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## **Solid-phase microextraction-liquid chromatography-mass spectrometry applied to the analysis of insecticides in honey**

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**Solid-phase microextraction-liquid chromatography-mass spectrometry applied to the analysis of insecticides in honey**

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4 **Solid-phase microextraction-liquid chromatography -mass**  
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7 **spectrometry applied to the analysis of insecticides in honey\***  
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14 **C. Blasco, G. Font and Y. Picó\*\***  
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39 \* Paper presented at the 2<sup>nd</sup> International Symposium on 'Recent Advances in Food  
40 Analysis, Prague November 2005  
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For Peer Review Only

**Abstract**

An approach based on solid-phase microextraction-liquid chromatography-mass spectrometry (SPME-LC-MS) has been developed for determining 12 insecticides (bromophos ethyl, chlorpyrifos methyl, chlorpyrifos ethyl, diazinon, fenoxycarb, fonofos, phenthoate, phosalone, pirimiphos methyl, profenofos, pyrazophos, and temephos) in honey. The influence of several parameters on the efficiency of the SPME was systematically investigated. Under optimal conditions, the procedure provided excellent linearity ( $>0.990$ ), detection and quantification limits (between  $0.001$  and  $0.1 \mu\text{g g}^{-1}$  and between  $0.005$  and  $0.5 \mu\text{g g}^{-1}$ , respectively), and precision ( $<19\%$  at the quantification limits and from  $6$  to  $14\%$  at ten times higher concentrations). However, recoveries were not so good, ranging from  $19$  to  $92\%$ . Honey samples were found which were contaminated with bromophos ethyl, diazinon, fonofos, pirimiphos ethyl, pyrazophos and temephos at estimated concentrations from  $6.2 \pm 1.2$  to  $19 \pm 3 \text{ ng g}^{-1}$ .

**Keywords:** *Solid-Phase Micro Extraction; Liquid Chromatography-Mass Spectrometry; Organophosphorus insecticides; Carbamate insecticides; Honey; Food Analysis.*

## Introduction

Honey is produced when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. The definition of honey stipulates a pure product absent of residues, which include, but are not limited to pesticide residues. However, the legislation is complex and is not always clear and consistent (Herrera et al. 2005). The European Union (EU) and United States (USA) have a very similar position, up to now, only three Maximum Residue Limits (MRLs) have been defined for acaricides —amitraz, coumaphos, and cymiazole—, which are directly applied within the beehive to control the parasitic mite *Varroa jacobsoni* (Commission Regulation (EC) No.2377/90 1990; US Environmental Protection Agency (EPA) 2005). There is no legislation relating to other pesticide residue limits in honey but countries such as Germany, The Netherlands and Italy have established those MRLs. In some cases, these tolerances are specific to particular substances and in other cases, there are a general limit of  $10 \mu\text{g kg}^{-1}$  or  $50 \mu\text{g kg}^{-1}$  for any of these residues (Piro & Multinelli 2003). Consumers are extremely sensitive to the issue of pesticide residues in food, particularly, for a product such as honey that is regarded as natural and pure should be as free from residues as possible. In addition, according to the Directive 96/23/EC, which contains guidelines for residues control in animals and in their products (honey included), there is a defined set of substance categories that must be monitored comprising, among others, organophosphorus compounds (Council Directive 96/23/EC 1996). In this way, honey and other bee products can concentrate pesticide residues used in the area close to the beehives because bees travel long distances to collect nectar and pollen, constituting excellent bioindicators of the environmental contamination in the surrounding area (Ghini et al. 2004).

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6 The determination of pesticides is generally performed by mass spectrometry (MS)  
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8 coupled to gas (GC-MS) or liquid chromatography (LC-MS) because it offers the  
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10 possibility of confirming the identity of their residues (Fernandez et al. 2002a;  
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12 Mastovska & Lehotay 2003; Hogendoorn & van Zoonen 2000; van der Hoff & van  
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14 Zoonen 1999; Pico et al. 2004). The development of chromatographic (gas and/or  
15  
16 liquid) methods implies optimization of a wide variety of preliminary steps, e.g.  
17  
18 isolation of the analytes and interfering compounds removal (clean up/extraction) prior  
19  
20 the separation. Liquid-solid extraction (LSE) (Blasco et al. 2004b; Jimenez et al. 2002),  
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22 supercritical fluid extraction (SFE) (Rissato et al. 2004), solid-phase extraction (SPE)  
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24 (Herrera et al. 2005; Albero et al. 2004; Cossu & Alamanni 2003; Blasco et al. 2003;  
25  
26 Fernandez et al. 2002b) matrix solid-phase dispersion (MSPD) (Albero et al. 2001),  
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28 solid-phase microextraction (SPME) (Volante et al. 2001; Yu et al. 2004; Blasco et al.  
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30 2004a) and stir-bar sorptive extraction (SBSE) (Blasco et al. 2004a) are the most  
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32 common techniques used to obtain suitable extracts for analysis.  
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41 SPME is frequently chosen by many analysts as an efficient technique for sample  
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43 preparation because it is ideally suited for coupling with MS, constituting one of the  
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45 tools that helps to improve the usefulness of mass spectrometric techniques for  
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47 screening and quantification of pesticides in food samples (Zambonin 2003; Rissato et  
48  
49 al. 2004; Vas & Vekey 2004). Most SPME procedures to determine pesticides in honey  
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51 have been performed in combination with gas chromatography (GC) where, after the  
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53 extraction step, the analytes are thermally desorbed into the injector of the  
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55 chromatograph (Volante et al. 2001; Yu et al. 2004). The SPME of antivarroa residues  
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57 using 100 µm thickness polydimethylsiloxane (PDMS) fibers was discussed (Volante et  
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3 al. 2001) demonstrating that it can be a useful tool for the rapid screening of residues in  
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6 honey. Organophosphorus insecticide residues were extracted using five fiber coatings  
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8 (30  $\mu\text{m}$  thickness PDMS, 85  $\mu\text{m}$  thickness polyacrilate (PA), 65  $\mu\text{m}$   
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10 polydimethylsiloxane divinylbenzene (PDMS/DVB) and sol-gel-derived bis-  
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12 benzocrown ether/hydroxyl-terminated silicone oil (OH-TSO)) and found that the OH-  
13  
14 TSO showed excellent extraction characteristic for organophosphorus (Yu et al. 2004).  
15  
16 SPME-GC is, however, generally limited to the analysis of volatile and thermally stable  
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18 compounds (Piro & Multinelli 2003; Council Directive 96/23/EC 1996). A typical  
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20 alternative to overcome these limits is interfaced SPME with liquid chromatography  
21  
22 (LC) (Vas & Vekey 2004). The desorption step marks the difference between SPME-  
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24 GC and SPME-LC because for the latter it is carried out by liquid removal. SPME and  
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26 SBSE in combination with LC-MS were evaluated to determine six OPPs in honey,  
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28 desorbing the compounds in 1 mL of methanol and injecting them in the LC. SBSE  
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30 showed higher concentration capability and greater accuracy and sensitivity (Blasco et  
31  
32 al. 2004a). However, SPME could present two advantages that have not been exploited  
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34 to determine residues in honey yet. One would be the use of a more proper fiber coating  
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36 (could markedly increase the method efficiency) and the other would be the capability  
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38 of desorbing the analytes directly in the LC system (could improve sensitivity more than  
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40 100 hundred times).  
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51 The present study was aimed at checking the reliability of these two possibilities. For  
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53 this purpose, an SPME-LC-MS method for the simultaneous determination of eleven  
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55 organophosphorus and one carbamate pesticides (bromophos ethyl, chlorpyrifos methyl,  
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57 chlorpyrifos ethyl, diazinon, fenoxycarb, fonofos, phenthoate, phosalone, pirimiphos  
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59 methyl, profenofos, pyrazophos, and temephos) in honey was developed using SPME  
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3 coupling to LC-MS by means of the commercially standard six port LC injector, with a  
4 special fiber-desorption chamber. The SPME conditions (extraction and desorption)  
5 were optimized. Finally, the potential of the method was demonstrated by the analysis  
6 of honey samples. As target analytes, organophosphorus and carbamate insecticides  
7 were selected instead of the acaricides directly applied on beehives because the formers  
8 were used widely in the agricultural crops. The presence of these insecticides  
9 establishes how much honey is affected by environmental contamination. The selection  
10 of the particular organophosphorus (and one carbamate) was carried out in basis of their  
11 application to the surrounding area of the beehives. Their intense application was  
12 established after a survey among cooperatives, farmers and pesticides vendors of the  
13 geographical area.  
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## 34 **Experimental**

### 35 *Chemicals*

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37 Pesticide standards (bromophos ethyl, chlorpyrifos methyl, chlorpyrifos ethyl, diazinon,  
38 fenoxycarb, fonofos, phenthoate, phosalone, pirimiphos methyl, profenofos,  
39 pyrazophos, and temephos) were purchased from Sigma-Aldrich (Madrid, Spain).  
40 HPLC-grade methanol was purchased from Merck (Darmstadt, Germany) and sodium  
41 chloride (analysis grade) was supplied by Scharlau (Barcelona, Spain). The individual  
42 stock solutions were prepared in methanol at a concentration of 1000 mg L<sup>-1</sup> and stored  
43 at 4°C. Standard working solutions at various concentrations were daily prepared in  
44 ultrapure water obtained from Milli-Q SP Reagent Water System (Millipore, Bedford,  
45 MA, USA).  
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### *Apparatus*

The SPME interface (Supelco, Bellefonte, PA, USA) consisted of a standard six-port reodyne valve equipped with a desorption chamber (total volume = 60  $\mu\text{L}$ ), installed between the autosampler of the LC and the analytical column. The LC system, a Hewlett Packard (Palo Alto, CA, USA) HP-1100 Series LC-MSD system, consisted of a HP-1100 autosampler with 200  $\mu\text{L}$  loop, a G1312 binary pump, a Luna  $\text{C}_{18}$  column (250 x 4.6 mm I.D, particle size 5  $\mu\text{m}$ ) protected by a Securityguard cartridge  $\text{C}_{18}$  (4 x 2 mm I.D), both from Phenomenex (Madrid, Spain), and diode array UV-Vis detector that can be coupled in series to a single quadrupole MS analyzer with an APCI interface usable in either positive ionization (PI) or negative ionization (NI) modes, controlled by HP Chemstation software version A.06.01.

### *Chromatographic and detection conditions*

The mobile phase was a methanol/water gradient at a flow-rate of 0.7  $\text{mL min}^{-1}$ . The gradient was 70% 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 min 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  $^{\circ}\text{C}$ ; nebulizer gas, nitrogen at a pressure of 60 psi; drying gas, also nitrogen, at a flow rate of 4  $\text{L min}^{-1}$  and temperature of 350  $^{\circ}\text{C}$ ; capillary voltage, 3500 V; and corona current 25  $\mu\text{A}$ . The chromatograms were recorded in full-scan and selected-ion monitoring (SIM) modes. Full scan conditions were  $m/z$  ranged from 50 to

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3 400, with a scan time of 0.75s. Time-scheduled SIM using five windows was  
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5 developed, as it is shown in Table I.  
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#### 10 11 *Solid-phase microextraction (SPME)* 12

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14 A SPME holder assembly for automated sampling and several replaceable fibers were  
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16 obtained from Supelco (Bellefonte, PA, USA). Silica fibers with four different coatings,  
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18 85  $\mu\text{m}$  thick polyacrilate (PA) film, 7, 30 and 100  $\mu\text{m}$  polydimethylsiloxane (PDMS),  
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20 60  $\mu\text{m}$  polydimethylsiloxane/divinylbenzene (PDMS/DVB) and 50  $\mu\text{m}$   
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22 carbowax/templated resins (CW/TPR) were employed for comparative studies. The new  
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24 fibers were conditioned in methanol by stirring for 30 min and the used ones were  
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26 cleaned in methanol by stirring for 15 min before extraction. The SPME device has  
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28 been described elsewhere (Zambonin 2003; Wardencki et al. 2004; Vas & Vekey 2004).  
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30 Spiked samples were prepared by adding 1.5 g of honey with different volumes of the  
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32 working solutions into 6 mL clear vials (Análisis Vinicos, Tomelloso, Spain) and by  
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34 keeping for 1 h at room temperature to ensure the appropriate distribution of the  
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36 insecticides in honey. Then, the honey was added with 5 mL of water and stirring for 10  
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38 min to appropriately dissolve the honey. The extraction was carried out for 120 min  
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40 under magnetic stirring in order to improve mass transfer to the aqueous sample into the  
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42 fiber coating. Desorption was performed in static mode placing the fiber into the  
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44 desorption chamber, which has been previously filled with methanol-water (70:30 v/v)  
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46 for 15 min. To avoid possible memory effects, the fiber was left continuously exposed  
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48 to the mobile phase flow during the analysis. The injection was recorder programming  
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50 the autosampler for a blank run, and using the handle of the SPME interface as external  
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### *Honey samples*

Twenty six honey samples were taken from local markets of Valencia, those samples were from different floral origins, thyme, multi flowers, rosemary, heather, lavender, orange blossom, lemon, acorn and eucalyptus. Honeys were locally produced. These samples were stored in their original containers (always glass jars) at room temperature in a dark place.

## **Results and discussion**

### *Organophosphorus Analysis*

The twelve organophosphorus insecticides studied can be separated and simultaneously detected by liquid chromatography-mass spectrometry using atmospheric pressure chemical ionization (APCI) and negative ionization mode (NI). In many application dealing with pesticide residue analysis, electrospray in positive ionization mode was used (Alder et al. 2006; Pico et al. 2004). However, the literature shows that the best sensitivity is obtained either by electrospray or APCI, depending on the make of the instrument and design of the ionization source (Pico et al. 2004). Different studies that compares the behaviour of different pesticides, including organophosphorus in both ionization source, using the same design as that applied in the present study show that organophosphorus pesticides are more sensitive by APCI than ES (Fernandez et al. 2002b; Blasco et al. 2003; Fernandez et al. 2000; Fernandez et al. 2003; Thurman et al. 2001). The same studies compared sensitivity in positive and/or negative ionization modes showing that carbamates were not detected with the ESI source in the NI mode at acceptable levels (Fernandez et al. 2000; Fernandez et al. 2003). Organophosphorus insecticides ionized in both PI and NI. The most sensitive ionization mode depends on the structure of each particular compound (Fernandez et al. 2002b; Blasco et al. 2003;

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3 Thurman et al. 2001). These previous works cover the pesticides selected in the present  
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5 study and clearly shows that best agreement to determine simultaneously all these  
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7 compounds with proper sensitivity is the negative ionization mode (Fernandez et al.  
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9 2002b; Blasco et al. 2003; Fernandez et al. 2000; Fernandez et al. 2003).

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15 Few publications involving SPME for the purpose considered here have been found in  
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17 the literature (Yu et al. 2004; Volante et al. 2001; Capillo et al. 2006), despite the fact  
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19 that honey is an obvious subject for using SPME because its solubility in water. None of  
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21 the above articles used LC-MS, even through the last reviews published on the subject  
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23 clearly demonstrate that LC is currently competing with GC for the status of reference  
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25 analytical technique to determine pesticide residues. LC has been found be able to  
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27 determine more pesticides and at much higher sensitivity than GC. This comment is  
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29 also valid for organophosphorus pesticides, which are most often analyzed by GC  
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31 methods up to now (Alder et al. 2006).

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39 The results obtained are in agreement with other LC-MS studies of organophosphorus  
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41 pesticides, which demonstrated that despite the organophosphorus pesticides are  
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43 compounds that can be determined by gas chromatography, they can be also identified  
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45 and quantified by LC-MS with almost the same sensitivity (Hogendoorn & van Zoonen  
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47 2000; Pico et al. 2004; Cossu & Alamanni 2003; Fernandez et al. 2002b; Blasco et al.  
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49 2004a).

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55 The ions used in selected ion monitoring (SIM) have been reported in Table I. The most  
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57 intense ion was used for quantification and the second and third ion for confirmation, to  
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59 meet the criteria established by the EU to confirm the identity of a pesticide (European  
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3 Commission Directorate General Health and Consumer Protection 2004). According to  
4 this criterion, the presence of a target pesticide in a given matrix is demonstrated if,  
5 compared with a reference standard analyzed in the same experimental conditions, (i)  
6 the LC retention time agrees within 2 %, and (ii) the abundance of signals of  
7 quantification ion plus two confirmation ions agree within 20 %. Fenoxycarb and  
8 temephos gave an in-source collision induced dissociation spectra (CID) with only one  
9 confirmation ion. The in-source CID spectra of pesticides extracted from honey and  
10 directly desorbed from the fiber displayed an abundance of the ions signals within the  
11 reported criterion. However, it should be noted that differences, although within the  
12 confirmation criteria, were more accentuated for the confirmation ions with low relative  
13 abundance.

#### 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 *Optimization of the SPME sample treatment*

33 Conditions for the SPME absorption of the pesticides were optimized in honey samples  
34 spiked at  $0.1 \mu\text{g g}^{-1}$  of each studied pesticide and desorbed off-line. Initial conditions  
35 were 1 g of honey samples was dissolve in 5 mL of water by stirring for 10 min. The  
36 water volume was the only condition that was maintained through the experiments,  
37 because it is the minimum volume to be able to dissolve properly the sample. Then, the  
38 fiber was immersed in the sample for 90 min. The SPME-LC off-line desorption of  
39 pesticides in honey samples was achieved by exposing the fiber to 1 mL of methanol for  
40 15 min in an ultrasonic bath. This volume enables the fiber coating to be immersed  
41 completely in the solvent.

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58 Extraction efficiencies of three PDMS of different thick coatings, PA, CW/TPR, and  
59 PDMS/DVB fibers, were evaluated and compared in order to select the best fiber. The  
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3 relative extraction efficiency of the target analytes on each fiber are reported in Fig. 1,  
4 showing that organophosphorus insecticides were extracted to a different extent  
5 depending on the nature of the polymeric coating. The PDMS/DVB fiber was the most  
6 effective in concentrating pesticides, and PA coating was the less effective. The addition  
7 of saturate NaCl (up to  $350 \text{ mg mL}^{-1}$ ) to the extraction solution, which changes the ionic  
8 strength of the aqueous solution and may change the partitioning of the analytes into the  
9 fiber, was evaluated for the six fibers. There was no measurable effect on the recoveries,  
10 except a modest improvement of the extraction efficiency of PA for profenofos. The  
11 PDMS/DVD coating was selected for further experiments because it gave satisfactory  
12 extraction efficiencies for all the pesticides which were studied and the adjustment of  
13 the ionic strength was not further considered.  
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32 Suspended matter as well as dissolved compounds could be responsible for interference  
33 on sample extraction by SPME by the absorption of analytes, forming micelles and/or  
34 interfering with diffusion (hindering to analytes to reach the fiber). The results of  
35 recovery test using different amounts of sample are shown in Fig. 2. The recovery was  
36 much better using low amounts of samples, but acceptable values (between 19 and 85  
37 %) are achieved using 1.5 g of sample. This was a good compromise between recovery  
38 and amount of sample to be processed, which should be great for a trace analysis to  
39 obtain appropriate limits of detection.  
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53 Fig. 3 shows the extraction efficiency profiles obtained by plotting the peak area versus  
54 the extraction times. As can be seen, extraction times  $> 120 \text{ min}$  were necessary to reach  
55 the equilibrium. After 120 min,  $\geq 85 \%$  of the equilibrium concentration was reached.  
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60 This extraction time was considered an appropriate conciliation between extraction

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3 efficiency obtained and analysis time. Under the equilibrium conditions, SPME  
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5 partitioning has been correlated with octanol-water distribution coefficients ( $K_{ow}$ ); log  
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7  $K_{ow}$  values ranged from 3.3 (diazinon) to 6.5 (bromophos ethyl). However, honey  
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9 matrix decreases the extraction recovery, which indicates a disturbance of the extraction  
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11 process. In this way, the experimental results obtained for extraction recovery do not  
12  
13 correspond with log  $K_{ow}$  values (i.e. diazinon vs bromophos-ethyl). One reported  
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15 explanation (Yu et al. 2004) is that compared with water, diffusion coefficients are  
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17 smaller in the more viscous sugar solutions. However, the lower diffusion coefficients  
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19 would affect the rate for reaching equilibrium but not the distribution between sample  
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21 and fiber. Another more realistic explanation is that suspended matter and dissolved  
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23 compound from the matrix block the analytes by different mechanisms decreasing the  
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25 recoveries, as has been discussed in the optimization of the amount of sample (Albero et  
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27 al. 2004; Albero et al. 2001; Blasco et al. 2004a).

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36 The desorption conditions by the SPME-LC commercial interface were optimized to  
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38 obtain the best possible detection limits. The optimization of the desorption conditions  
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40 is the most critical step in SPME-LC. Dynamic desorption mode, which ensures  
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42 quantitative recoveries, causes a significant increase of peak width and peak asymmetry  
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44 compared to conventional loop injection (20  $\mu$ L), deteriorating the chromatographic  
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46 efficiency and resolution. Thus, a static desorption technique was evaluated as a  
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48 possible alternative. Desorption was performed with different methanol water mixtures.  
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51 Fig. 4 displays the dynamic and static desorption as well as the effect of methanol  
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53 percentage in the desorption mixture when the static mode is used. The best resolution  
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55 was achieved using methanol/water (70:30, v/v). Other proportions provided wide and  
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57 badly resolved peaks. The dynamic mode gave bad peak-shapes in spite of the initial  
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3 composition of the mobile phase containing the optimal proportion of methanol. The  
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5 fiber was soaked in the static mobile phase contained in the desorption chamber (60  $\mu\text{L}$   
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7 volume) for a variable period of time before injection into the LC column. The best  
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9 conditions were reached after 15 min of static desorption followed by injection into the  
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11 mobile phase stream. The peak shape observed for conventional loop injection was now  
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13 quite well preserved.  
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#### 16 17 18 19 20 *Performance of the SPME-LC-MS method*

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22 Recovery, repeatability, and detection and quantification limits using fortified honey  
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24 samples are reported in Table II. Recoveries of the 12 organophosphorus insecticides  
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26 were in the range of 19-92 % (n=5). The intraday precision was better than 19 % at the  
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28 LOQ levels and between 6-14 % at 10 LOQ levels. Limits of detection (LODs) were  
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30 between 0.001 and 0.1  $\mu\text{g g}^{-1}$  and limits of quantification (LOQs) between 0.005 and 0.5  
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32  $\mu\text{g g}^{-1}$ . LODs and LOQs have been dictated by the signal intensity of the less abundant  
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34 ion to ensure a proper confirmation of the pesticide identity prior to quantify it.  
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41 The whole analytical procedure using SPME combined with LC-MS was tested for  
42  
43 linearity in the range of LOQs to 100 times LOQs. As shown in Table III, a linear  
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45 relationship was obtained for each pesticide in this range (six points calibration) from  
46  
47 water and honey by SPME-LC. Great differences in the regression equations between  
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49 water and honey were found, which is reasonable since the differences in the recovery  
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51 between water and honey are also important.  
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57 The proposed method showed appropriate linearity, precision (relative standard  
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59 deviation (RSDs) less than 19 %), and high sensitivity, in-line with the international  
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3 criteria for validation (European Commission Directorate General Health and Consumer  
4 Protection 2004; European Commission Directorate General Health and Consumer  
5 Protection 2000).  
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12 SPME of pesticides in honey samples showed, in most cases, low recoveries that can be  
13 explained by the complexity of the matrix and the interactions between analytes and  
14 matrix components, as it has previously been commented. SPME of organophosphorus  
15 pesticides in fruit and fruit juices without clarify gave small recoveries (<10 %) (Yu et  
16 al. 2004; Blasco et al. 2004a). Low recovery does not necessarily imply insufficient  
17 precision of the method. However, the matrix variability forces to construct the  
18 calibration curve by using blank honey samples (free from the analytes to be quantified)  
19 spiked with analytical standards of the target pesticides at different concentrations.  
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34 The optimized procedure was applied to the analysis of several real samples of different  
35 origin. The samples were analyzed in triplicate and each of the replicates was injected  
36 twice. Table IV shows the pesticides and the concentrations found. Of the 26 samples  
37 analyzed, pesticide residues were detected in six samples (25 %). Diazinon was the  
38 most frequently detected pesticide. The detection of several of the investigated  
39 compounds in real samples confirms the need for monitoring these residues in honey  
40 and other bee products. These finding are in concordance with the most recent studies of  
41 pesticide residues content in Spanish honey samples (Blasco et al. 2004b; Jimenez et al.  
42 2002; Herrera et al. 2005; Albero et al. 2004).  
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3 Fig. 5 illustrates a typical chromatogram of an unspiked honey sample, a spiked honey  
4 sample at LOQ levels, and a real sample containing diazinon. The peaks showed up  
5 nicely, and the background noise was low.  
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### 10 11 12 *Comparison of methods*

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14 For analyzing pesticide residues in honey, SPME has been already tested in a few  
15 studies with recoveries comparable to those obtained in the present study (Capillo et al.  
16 2006; Volante et al. 2001; Yu et al. 2004). The significantly lower recoveries in honey  
17 solution compared to water were also noted in these studies (Capillo et al. 2006;  
18 Volante et al. 2001; Yu et al. 2004). Traditional sample treatment methods are almost  
19 invariably based on solvent and solid-phase extraction followed, sometimes, by various  
20 cleanup steps. These method provided higher recoveries (Blasco et al. 2003; Fernandez  
21 et al. 2002b; Fernandez et al. 2002a) but SPME is a relatively easy and fast technique  
22 for the analysis of pesticides present in trace amounts and presents the advantage of  
23 avoiding the use of organic solvents (Capillo et al. 2006; Volante et al. 2001; Yu et al.  
24 2004).

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44 Most SPME were carried out in combination with GC with different detectors (Capillo  
45 et al. 2006; Yu et al. 2004; Volante et al. 2001). However, the method described here is  
46 based on separation and determination by LC-MS, which allows to increase the number  
47 of pesticides analyzable because LC-MS is able to determine any compound  
48 independently of its volatility and thermolability (Pico et al. 2004).  
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There is one previous study that compares SBSE and SPME for determining six  
organophosphorus pesticide residues. In that study, SPME enrichment was performed

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3 using a poly(dimethylsiloxane) (PDMS) fiber to compare both techniques in the same  
4 conditions (Blasco et al. 2004a). However, PDMS is not the optimum coating to adsorb  
5 organophosphorus pesticides because recoveries are between 3.2 to 7.6 %. In the  
6 present work, the fiber coating selected was tested and the method extracts substantially  
7 large amount of the analytes from honey (from 19 to 92 %). In addition, the previous  
8 study desorbs the analytes from the fiber in 1 mL of methanol and 5  $\mu\text{L}$  of this extract  
9 were injected in the LC-MS systems, attaining LOQs between 0.8 and 2  $\mu\text{g g}^{-1}$ . The  
10 present study directly desorbs all the analytes adsorbed in the fiber in the LC systems by  
11 means of a commercial six port-valve device. This system allows to obtain LOQs from  
12 0.005 to 0.5  $\mu\text{g g}^{-1}$ , which is around hundred times lower for most of the studied  
13 compounds. These low LOQs document the effectiveness of this method in measuring  
14 trace levels of pesticide residues.  
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## 34 **Conclusions**

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36 A method combining SPME with LC-MS that directly desorbs the analytes from the  
37 fiber to the LC system by means of a commercial device has been developed for first  
38 time. A PDMS/DVB coated fiber ensures good extraction efficiency, whereas the static  
39 desorption mode ensures the best compromise in terms of analyte transfer efficiency and  
40 preservation of chromatographic efficiency. When SPME and analysis by LC-MS are  
41 combined, a highly sensitive and selective analytical procedure is obtained for  
42 multiresidue screening of pesticides in complex food samples such as honey. Additional  
43 advantages, derived from easy removal of sugar and extraction solvents providing  
44 enhanced protection to the LC-MS system, are also characteristics of the proposed  
45 procedure.  
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3 Data obtained in real samples indicate the importance of analytical control methods for  
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5 honey and the need of further studies of contaminant sources in relation to food safety,  
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7 the protection of the environment, and the management of agricultural resources.  
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### 10 11 12 **Acknowledgements**

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16  
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**Figure captions.**

Figure 1. Relative extraction efficiencies (recovery %) of the studied insecticides by each fiber (1 g honey/5 mL water).

Figure 2. Relative extraction efficiencies (recovery %) of the studied insecticides using different amount of honey using the PDMS/DVB fiber.

Figure 3. Variation of the peak areas with the extraction time using the PDMS/DVB (1.5 g/5 mL water).

Figure 4. Chromatograms obtained from different desorption modes and mixtures. (A) dynamic mode, (B) static mode using methanol-water (90:10 v/v) as desorption mixture, (C) static mode using methanol-water (70:30, v/v) as desorption mixture. Peak identification: (1) fenoxycarb, (2) phenthoate, (3) fonofos, (4) diazinon, (5) Phosalone, (6) pyrazophos, (7) chlorpyrifos methyl, (8) profenofos, (9) pirimifos methyl, (10) temephos, (11) bromophos ethyl, (12) chlorpyrifos ethyl. Honey samples spiked at 10 times LOQ.

Figure 5. Chromatograms of (A) honey spiked at LOQ levels, (B) unspiked honey that does not contain any of the studied pesticides, and (C) sample that contains 17.2 ng g<sup>-1</sup> of diazinon. Peak identification as in Figure 4.

Figure 1. Relative extraction efficiencies (recovery %) of the studied insecticides by each fiber(1 g honey/5 mL water).

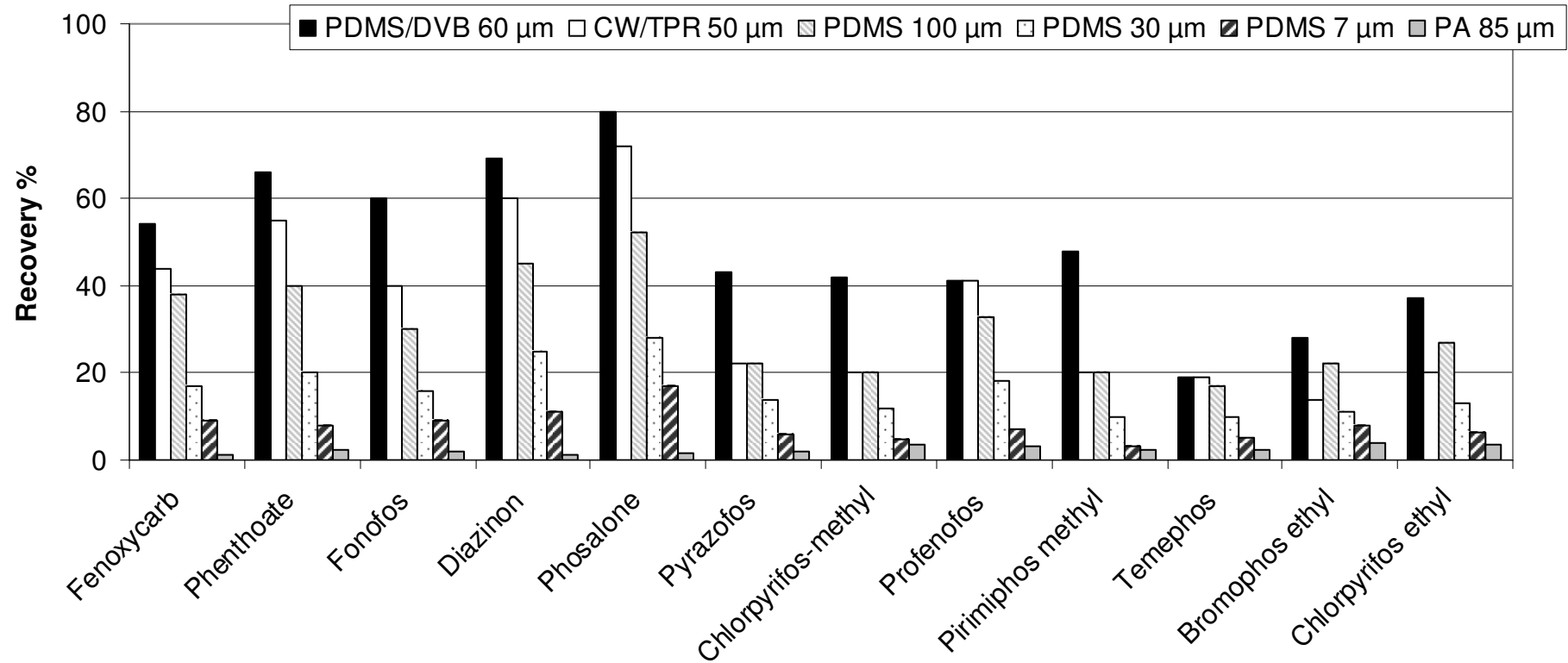


Figure 2. Relative extraction efficiencies (recovery %) of the studied insecticides by different amount of honey using the PDMS/DVB fiber.

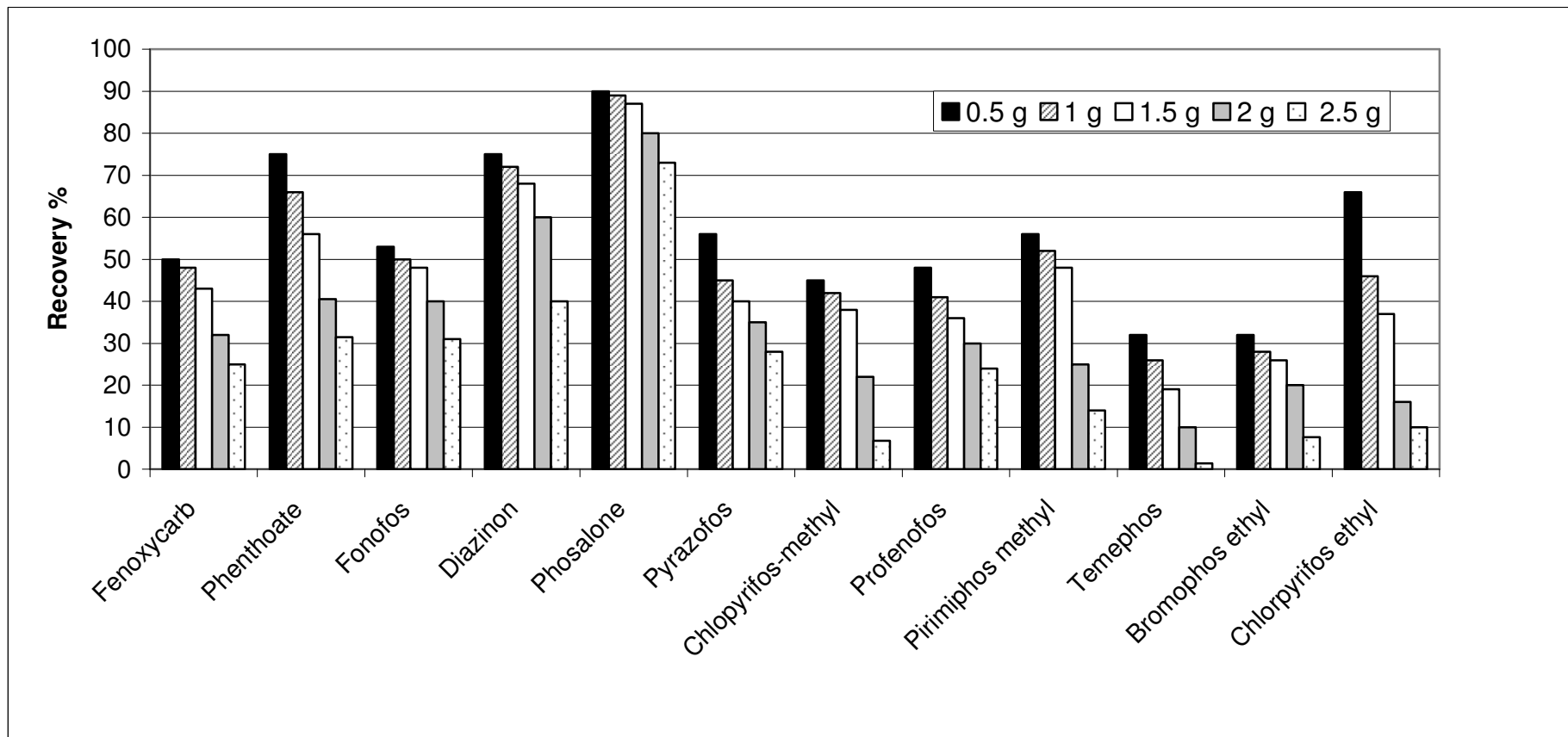


Figure 3. Variation of the peak areas with the extraction time using the PDMS/DVB (1.5 g honey/5 mL water).

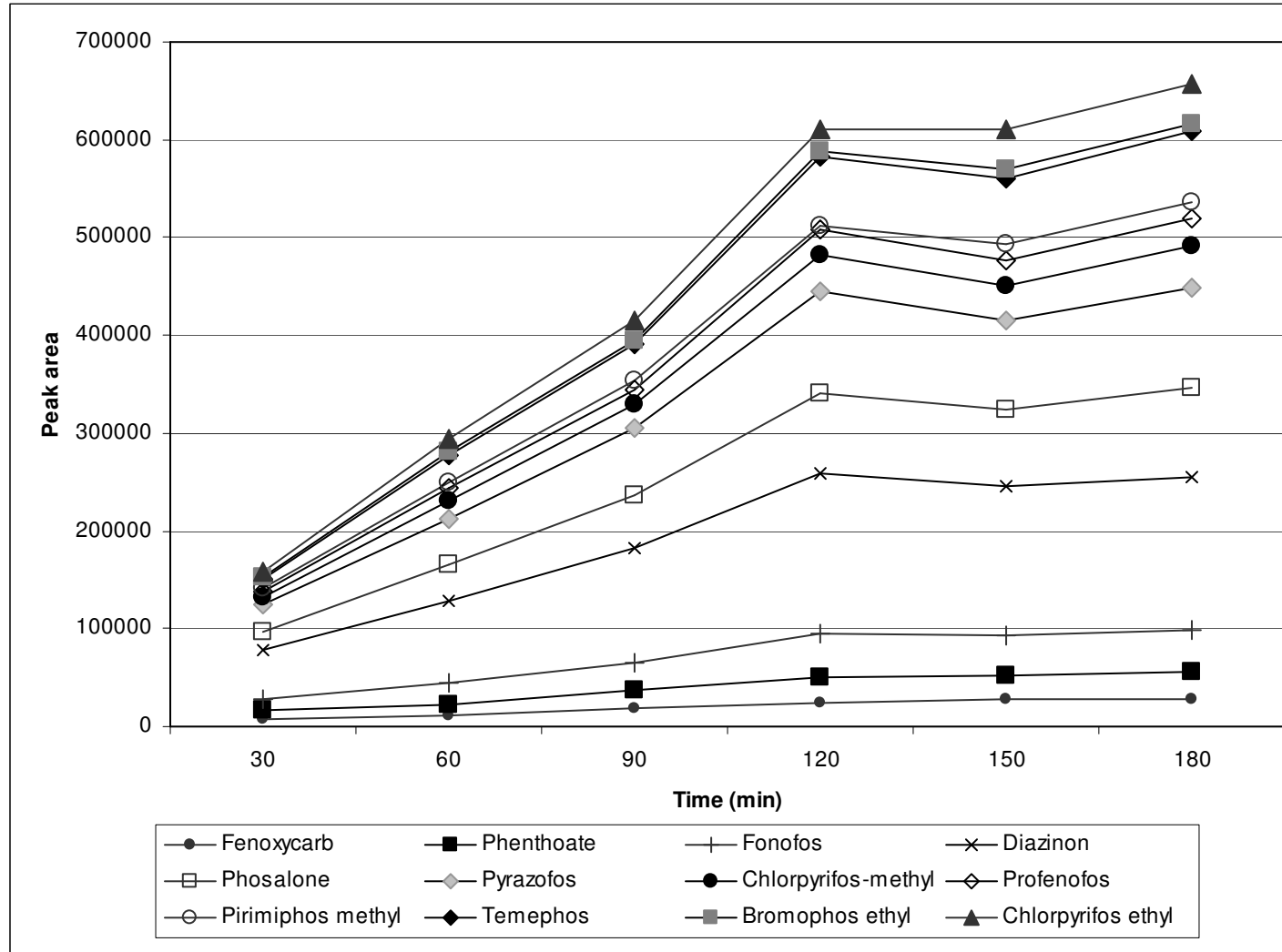


Figure 4. Chromatograms obtained from different desorption modes and mixtures. (A) dynamic mode, (B) static mode using methanol-water (90:10 v/v) as desorption mixture, (C) static mode using methanol-water (70:30, v/v) as desorption mixture. Peak identification: (1) fenoxycarb, (2) phenthoate, (3) fonofos, (4) diazinon, (5) Phosalone, (6) pyrazophos, (7) chlorpyrifos methyl, (8) profenofos, (9) pirimifos methyl, (10) temephos, (11) bromophos ethyl, (12) chlorpyrifos ethyl. Honey samples spiked at 10 times LOQ.

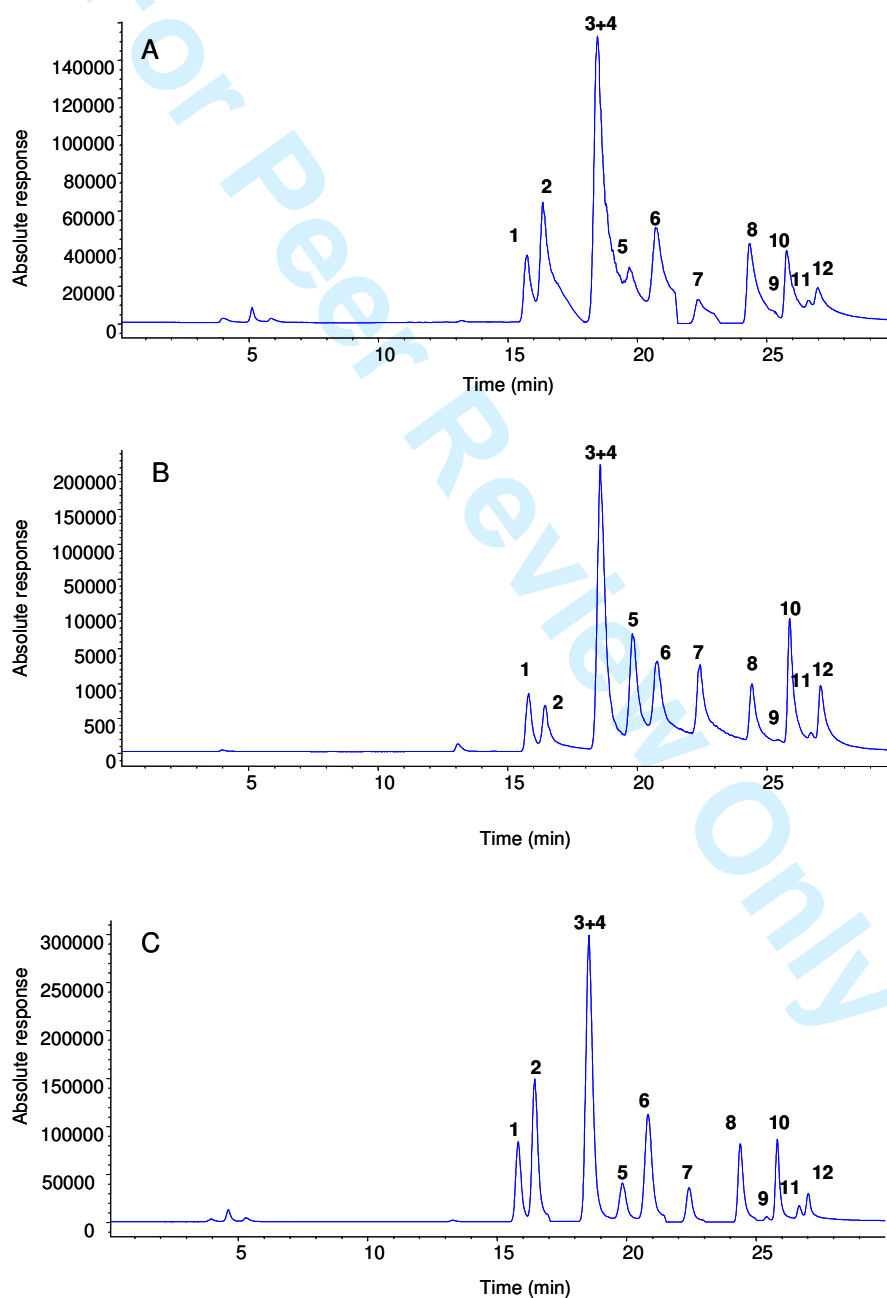


Figure 5. Chromatograms of (A) honey spiked at LOQ levels, (B) unspiked honey that does not contain any of the studied pesticides, and (C) sample that contains 17.2 ng g<sup>-1</sup> of diazinon. Peak identification as in Figure 4.

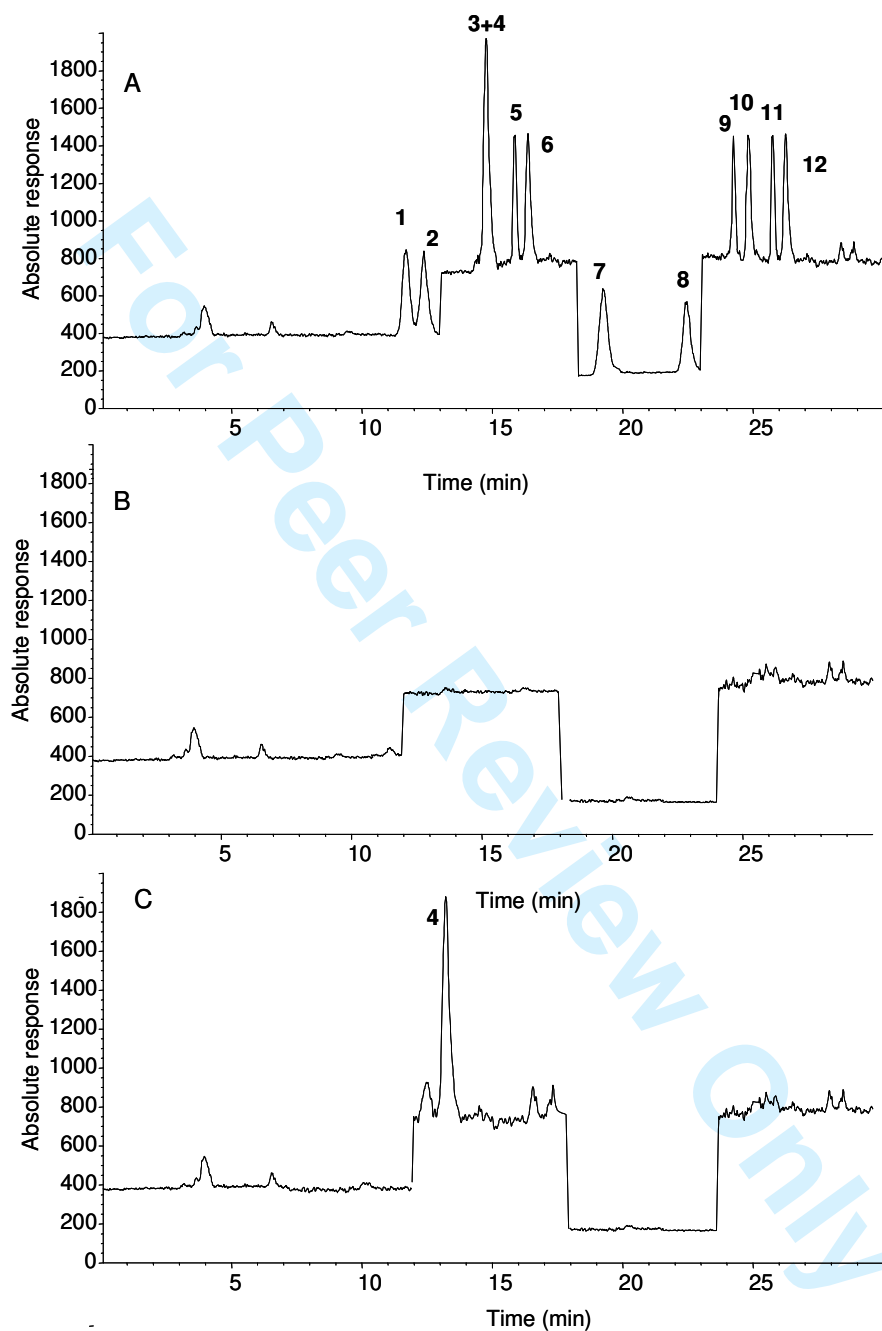


Table I. Time scheduled SIM conditions for monitoring the studied insecticides.

Pesticide	Time window (min)	Compound retention time (min)	Quantification ion, <i>m/z</i> (relative abundance)	Confirmation ions, <i>m/z</i> (V) (relative abundance)	Fragmentor	Dwell time (ms)
Fenoxycarb	0-18	16.154	185 (100)	300 (15)	40	199
Phenthoate		16.796	319 (100)	110 (70), 157 (12)		199
Fonofos	0-22	19.016	153 (100)	137 (52), 109 (68)	60	98
Diazinon		19.016	275 (100)	159 (40), 151 (40)		98
Phosalone		20.317	338 (100)	185 (80), 142 (40)		98
Pyrazophos		21.215	372 (100)	157 (24), 125 (62)		98
Chlorpyrifos methyl	22-24	22.735	302 (100)	304 (66), 141 (70)	60	400
Profenofos	24-25	24.559	207 (100)	343 (50), 345 (63)	40	400
Pirimiphos ethyl	25-30	25.589	304 (100)	180 (50), 169 (10)	70	98
Temephos		26.050	451 (100)	217 (17)		98
Bromophos ethyl		26.873	351 (100)	363 (30), 239 (30)		98
Chlorpyrifos ethyl		27.228	330 (100)	332 (66), 169 (73)		98

Table II. Performance of the SPME-LC-MS method for the studied insecticides in honey samples

Compound	LOD ( $\mu\text{g/g}$ ) <sup>1</sup>	LOQ ( $\mu\text{g/g}$ ) <sup>2</sup>	Recovery (%) ( $X \pm \text{RSD}, n=5$ )	10 LOQ ( $\mu\text{g/g}$ )	Recovery (%) ( $X \pm \text{RSD}, n= 5$ )
noxy carb	0.03	0.1	44 $\pm$ 12	1	47 $\pm$ 9
enthoate	0.007	0.02	56 $\pm$ 10	0.2	59 $\pm$ 8
no Fos	0.003	0.01	50 $\pm$ 14	0.1	56 $\pm$ 10
azinon	0.004	0.01	70 $\pm$ 8	0.2	69 $\pm$ 6
osalone	0.07	0.2	89 $\pm$ 6	2	92 $\pm$ 6
razophos	0.001	0.005	43 $\pm$ 10	0.05	45 $\pm$ 8
ldpyrifos methyl	0.1	0.5	38 $\pm$ 15	5	40 $\pm$ 12
ofenofos	0.02	0.05	41 $\pm$ 13	0.5	43 $\pm$ 10
rimphos ethyl	0.003	0.01	48 $\pm$ 12	0.1	50 $\pm$ 8
mephos	0.001	0.005	19 $\pm$ 19	0.05	21 $\pm$ 14
onophos ethyl	0.003	0.01	28 $\pm$ 17	0.1	34 $\pm$ 11
ldpyrifos ethyl	0.003	0.01	37 $\pm$ 16	0.1	43 $\pm$ 11

<sup>1</sup>LODs (S/N=3) were calculated considering the ion of those reported in Table I giving the worst S/N for each analyte.

<sup>2</sup>LOQs (lower amount of pesticide that provided acceptable recoveries and repeatabilities (< 20 %)) were calculated for the lowest of the confirmation ions

Table III. Data on regression equations for the 12 pesticides extracted from water and honey by SPME/LC/MS

	Concentration added ( $\mu\text{g g}^{-1}$ )*	Water Equation**	Correlation coefficient ( <i>r</i> )	Honey Equation**	Correlation coefficient
carb	0.1-10	$y = 12244 x + 769$	0.998	$y = 4308 x + 3664$	0.992
ate	0.02-2	$y = 343395 x + 16872$	0.998	$y = 101303 x + 107803$	0.998
	0.01-1	$y = 110795 x + 1219$	0.999	$y = 54482 x + 557$	0.999
n	0.015-1.5	$y = 485473 x + 52649$	0.993	$y = 354362 x + 20095$	0.999
ne	0.2-20	$y = 262085 x + 50933$	0.999	$y = 75079 x + 2053$	0.994
hos	0.005-0.5	$y = 1954618 x + 4620$	0.999	$y = 837433 x + 1476$	0.993
trifos methyl	0.5-50	$y = 115148 x + 12109$	0.998	$y = 86076 x + 5643$	0.999
fos	0.05-5	$y = 97272 x + 17236$	0.997	$y = 38788 x + 8671$	0.994
nos ethyl	0.0125-1.25	$y = 937249 x + 85186$	0.992	$y = 480939 x + 10212$	0.999
os	0.005-5	$y = 35756 x + 415185$	0.990	$y = 8286 x + 38262$	0.999
hos ethyl	0.01-10	$y = 215350 x + 3869.6$	0.999	$y = 68102 x + 13300$	0.999
trifos ethyl	0.01-10	$y = 344646 x + 135280$	0.999	$y = 179149 x + 53872$	0.999

\* Concentration in honey samples that correspond at an amount of pesticide between 7.5 ng and 15  $\mu\text{g}$  dissolve in 5 mL of water.

\*\*The data were subjected to linear regression analysis of peak area (*y*) of the compound against the spiked concentration (*x*). For the equations, 6 plots (each point represents the mean of duplicate determinations) with different concentrations for each compound were used.

Table IV. Pesticide levels found in honey samples

Sample	Pesticide	Level (ng g <sup>-1</sup> ) ( $\bar{X} \pm \text{SD}$ , $n=3$ )
Orange blossom 1	Temephos	7.2 ± 0.9
Orange blossom 2	Fonofos	15 ± 2.2
	Pyrazophos	6.2 ± 1.2
Rosemary	Diazinon	17.2 ± 2.8
Lemon 1	Diazinon	12.4 ± 1.1
Lemon 2	Pirimiphos ethyl	19 ± 3.0
Lavander	Bromophos ethyl	11.5 ± 1.2