covered by recent reviews [20,21], SPME bibliography for pesticide analysis in honey is still scarce and restricted to GC desorption [22–24]. Jiménez et al. [22] examined different SPME fiber coatings for the extraction of pesticide residues in honey, being the 100 μm PDMS the selected one for the less polar analytes. The same fiber coating was applied for determining acaricides in honey by GC–MS analysis [23,24]. Although the precision and accuracy was unsatisfactory with some of the analytes, these studies concluded that the method proposed is a useful tool for rapid screening of pesticides in honey. In several other works, the feasibility of SBSE to determine pesticides in fruit and vegetables has been successfully tested [25–27] but no application of SBSE to analyze them in honey has been reported. SPME and SBSE have been compared for the analysis of different compounds as organochlorine pesticides in strawberry [27], volatiles in malt [28] or in Arabica roasted coffee [29] and polycyclic aromatic hydrocarbons in water [30]. All the studies reach the same conclusion, the SBSE concentration capability was better that those presented by SPME because the film of PDMS phase that covers the bar is thicker. Therefore, SPME is considered ideally suited for the detection of compounds that present high concentration whereas SBSE is the method of choice for trace and ultratrace analysis. SPME directly coupled to LC (on-line coupled) leads to further increase of sensitivity.

The present study compares SBSE and SPME for extracting chlorpyriphos methyl, diazinon, fonofos, phenthoate, phosalone, and pirimiphos ethyl from honey. The enrichment is performed on PDMS coated and the determination is carried out by LC–atmospheric pressure chemical ionization (APCI) MS injecting 5 μl. The extraction efficiencies were studied to adjust the following parameters: volume of aqueous solution required for the extraction, samples dilution (water/honey proportion), time necessary to achieve the equilibrium, ionic strength (salting out effect), and elution solvent, to compare both procedures under identical conditions. Validation parameters such as linearity, precision, limits of detection and quantification were determined and discussed. Finally, the procedures were applied for the determination of OPPs in honey samples.

2. Experimental

2.1. Chemicals

Pesticide standards (chlopyriphos methyl, diazinon, fonofos, phenthoate, phosalone, and pirimiphos ethyl) were obtained from Sigma–Aldrich (Madrid, Spain). HPLC-grade methanol was purchased from Merck (Darmstadt, Germany) and sodium chloride (analysis grade) was supplied by Scharlau (Barcelona, Spain). The individual stock solutions were prepared in methanol at a concentration of 1000 mg l^{-1} and stored at 4 °C. Standard working solutions at various concentrations were daily prepared in ultrapure water obtained from Milli-Q SP reagent water system (Millipore, Bedford, MA, USA).

A SPME holder for automated sampling and a kit of SPME fiber assembly consisting of three 1-cm long fibers coated with 100-μm thick PDMS were obtained by Supelco (Bellefonte, PA, USA). The new fibers were conditioned in methanol for 30 min by stirring, and the used ones were cleaned in methanol by stirring for 15 min before extraction.

The stir bars (Twister) were from Gerstel (Mülheim, Germany) with a length of 10 mm and coated with a 1 mm PDMS layer (volume: 55 μl). After desorption, stir bars were conditioned into a vial containing 15 ml of methanol, and treated for 5 min by sonication, then the solvent was rejected and the procedure was repeated three times.

2.2. Solid-phase microextraction

2.5 g of honey was placed into a 50 ml glass beaker, diluted 1/10 ratio with water and homogenized over 15 min using a magnetic stirring bar. The fiber was immersed in the aqueous sample for 120 min under stirring at 900 rpm. Subsequently, the fiber was withdrawn into the holder needle and immediately introduced into a 2 ml vial filled with 1 ml of methanol and desorbed for 15 min under stirring. Five microliters of this extract were injected into the LC–MS system.

2.3. Stir bar sorptive extraction

Honey solution was prepared as described above. A stir bar coated with PDMS was placed in the honey solution and the sorption was carried out for 120 min while stirring at 900 rpm. After extraction, the stir bar was removed from the aqueous sample with tweezers and the analytes desorbed into 2 ml vial filled with 1 ml of methanol. Desorption of the pesticides was performed agitating for 15 min. Five microliters of this extract were injected into the LC–MS system.

2.4. Liquid chromatograph with mass spectrometry

The LC–MS was performed in a Hewlett-Packard (Palo Alto, CA, USA) HP-1100 series LC–MSD system consisting of an LC connected to a single quadrupole MS analyzer with an APCI interface usable in either positive ionization (PI) or negative ionization (NI) modes. An HP Chemstation software version A.06.01 was used for LC–MS control and signal acquisition.

The LC separation was carried out on a Luna C_{18} column (250 mm × 4.6 mm i.d., particle size: 5 μm) protected by a Securityguard cartridge C_{18} (4 mm × 2 mm i.d.), both

Table 1
Time scheduled SIM conditions for monitoring OPPs pesticides

Pesticide	Time (min)	Quantification ion, m/z (relative abundance)	Confirmation ions, m/z (relative abundance)	Fragmentor (V)	Dwell time (ms)
Phenthoate	0.00–12.00	319.0 (100)	110 (70), 157 (12)	60	132
Fonofos	12.00–15.00	153.0 (100)	137 (52), 109 (68)	60	40
Diazinon		275.0 (100)	169 (40), 151 (40)		
Phosalone		338.0 (100)	185 (80), 142 (40)		
Chlorpyriphos methyl	15.00–20.00	302.0 (100)	157 (24), 125 (62)	60	132
Pirimiphos ethyl	20.00–30.00	304.0 (100)	180 (50), 169 (10)	60	132

from Phenomenex (Madrid, Spain). For the separation of OPPs, the mobile phase was a methanol/water gradient at a flow-rate of 0.7 ml min^{-1}. The gradient was 80% methanol from 0 to 15 min, followed by a linear gradient to 90% from 15 to 20 min, then increased again linearly to 95% from 20 to 25 min, and finally, maintained at 95% methanol from 25 to 30 min and re-equilibrates to the initial conditions in 10 min.

Optimum operating parameters of the APCI interface in NI mode were: vaporizer temperature, 450 °C; nebulizer gas, nitrogen at a pressure of 60 psi (1 psi = 6894.76 Pa); drying gas, also nitrogen, at a flow rate of 4 l min^{-1} and temperature of 350 °C; capillary voltage, 3500 V; and corona current, 25 μA. The chromatograms were recorder in full-scan and selected-ion monitoring (SIM) modes. Full scan conditions were: m/z ranged from 50 to 400, with a scan time of 0.75 s. Time-scheduled SIM using four windows was developed. The most intense ion was used for quantification and the second and third ion for confirmation, as it is shown in Table 1.

3. Results and discussion

3.1. Optimization

Sorptive enrichment in aqueous media is an equilibrium, therefore extraction is significantly influenced by aqueous volume, extraction and desorption time, desorption solvent and ionic strength. A set of experiments to determine the effect of these parameters in the recoveries of the six OPPs was designed. Honey was spiked with 100 μl of a working solution that contains 50 μg ml^{-1} of diazinon, chlorpyriphos methyl, and pirimiphos ethyl, 100 μg ml^{-1} of fonofos, 20 μg ml^{-1} of phosalone and 10 μg ml^{-1} of phentoate and allowed to stand at room temperature for 1 h.

Different water volumes (2–50 ml) were tested as it is shown in Fig. 1. The lower the sample volume is, the higher the recovery obtained. Although theoretical principles and extractives phases are identical, substantially differences between both methods were observed, SBSE recoveries ranged from nearly to 100% using 2 ml of water sample to 40% using 50 ml. However, SPME recoveries were from 20% using 2 ml to 5% using 50 ml of aqueous solution. In both cases, recoveries decrease considerably for volumes higher than 10 ml but differences of recoveries are not so accused between 10 and 25 ml. A water volume of 25 ml was selected for further experiments as a compromise to attain appropriate sensitivity with a water volume that achieves the dissolution of an appropriate quantity of honey.

The influence of honey matrix on the extraction efficiency of SPME and SBSE, was checked diluting different amounts of honey in 25 ml of water. Fig. 2 displays the results in terms of recovery for SPME and SBSE. Honey reduced the recovery obtained by SPME for all pesticides, on the contrary it scarcely affected SBSE. This is an interesting feature that underlines the potential of SBSE versus SPME. The amount of 2.5 g of honey was used for the following experiments, since it provided acceptable recoveries and good sensitivity.

Different extraction times were studied to obtain the sorption time profiles, which are presented in Fig. 3. The time required for full equilibration using SPME was 90 min for phenthoate, phosalone, diazinon and fonofos and 120 min for pirimiphos ethyl and chlorpyriphos methyl. The extraction time for SPME was set at 120 min to obtain the highest possible recoveries since they are, in any case, quite low given the small volume of polymeric coating (the volume of PDMS coated onto the fiber is 0.6 μl). A 120 min extraction time was also selected for SBSE to avoid unreasonable analysis time. Equilibrium was not attained for any of the studied pesticides because the higher thickness of the PDMS coating (55 μl). However, quantitative analysis can be carried out because the samples are extracted exactly the same time and analytical sensitivity is rather satisfactory.

Extraction efficiencies for a wide variety of compounds (depending on the polarity) can be improved increasing ionic strength since high ionic strength reduces their water solubility [31]. This effect was tested adding 30% (w/w) of sodium chloride, which is much closer to the saturated solution. On the contrary, for the studied compounds that are quite apolar, recoveries decreased with increasing the ionic strength. This decrease in the recovery is caused by the influence of salt on the polarity of the sample—lowering it—that, in this case, reduces the equilibrium constant between the sample and the PDMS phase, i.e. the affinity of the target organophosphorus towards the PDMS coating [31].

Table 2 shows the effect of desorption solvent and desorption time on the recoveries. Methanol and acetonitrile were tested at different times. Both solvents gave similar results but methanol was selected for further experiments because

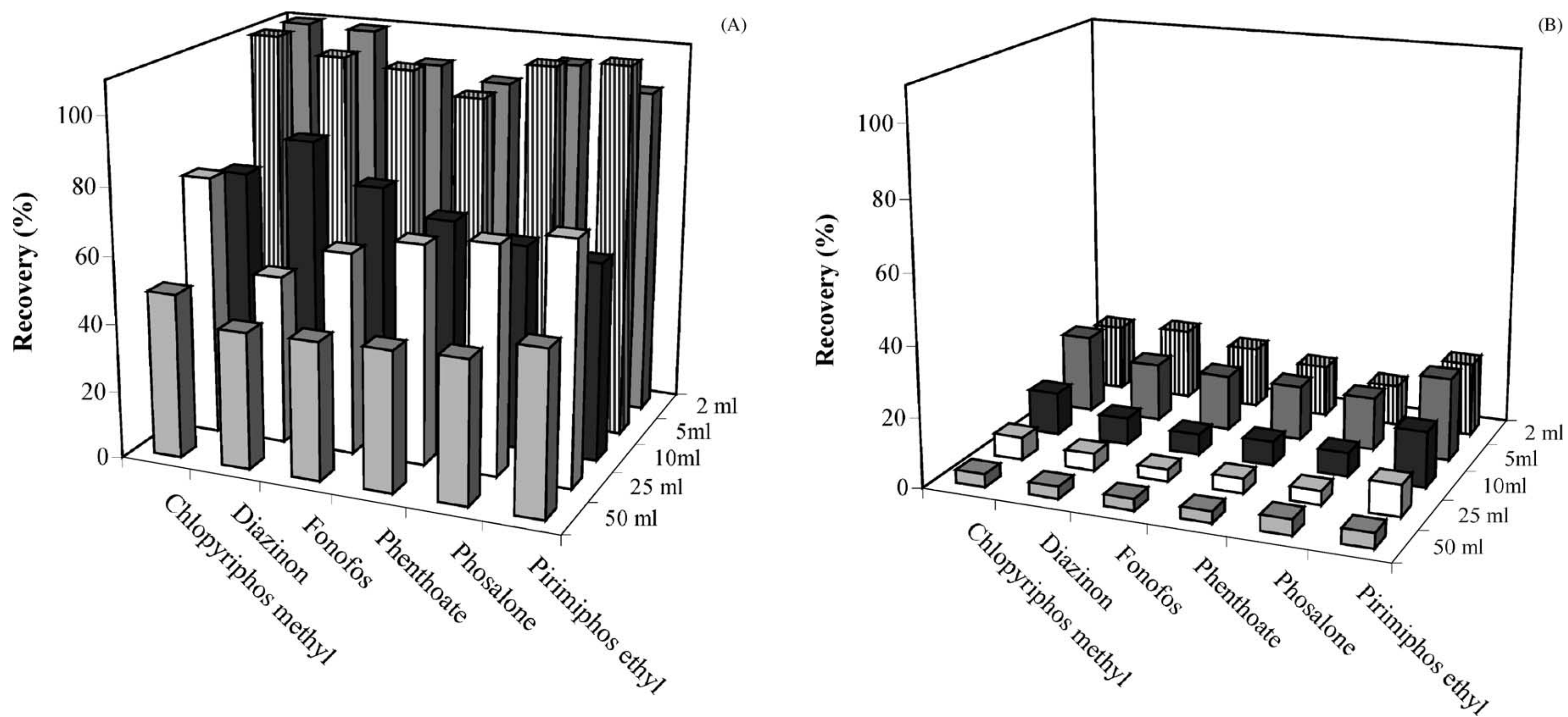

Fig. 1. Influence of the water volume on the extraction efficiency: (A) SBSE and (B) SPME.

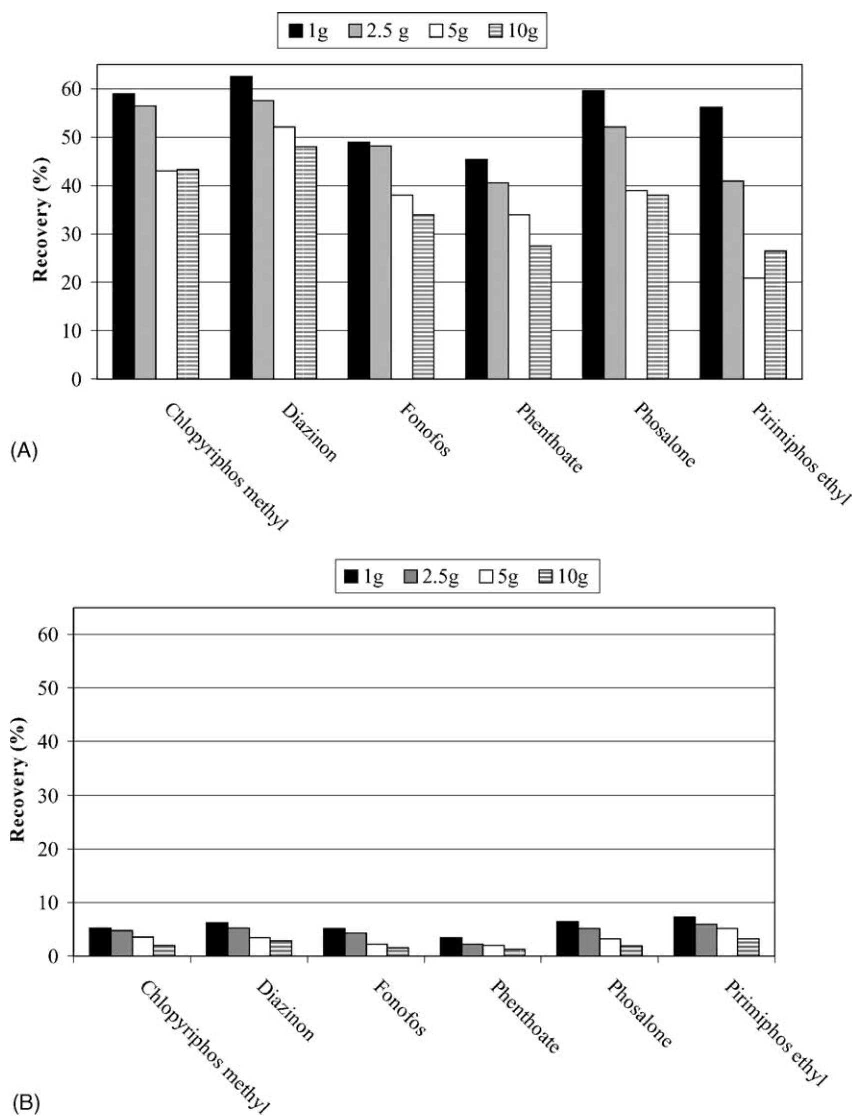

Fig. 2. Effect on pesticide recoveries of different amount of honey: (A) SBSE and (B) SPME.

it is used as mobile phase. The desorption time has a strong influence on the recoveries. In SPME, recoveries were increased gradually from 5 to 10 min and remained almost constant from 15 to 20 min, whereas in SBSE the fully desorption of analytes was achieved at 15 min.

3.2. Validation

The linearity was evaluated at five concentrations, from the LOQ to 100 times the LOQ. Concentrations range, regression equations and correlation coefficients for the six OPPs are given in Table 3, showing correlation coefficients higher than 0.998 for SBSE and 0.994 for SPME. These coefficients (0.99) are relatively poor compared to conventional calibration technique (0.999) because the extraction is included as it has been previously reported [14,19,29]. The slopes of the regression equations are relatively constant for honey of different floral origins.

The detection limits (LODs) were calculated as three times the standard deviation of the slope of the calibration curve. LODs obtained by SBSE were 0.08 mg kg^{-1} for chlopyriphos methyl and pirimiphos ethyl, 0.1 mg kg^{-1} for diazinon and fonofos and 0.01 mg kg^{-1} for phenthoate and phosalone. LODs achieved by SPME were 0.5 mg kg^{-1} for chlopyriphos methyl, phosalone, pirimiphos ethyl, 1 mg kg^{-1} for diazinon and fonofos and 0.3 mg kg^{-1} for phenthoate. Table 4 shows the mean recovery and precision obtained from spiked samples at the LOQ levels and at 10 times the LOQ levels. LOQs were calculated according to the European Union Guidelines as the lower concentration that provides repeatabilities lower than 20%. Table 4 reports LOQs ranging from 0.04 to 0.4 mg kg^{-1} by SBSE, and from 0.8 to 3 mg kg^{-1} by SPME. Stir bar LOQs are between 7 and 20 times lower compared to those from the SPME fiber. Recoveries of SBSE were between 40% for pirimiphos ethyl and 64% for fonofos, with relative standard deviation

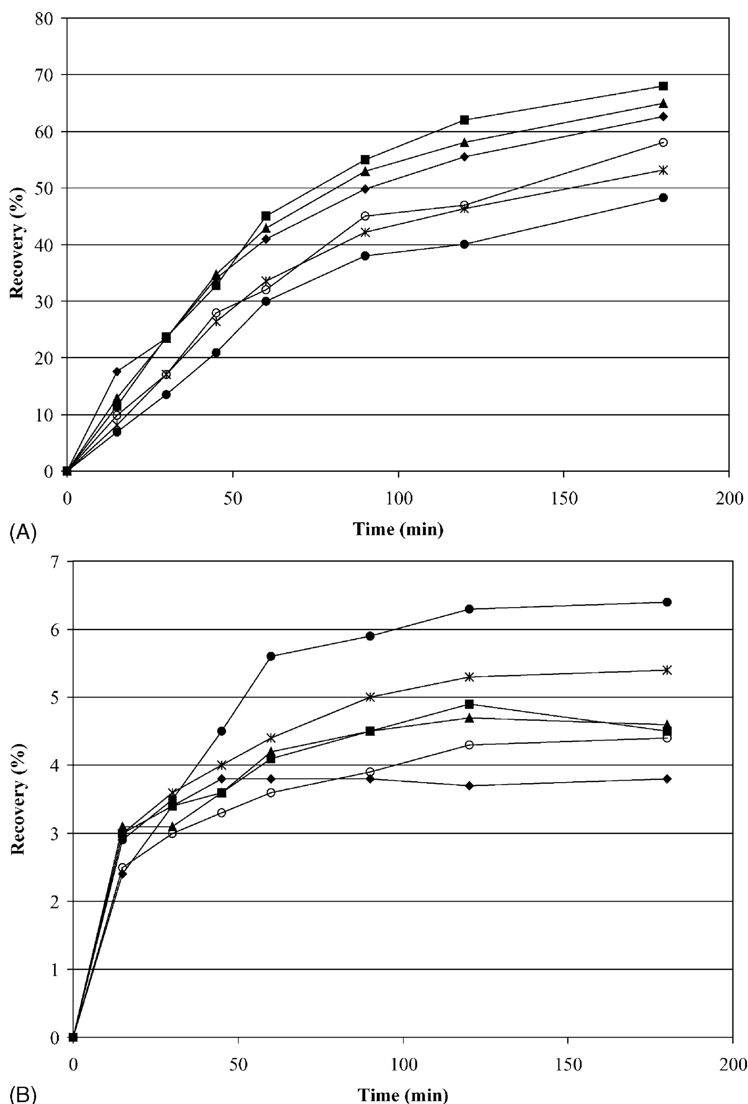

Fig. 3. Sorption time profile of (◆) phenthoate, (■) diazinon, (▲) fonofos, (○) phosalone, (✱) chlopyriphos methyl and (●) pirimiphos ethyl by (A) SBSE and (B) SPME.

(RSD) <9%. These recoveries are generally one order of magnitude higher than those obtained by SPME, ranging from 3.6% for phenthoate to 7.6% for pirimiphos ethyl, with RSDs < 10%. The low recoveries and worst LOQs obtained by SPME, compared to those from the SBSE, can be explained because the extraction procedures are based on reaching equilibrium and the lower volume of PDMS coating (0.6 against 55 μl).

Table 2
Recoveries (%) obtained for the studied OPPs in honey by SPME and SBSE depending on the desorption solvent and desorption time

Compound	SBSE								SPME							
	Acetonitrile time (min)				Methanol time (min)				Acetonitrile time (min)				Methanol time (min)			
	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Chlopyriphos methyl	29	34	40	41	31	39	40	39	2.1	3.5	3.9	4.1	2.5	3.1	3.5	4.5
Diazinon	27	43	50	49	33	45	52	53	1.9	2.1	2.3	2.2	1.7	2.9	4.0	4.2
Fonofos	31	37	49	50	39	45	58	57	2.3	2.9	3.1	3.2	2.1	3.1	3.2	3.3
Phenthoate	35	40	55	55	39	43	55	54	1.7	2.5	3.5	3.1	2.9	3.0	3.2	3.4
Phosalone	29	37	47	48	33	44	47	49	2.6	3.6	4.7	5.1	2.6	3.9	4.2	4.2
Pirimiphos ethyl	33	40	42	42	29	35	39	39	3.4	5.2	6.8	7.0	3.8	5.7	6.2	6.1

Table 3
Regression data and equations for the six OPPs extracted from honey

Compound	SBSE			SPME		
	Concentration range ($mg\,kg^{-1}$)	Equation	Correlation Coefficient (r)	Concentration range ($mg\,kg^{-1}$)	Equation	Correlation Coefficient (r)
Chlopyriphos methyl	0.2–20	$y = 6236x + 1698$	0.998	2–200	$y = 286x + 304$	0.998
Diazinon	0.4–40	$y = 3840x + 1002$	0.997	3–300	$y = 172x + 262$	0.999
Fonofos	0.2–20	$y = 7019x + 1740$	0.998	3–300	$y = 290x + 237$	0.999
Phenthoate	0.04–4	$y = 30693x + 821$	0.996	0.8–80	$y = 1187x + 341$	0.998
Phosalone	0.08–8	$y = 15073x + 540$	0.997	2–200	$y = 567x + 389$	0.994
Pirimiphos ethyl	0.2–20	$y = 3648x + 1554$	0.998	2–200	$y = 215x + 393$	0.996

Table 4
Recovery and relative standard deviation (RSD) of the six OPPs in honey samples at the two spiked levels

Compound	SBSE			SPME		
	Concentration[a] ($mg\,kg^{-1}$)	Recovery (%)	RSD (%)	Concentration[b] ($mg\,kg^{-1}$)	Recovery (%)	RSD (%)
Chlopyriphos methyl	0.2	42.1	7.9	2	4.5	3.1
	2.0	47.2	6.2	20	5.6	4.4
Diazinon	0.4	63.0	7.9	3	5.1	9.0
	4.0	58.0	9.5	30	4.6	8.4
Fonofos	0.2	64.0	5.0	3	3.6	4.4
	2.0	66.0	8.4	30	3.9	8.1
Phenthoate	0.04	57.0	8.3	0.8	3.6	8.9
	0.4	54.8	6.8	8	3.3	9.9
Phosalone	0.08	58.7	6.8	2	5.0	5.3
	0.8	52.0	7.5	20	4.1	6.0
Pirimiphos ethyl	0.2	40.6	6.9	2	7.6	6.6
	2.0	46.3	8.2	20	7.3	7.3

[a] The lowest concentration is the LOQ obtained by SBSE.
[b] The lowest concentration is the LOQ obtained by SPME.

Accuracy obtained by both methods in honey is presented in Table 5. The accuracy ranged from 75 to 111%, with a precision lower than 10%, by SBSE and from 52 to 75% with a precision lower than 10%, by SPME. Although precision was similar in both methods, the higher accuracy with SBSE, especially for fonofos, can be attributed to the superior recoveries.

Chromatograms of the SBSE–LC–MS analysis of an unspiked honey sample and spiked honey at 10 times the LOQ levels are illustrated in Fig. 4A and B, and the chromatograms of the SPME–LC–MS analysis of an unspiked honey sample and of a spiked honey at twice the LOQ levels showed in Fig. 5A and B. As it can be observed in both cases, the lack of interfering peaks and the low background noise provided unequivocal determination of the studied pesticides. Unequivocal identification criteria was based on: (a) the chromatographic retention data, and (b) the relative peak heights of the three characteristic masses in the sample peak, which must be within ±20% of the relative intensity of these masses, on the mass spectrum of the standard analyzed in the LC–MS system.

Table 5
Precision and accuracy for the six OPPs from honey by SBSE and SPME

Compound	SBSE				SPME			
	Concentration added ($mg\,kg^{-1}$)	Concentration found ($mg\,kg^{-1}$)	Accuracy (%)	RSD (%)	Concentration added ($mg\,kg^{-1}$)	Concentration found ($mg\,kg^{-1}$)	Accuracy (%)	RSD (%)
Chlopyriphos methyl	2.0	2.23 ± 0.02	111	5.3	20.0	13.20 ± 0.03	66	10.2
Diazinon	2.0	1.82 ± 0.05	90	10.2	20.0	14.62 ± 0.06	73	10.5
Fonofos	4.0	3.71 ± 0.07	92	8.6	40.0	20.85 ± 0.09	52	9.2
Phenthoate	0.4	0.38 ± 0.02	96	6.9	4.0	2.88 ± 0.02	72	8.3
Phosalone	0.8	0.63 ± 0.01	75	6.6	8.0	4.88 ± 0.01	61	10.0
Pirimiphos ethyl	2.0	1.88 ± 0.02	94	7.4	20.0	15.05 ± 0.06	75	14.1

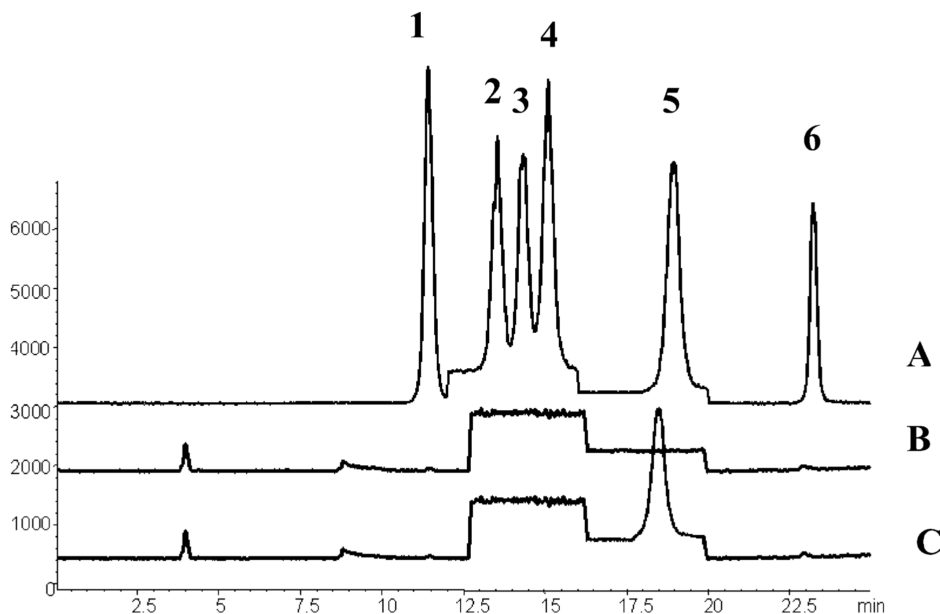

Fig. 4. SBSE–LC–MS chromatograms in SIM mode of (A) untreated honey sample spiked at 10 times the LOQ, (B) untreated honey sample, and (C) contaminated honey sample with $2.2 \pm 0.22\,mg\,kg^{-1}$ of chlorpyriphos methyl. Peaks: 1 = phenthoate, 2 = fonofos, 3 = diazinon, 4 = phosalone, 5 = chlorpyriphos methyl, and 6 = pirimiphos ethyl.

3.3. Application

SPME and SBSE procedures were applied for determining six OPPs in 15 commercially honey samples from various floral origins (rosemary, lavender, lime, citrus, and multi-flower) produced in the Valencian Community. Only chlorpyriphos methyl was detected in one sample of multi-flower honey. This sample was extracted by triplicate and each replicate was injected twice. The mean concentration value and the standard deviation were $2.2 \pm 0.22\,mg\,kg^{-1}$ by SBSE and $2.0 \pm 0.28\,mg\,kg^{-1}$ by SPME. Fig. 4C show the chromatogram of the sample extracted by SBSE and Fig. 5C displays the chromatogram of the sample obtained by SPME. Good agreement was obtained by both procedures.

3.4. Comparison

SBSE recoveries are between 10 and 20 times higher than those obtained by SPME fiber, because to the thicker PDMS coating. The linearity of the calibration curves, constructed from the analysis of spiked samples, was satisfactory in both

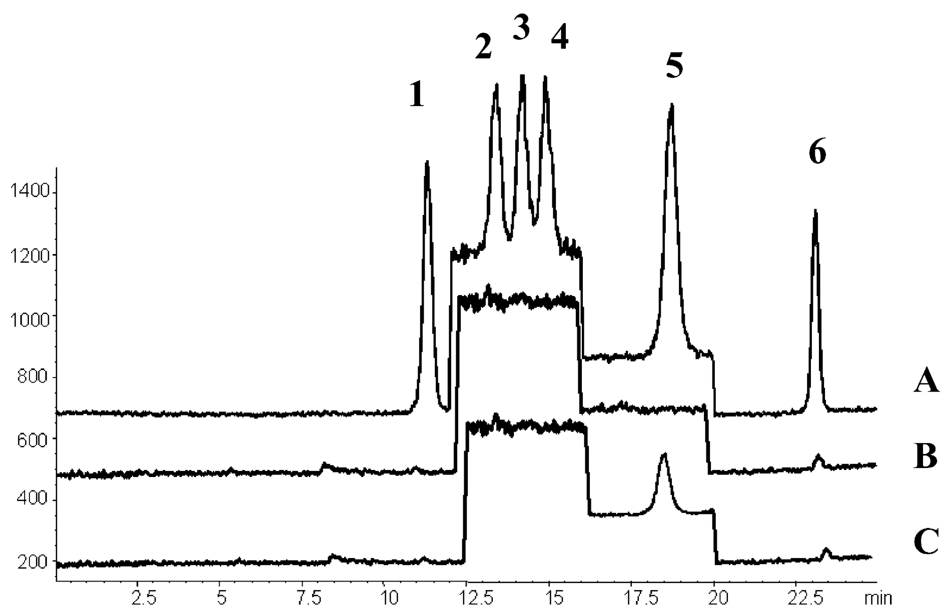

Fig. 5. SPME–LC–MS chromatograms in SIM mode of (A) untreated honey sample spiked at twice the LOQ, (B) untreated honey sample, and (C) sample containing $2.0 \pm 0.28\,mg\,kg^{-1}$ of chlorpyriphos methyl. Peaks identification as in Fig. 4.

methods. SBSE showed better sensitivity than SPME (between 5 and 20 times), and it can be still improved processing larger quantities of honey. SBSE provided also better accuracy. However, SPME presents some advantages with respect to SBSE, which can be hardly deduced from the data presented. Recoveries obtained by SPME could be further increased when using different types of commercially available fibers. Up to now, the stir bar offer a limited enrichment capability of polar pesticides because is only available with PDMS coating. It is also extremely difficult to obtain commercially stir bars compared to fibers. Another advantage is the possibility of automating most parts of the manual experimental SPME setup used in this report and the capability of desorbing the analytes directly in the LC, which would increase about 100 times the sensitivity of SPME. However, the results presented indicate the potential of SBSE for determining OPPs pesticides in honey. In a nearby future it is expected that new types of materials will be developed to cover the stir bar allowing the analysis of a major number of substances.

4. Conclusions

SPME and SBSE in combination with LC–MS enables selective and sensitive analysis of chlopyriphos methyl, diazinon, fonofos, phenthoate, phosalone, and pirimiphos ethyl in honey. Both techniques are simple, economical, do not require any preliminary sample preparation step and reduce the volume of (toxic) solvents used. Honey matrix scarcely influence SBSE but has a significant effect in SPME. Linearity and precision obtained by SBSE and SPME are similar but SBSE has demonstrated to be more accurate and sensitive than SPME.

Acknowledgements

This work has been supported by the Spanish Ministry of Science and Technology together with the European Regional Developments Funds (ERDF) (project No. AGL2003-01407) and the Integrated Actions Program between Spain and Portugal (HP2001-0009), and Spain and Italy (HI02-52).

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