Analysis of Organophosphorus Pesticides in Honeybee by Liquid Chromatography−Atmospheric Pressure Chemical Ionization−Mass Spectrometry

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Pesticides applied in extended agricultural fields may be controlled by means of bioindicators, such as honeybees, in which are the pesticides bioaccumulate. Liquid chromatography−atmospheric pressure chemical ionization−mass spectrometry (LC-APCI-MS) experiments with positive (PI) and negative (NI) ion modes were optimized for the analysis of 22 organophosphorus pesticides in honeybee samples. The extraction required 3 g of sample, which was extracted with acetone. The extract was purified with coagulating solution and reextracted with Cl_2CH_2. Pesticides studied could be detected by both ionization modes except for parathion, parathion-methyl, and bromophos, which did not give signals in PI mode, and triazophos, which was not detected in NI mode. Fragmentation voltage and vaporizer temperature were optimized to achieve the highest sensitivity. The spectra profile of each pesticide in PI mode showed the $[M + H]^+$ ion as the main signal, whereas in NI mode only fragment ions were shown. The detection limit obtained in selected ion monitoring mode ranged from 1 to 15 μg kg^{-1}. The average recoveries from spiked honeybees at various concentration levels (0.5−5 mg kg^{-1}) exceeded 65% with relative standard deviations of 4−15%. The method was applied to real samples, in which residues of coumaphos and dimethoate were detected.

Keywords: *Mass spectrometry; organophosphorus pesticides; liquid chromatography; atmospheric pressure chemical ionization; honeybee*

INTRODUCTION

Determination of organophosphorus (OPP) pesticide residues in food and vegetables is a matter of public concern because these pesticides are the most widely applied and their residues constitute a potential risk to human health (*1*). Honeybees (*Apis mellifera*) are subjected to an intensive and continuous hazard from pesticide poisoning; during the pollination of agricultural crops, they are in contact with these compounds, which are retained and bioaccumulated in their bodies. Because of this, pesticide residues found in bees reflect the type of pesticides applied in the cultivated fields that surround their hives (*2*, *3*). Moreover, some OPPs such as coumaphos and malathion are used against *Varroa jacobsoni*, a parasitic mite that affects honeybee colonies. To determine OPP residues in bees, a reliable and sensitive method for detecting these pesticides is necessary.

Liquid chromatography (LC) has been mainly focused on thermolabile, polar, and low-volatile pesticides that are not suitable for gas chromatography (GC) analysis. However, recent developments in detection and column material technology have enlarged LC's scope to other analytical fields as typical "GC pesticides" (*4*). Among the commercially available interfaces for coupling mass spectrometry (MS), atmospheric pressure chemical ionization (APCI) has become accepted as a robust, sensitive, and versatile interface for the analysis of different compounds (*5*). APCI is a soft ionization technique, which yields the quasi-molecular ions providing molecular weight information with an excellent sensitivity. In addition, structural information can be obtained by collision-induced dissociation (CID), and it makes possible the identification with high specificity.

These features have made LC-APCI-MS a key element in the identification of pesticides and their degradation products in different matrices such as biological samples (*6*), fruits and vegetables (*7*, *8*), and ground water (*9*, *10*).

Honeybees are considered to be a complex matrix because the presence of wax residues adhered to their bodies may lead to important chromatographic interferences. Different procedures have been reported for pesticide analysis in honeybees. Liquid−liquid extraction (LLE) is still the preferred technique (*11*, *12*) in contrast to solid phase extraction, matrix solid phase dispersion (*13*), or supercritical fluid extraction (*14*), which have been scarcely reported. The extracts obtained have been properly analyzed using either GC with specific detectors such as electron capture detection (ECD), nitrogen phosphorus detection (NPD), or mass spectrometry (MS) or LC using UV or fluorescence detection. Despite all of the advantages mentioned above, the capabilities of the LC-MS, and the fact that the methods based on LC-MS determination have been recommended by the U.S. FDA, analysis of pesticides in honeybees using LC-MS has not been described until now.

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Table 1. Classification of OPP Pesticides According to Their Chemical Structures and Ionization Modes in LC-APCI-MS

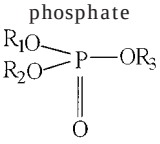
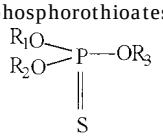
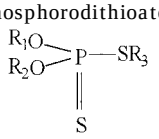
	pesticides		
	phosphate 	phosphorothioates 	phosphorodithioates 
mode of detection	O	S	S
only in PI mode		triazophos	
only in NI mode		parathion parathion-methyl bromophos	
more sensitive in PI mode	heptenophos	diazinon pirimiphos-methyl pirimiphos-ethyl quinalphos vamidothion	dimethoate malathion phosmet phenthoate fonofos
more sensitive in NI mode	paraoxon	coumaphos pyrazophos chlorpyriphos-methyl	azinphos-ethyl phenthoate methidathion phosalone

Table 2. Selected Ion Program for OPP Pesticides in SIM Mode

	positive mode			negative mode			
retention window (min)	selected ion monitoring (m/z)	dwell time (ms)	fragmentor (V)	selected ion monitoring (m/z)	dwell time (ms)	fragmentor (V)	pesticides
0−5.2	230, 288	199	40	214, 272	199	50	dimethoate, vamidothion
5.2−10	248	400	60	232	400	30	paraoxon
10−17.5	145, 160, 251	132	30	287, 157, 235	132	60	methidathion, phosmet, heptenophos
17.5−20.5	285	400	70	157, 138	199	80	malathion, parathion-methyl
20.5−23	314	400	20	185	400	50	triazophos, azinphos-ethyl
23−30	346	400	40				
30−37	299, 321	199	40	169, 319, 262	132	50	quinalphos, phenthoate, parathion
37−44.5	305, 247, 363	132	80	275, 153, 361	132	40	diazinon, fonofos, coumaphos
44.5−52	306, 368, 374, 322	98	40	290, 338, 372, 302	98	50	pirimiphos-methyl, phosalone, pyrazophos, chlorpyriphos-methyl
52−56	334	400	60	304, 351	199	50	pirimiphos-ethyl, bromophos

This paper reports a method for determining OPP pesticides in honeybees. It involves a rapid LLE procedure and LC coupled, via an APCI source, to mass selective detection. This work also discusses the APCI-MS spectra characteristic of the 22 OPP pesticides studied.

MATERIALS AND METHODS

Reagents and Chemicals. Pesticides (listed in Table 1) were purchased from Aldrich (Madrid, Spain) with a minimum certified purity of 98%. Stock solutions of each OPP pesticide at 1000 mg L^{-1} were prepared in methanol and stored at 4 °C in stoppered glass bottles. Methanol, dichloromethane, and acetone for HPLC analysis were from Merck (Darmstadt, Germany). Deionized water of <18 MΩ·cm resistivity was obtained from a Milli-Q water purification system. Granular anhydrous sodium sulfate, sodium chloride, and orthophosphoric acid 85% (v/v) analysis grade were from Panreac (Barcelona, Spain). Diatomaceous earth was supplied by Sigma (Steinheim, Germany). Sample lyophilization was carried out with a Drywinner Heto1.0-60/CT 60 cooling trap (Allerod, Denmark).

Extraction Procedure. Three grams of lyophilized honeybees previously pounded in a glass mortar was placed in a 250 mL flask and shaken vigorously during 10 min with 100 mL of acetone. The mixture was filtered through a Büchner funnel packed with a layer of Celite (∼5 mm). A coagulate solution of 1% (w/v) ammonium chloride and 2% (v/v) orthophosphoric acid was added to the filtrate, allowed to stand for 30−40 min with occasional stirring, and then filtered. The filtrate was diluted with 200 mL of 2% aqueous NaCl (w/v) and extracted twice with 100 mL of dichloromethane. The organic extracts were passed through anhydrous sodium sulfate and evaporated to ∼10 mL in a rotary evaporator at 35 °C. Five milliliters of methanol was added, the mixture was evaporated to 5 mL using a gentle stream of nitrogen, and of this solution, 5 μL was injected into the LC-MS system.

Spiked samples were prepared by adding volumes between 300 and 150 μL of the standard working solution of 10 and 1 μg mL^{-1} to honeybee samples. They were allowed to stand at room temperature for 1 h.

LC-APCI-MS. The equipment used was a Hewlett-Packard (Palo Alto, CA) HP-1100 series LC-MS detector system equipped with an autosampler, a binary solvent pump, and an MS detector consisting of a standard API source that can be configured as APCI and ES.

The chromatographic separation was accomplished by water/methanol gradient at a flow rate of 1 mL min^{-1}. The gradient was programmed from 60 to 65% of methanol in 10 min, held during 30 min, and then raised to 80% of methanol in 5 min. The analytical column was a Spherisorb C_{18} (250 × 4.6 mm i.d., 5 μm particle diameter), and the guard column was a LiChrosorb RP-18 (10 × 4.6 mm, 5 μm), both from Supelco (Madrid, Spain).

The APCI source conditions in PI mode were as follows: vaporizer temperature, 350 °C; nebulization gas (nitrogen) pressure, 4.0 bar; drying gas (nitrogen) flow rate, 4 L min^{-1}; drying gas temperature, 350 °C; capillary voltage, 4000 V; and corona current, 4 μA. The experimental conditions of the APCI in NI mode were the same as those reported in PI mode but with a corona current of 25 μA.

Optimization of Analytical Parameters. Mass spectra were collected in full-scan mode from m/z 100 to 450 (cicle time = 0.42 s/cycle, interscan time = 0.1 s). The effect of vaporizer temperature and extraction voltage on ion abundance and fragmentation were studied by injecting 50 ng of each pesticide by flow injection analysis (FIA). Time-scheduled selected ion monitoring (SIM) of the most abundant ion of each compound used for quantification is shown in Table 2.

Table 3. Characterization of OPP Pesticides with LC-APCI-MS PI Mode at Different Fragmentor Voltages

				abundance (%)			
compound	MW	*m/z*	tentative identification	20 V	40 V	60 V	80 V
azinphos-ethyl	345	346	$[M + H]^+$	100	99	14	
		318	$[M - CH_2CH_3 + 2H]^+$		16	12	10
		289	$[M - (CH_2CH_3)_2 + 2H]^+$	4	68	62	44
		261	$[M - (CH_2CH_3)_2 - CO + 2H]^+$			24	40
		160	$[M - PS_2(OCH_2CH_3)_2]^+$	22	20		
		153	$[PS(OCH_2CH_3)_2]^+$			26	25
		132	$[C_6H_4N_3CH_2]^+$	34	100	100	100
		105	$[C_6H_4N_2 + H]^+$				25
chlorpyriphos-methyl	321	322	$[M + H]^+$	100	100	100	50
		125	$[PS(OCH_3)_2]^+$			30	100
coumaphos	362	363	$[M + H]^+$	100	100	100	100
diazinon	304	305	$[M + H]^+$	100	100	100	100
		277	$[M - CH_2CH_3 + 2H]^+$			6	11
		153	$[PS(OCH_2CH_3)]^+$	8	7	12	15
dimethoate	229	230	$[M + H]^+$	100	100	86	20
		199	$[PS_2(OCH_3)_2CH_2CO]^+$		30	100	100
		171	$[PS_2(OCH_3)_2CH_2]^+$				48
		125	$[PS(OCH_3)_2]^+$				50
fonofos	246	247	$[M + H]^+$	100	100	100	40
		137	$[PSO(CH_2CH_3)_2]^+$		8	66	100
		109	$[PSO(CH_2CH_3) + H]^+$			6	47
heptenophos	250	251	$[M + H]^+$	100	100	86	26
		127	$[M - PO_2(OCH_3)_2 + 2H]^+$	6	12	100	100
malathion	330	331	$[M + H]^+$	100	100	56	12
		285	$[M - OCH_3CH_2]^+$		42	100	55
		127	$[M - (CH_3O)_2PS_2 - C_2H_6O]^+$		10	80	100
methidathion	302	303	$[M + H]^+$	100	47		
		177	$[M - PS(O\ CH_3)_2]^+$	34	17	10	11
		145	$[M - PS_2(O\ CH_3)_2]^+$	22	100	100	100
		119	$[C_2H_3SN_2O_2]^+$	17	12		23
paraoxon-methyl	247	248	$[M + H]^+$	100	100	100	100
		141	$[C_6H_6OHNO_2]^+$	35	28	25	20
		109	$[PO(OCH_3)_2]^+$				10
phenthoate	320	321	$[M + H]^+$	100	100	60	
		275	$[M - OCH_2CH_3]^+$		28	80	20
		247	$[M - COOCH_2CH_3]^+$			94	90
		163	$[M - PS_2(OCH_3)_2]^+$			100	100
		135	$[M - PS_2(OCH_3)_2 - CH_2CH_3 + H]^+$				52
		125	$[PS(OCH_3)_2]^+$				32
phosalone	367	368	$[M + H]^+$	100	100	76	10
		322	$[M - OCH_2CH_3]^+$			12	10
		182	$[M - PS_2(OCH_2CH_3)_2]^+$		12	100	100
		153	$[PS(OCH_2CH_3)_2]^+$				16
		144	$[C_6H_3ClNH_4O]^+$		16	12	
phosmet	317	318	$[M + H]^+$	100	100	30	
		160	$[M - PS_2(OCH_3)_2]^+$	10	45	100	100
pyrazophos	373	374	$[M + H]^+$	100	100	100	100
		222	$[M - PS(OCH_2CH_3)_2 + 2H]^+$	10	11	14	17
pirimiphos-ethyl	333	334	$[M + H]^+$	100	100	100	100
		182	$[M - PS(OCH_2CH_3)_2 + 2H]^+$	10	10	12	18
pirimiphos-methyl	305	306	$[M + H]^+$	100	100	100	100
		182	$[M - PS(OCH_3)_2 + 2H]^+$	25	28	32	30
quinalphos	298	299	$[M + H]^+$	100	100	100	100
		271	$[M - CH_2CH_3 + 2H]^+$				15
triazophos	313	314	$[M + H]^+$	100	100	100	100
		162	$[M - PS(OCH_2CH_3)_2 + 2H]^+$	11	9	14	23
vamidothion	287	288	$[M + H]^+$	100	100	90	18
		180	$[M - PO(OCH_3)_2 + 2H]^+$	22	20	14	
		146	$[M - PSO(OCH_3)_2]^+$	3	19	100	100
		120	$[M - PSO(OCH_3)_2C_2H_4 + 2H]^+$	7	8	16	17

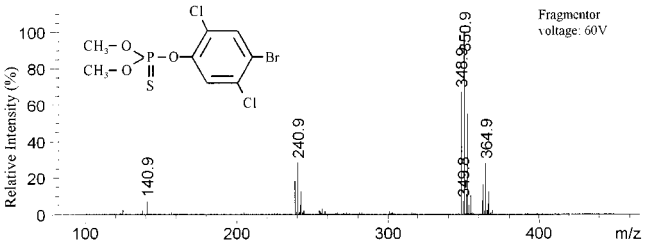


Figure 1. Mass spectra (PI mode) of bromophos.

RESULTS AND DISCUSSION

Optimization of APCI Interface. Table 1 reports the behavior of OPP pesticides studied in this work under PI and NI modes. At the concentration studied, triazophos has been the unique OPP pesticide that was not detected in NI mode, whereas parathion, parathion-methyl, and bromophos were detected only by NI mode. The other OPP pesticides studied can be detected using both ionization modes. In a previous work, Itoh et al. (*15*) described a similar behavior for a mixture of 21 OPP pesticides. Those authors did not find any relationship between structure and detectability by both ionization modes. However, they noted that pesticides which contain a nitro group with the molecule, such as parathion, parathion-methyl, and fenitrothion, were detected only with the NI mode. Our work partially confirmed the results reported by Itoh et al., but paraoxon, which also contains a nitro group, was detected in both ionization modes. The use of both ionization modes during the analysis of real samples represents a valuable tool for the identification and confirmation of the unknown compounds detected.

As is well-known (*16*), the fragmentation voltage and vaporizer temperature are the parameters with higher effect on sensitivity and molecule fragmentation. Table 3 shows the ions obtained under full-scan conditions using the PI mode at cone voltages of 20, 40, 60, and 80 V by FIA. At low fragmentor voltages, the most abundant ion is always the $[M + H]^+$ ion. Other ions observed were the characteristic fragments of the OPP group [153 $[PS(OCH_2CH_3)_2]^+$ and 125 $[PS(OCH_3)_2]^+$], the loss of these groups [$[M - PS_2(OCH_3)_2]^+$, $[M - PS(OCH_3)_2]^+$, $[M - PS(OCH_2CH_3)_2 + 2H]^+$, $[M - PO(OCH_3)_2 + 2H]^+$, $[M - PSO(OCH_3)_2]^+$], and the elimination of methyl or ethyl groups. Apart from those fragments, the presence of the isotopic signals typical of the halogens present in the ester bond provide additional information. This fact is especially relevant in the bromophos molecule, which has a maximum response peak of *m*/*z* 351 instead of *m*/*z* 349, which corresponds to $[M - CH_3]^-$ (see Figure 1); this is due to the presence of bromine and chlorine isotopes. Quantification was performed using the *m*/*z* 351 ion.

The NI operation is frequently used because it allows the detection of compounds such a acids or phenols. Figure 2 illustrates diffences between PI and NI modes for fonofos. As a general rule, higher fragmentations were obtained in NI mode with lower voltages compared to PI mode. Most of the pesticides studied obtained the higher response with fragment ions $[M - CH_3]^-$ and $[M - CH_2CH_3]^-$; only phentoate showed $[M - H]^-$ as the base peak. Moreover, characteristic fragments of OPP pesticides, 169 $[PSO(OCH_2CH_3)_2]^-$, 157 $[PS_2(OCH_3)_2]^-$, and 141 $[PSO(OCH_3)_2]^-$, are observed, as is shown in Table 4.

In PI mode, fragmentor voltage changes induce important fragmentation to azinphos, phenthoate, phosmet, and methidathion, whereas coumaphos, pirimiphos, and diazinon are scarcely affected, showing [M +

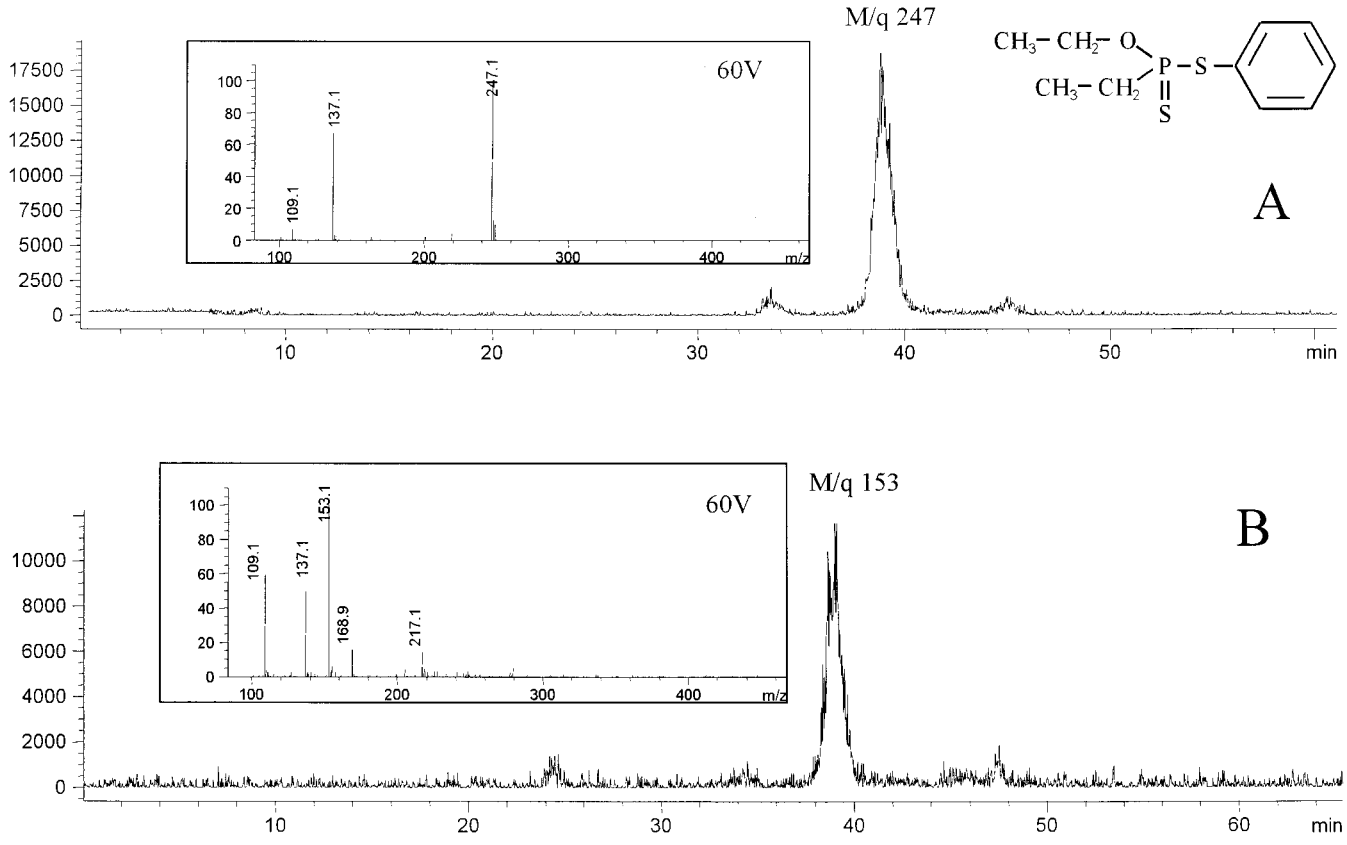


Figure 2. LC-APCI-MS chromatograms and mass spectra of fonofos in (A) PI mode and (B) NI mode.

Table 4. Characterization of OPP Pesticides with LC-APCI-MS NI Mode at Different Fragmentor Voltages

compound	MW	*m/z*	tentative identification	abundance (%) 20 V	40 V	60 V	80 V
azinphos-ethyl	345	185	$[PS_2(OCH_2CH_3)_2]^-$	100	100	100	100
		146	$[M - CH_2PS_2(OCH_2CH_3)_2]^-$	23	8	20	8
bromophos	364	363	$[M - H]^-$	25	26	30	34
		349	$[M - CH_3]^-$	100	100	100	100
		239	$[M - PS(OCH_3)_2]^-$	20	20	21	46
		141	$[PSO(OCH_3)_2]^-$	10	6	7	8
chlorpyriphos-methyl	321	302	$[M - Cl + O]^-$	100	100	100	100
		196	$[M - PS(OCH_3)_2]^-$	25	22	24	26
		157	$[M - PS(OCH_3)_2 - 2Cl + 2O + H]^-$			7	6
		141	$[OPS(OCH_3)_2]^-$	70	65	62	56
		126	$[M - PSO(OCH_3)_2 - 2Cl + O]^-$				5
coumaphos	363	361	$[M - H]^-$	100	100	100	100
		333	$[M - CH_2CH_3]^-$	92	100	95	97
		209	$[M - PS(OCH_2CH_3)_2]^-$	90	90	95	100
diazinon	304	303	$[M - H]^-$	14	15	16	13
		275	$[M - CH_2CH_3]^-$	100	100	100	100
		169	$[PSO(OCH_2CH_3)_2]^-$	32	40	40	50
		151	$[M - PS(OCH_2CH_3)_2]^-$	35	42	40	30
dimethoate	229	214	$[M - CH_3]^-$	100	100	100	100
		141	$[PSH_2(OCH_3)_2CH_2]^-$	50	50	47	60
		104	$[SCH_2CONHCH_3]^-$	12	12	12	18
fonofos	246	217	$[M - CH_2CH_3]^-$	22	16	14	
		169	$[PS_2O(CH_2CH_3)_2]^-$	40	38	16	13
		153	$[PSO_2(CH_2CH_3)_2]^-$	100	100	100	100
		137	$[PSO(CH_2CH_3)_2]^-$	50	66	52	45
		109	$[PSO(CH_2CH_3)]^-$	60	66	68	100
heptenophos	250	235	$[M - CH_3]^-$	100	100	100	100
		141	$[M - PO(OCH_3)_2]^-$	8	7		
malathion	330	329	$[M - H]^-$	33	52	21	
		315	$[M - CH_3]^-$	26	32	25	27
		205	$[M - PS(OCH_3)_2]^-$	14	16	7	11
		157	$[PS_2(OCH_3)_2]^-$	100	100	100	100
methidathion	302	287	$[M - CH_3]^-$	100	100	100	100
		157	$[PS_2(OCH_3)_2]^-$	85	70	70	75
		131	$[M - CH_2PS_2(OCH_3)_2]^-$	80	60	40	15
		117	$[C_2O_2N_2SH]^-$	21	10	10	10
paraoxon-methyl	247	232	$[M - CH_3]^-$	70	65	62	58
		138	$[M - PO(OCH_3)_2]^-$	100	100	100	100
		125	$[PO_2(OCH_3)_2]^-$	10	7	9	8
parathion-ethyl	291	262	$[M - CH_2CH_3]^-$	100	100	100	100
		169	$[PSO(OCH_2CH_3)_2]^-$	16	18	21	20
		138	$[M - PS(OCH_2CH_3)_2]^-$	22	25	39	68
parathion-methyl	263	248	$[M - CH_3]^-$	100	100	100	100
		138	$[M - PS(OCH_3)_2]^-$	25	30	44	86
phenthoate	320	319	$[M - H]^-$	100	100	100	18
		305	$[M - CH_3]^-$		10	10	
		195	$[M - PS(OCH_3)_2]^-$	7	8	12	11
		157	$[PS_2(OCH_3)_2]^-$			12	16
		125	$[PS(OCH_3)_2]^-$		8	70	100
		110	$[PSO_2CH_3]^-$				23
phosalone	367	338	$[M - CH_2CH_3]^-$	100	100	100	100
		185	$[PS_2(OCH_2CH_3)_2]^-$	90	80	80	100
		168	$[M - CH_2PS_2(OCH_2CH_3)_2]^-$	25	28	28	48
		142	$[C_6H_3ClNH_2O]^-$	40	40	40	18
phosmet	317	157	$[PS_2(OCH_3)_2]^-$	100	100	100	100
		146	$[M - CH_2 - PS_2(OCH_3)_2]^-$	60	70	70	90
pyrazophos	373	372	$[M - H]^-$	100	100	100	100
		220	$[M - PS(OCH_2CH_3)_2]^-$	19	23	27	31
pirimiphos-ethyl	333	304	$[M - CH_2CH_3]^-$	100	100	100	100
		180	$[M - PS(OCH_2CH_3)_2]^-$	42	45	50	50
		169	$[PSO(OCH_2CH_3)_2]^-$	10	10	10	8
pirimiphos-methyl	305	290	$[M - CH_3]^-$	100	100	100	100
		180	$[M - PS(OCH_3)_2]^-$	80	99	84	100

Table 4 (Continued)

compound	Pm	*m/z*	tentative identification	abundance (%) 20 V	40 V	60 V	80 V
quinalphos	298	269	$[M - CH_2CH_3]^-$	31	31	20	15
		169	$[PSO(OCH_2CH_3)_2]^-$	100	99	68	56
		145	$[M - PS(OCH_2CH_3)_2]^-$	100	100	100	100
vamidothion	287	272	$[M - CH_3]^-$	100	100	100	100
		141	$[PSO(OCH_3)_2]^-$	22	24	23	20
		118	$[SCHCH_3CONHCH_3]^-$	35	35	30	18

Table 5. Limits of Detection (LOD) and Quantitation (LOQ) in Both Ionization Modes and the Ion Used for Quantification

compound	positive mode SIM ion (*m/z*)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	negative mode SIM ion (*m/z*)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
azinphos-ethyl	346	10	32	185	8	25
bromophos		nd	nd	349	5	15
chlorpyriphos-methyl	322	10	32	302	7	21
coumaphos	363	6	18	361	4	12
diazinon	305	2	6	275	17	51
dimethoate	230	4	12	214	5	15
fonofos	247	2	6	153	15	45
heptenophos	251	4	12	235	30	90
malathion	285	6	18	157	13	42
methidathion	145	90	200	287	8	24
paraoxon	248	25	75	232	8	25
parathion-ethyl		nd	nd	262	2	7
parathion-methyl		nd	nd	138	1	4
phenthoate	321	60	180	319	8	24
phosalone	368	12	36	338	4	13
phosmet	160	15	45	157	20	60
pirimiphos-ethyl	334	1	4	304	5	15
pirimiphos-methyl	306	2	7	290	7	21
pyrazophos	374	3	9	372	1	3
quinalphos	299	4	12	169	10	30
triazophos	314	5	15		nd	nd
vamidothion	288	4	12	272	5	17

$H]^+$ as the main ion. To obtain molecular mass information, low voltages are preferred for pesticides that suffer an important fragmentation, but unaffected pesticides showed better sensitivity using higher voltages (optimum values are ~70 V). In NI mode fragmentor voltage changes have less influence in fragmentation than in PI mode. The fragmentor voltages selected for each group of ions in PI and NI modes in the routine determination are outlined in Table 2.

The optimum vaporizer temperature observed for most of the pesticides analyzed was from 400 to 450 °C except for heptenophos and quinalphos, which showed better response at 300 °C. A compromise should be achieved, and 350 °C was selected as vaporizer temperature. At the temperature studied no changes in the fragmentation were observed; only some pesticides present a slight variation in the relative abundance at 450 and 500 °C.

Extraction Procedure. The extraction procedure selected is based on a method previosly applied for the determination of OPP pesticides in vegetables by GC (*17*) with some modifications.

Interferences due to matrix effect were higher in NI mode that those observed in PI mode as has been reported by other authors (*10*). This is probably because of the presence of fatty acids of low molecular weight from wax and NI mode is more sensitive and selective to acid molecules. These substances have been previously identified by Bernal et al. (*18*) in larvae, and their origins were related to wax residues from hive combs adhering to the larvae. After the injection of five blank samples in both ionization modes, interference peaks corresponding to pesticide ions were observed; this problem was solved by changing the selected ion. The *m/z* 331 ion of malathion was changed to *m/z* 285 in PI mode, and the *m/z* 248 ion of parathion-methyl was changed to *m/z* 138 in NI mode. Other authors have observed enhancement or suppression effects on the ionization process (*8*). To evaluate the matrix effect, the response of standards prepared in the matrix was compared with the response of standards prepared in methanol. Enhancement or suppression effects were noticed for most compounds. Consequently, recovery results for LC-MS were calculated against standards prepared in residue-free matrix extracts.

Calibration graphs obtained from 0.01 to 5 μg mL^{-1} in both PI and NI modes revealed a very good response linearity of each compound over 2 orders of magnitude. The correlation factors ranged from 0.9927 to 0.9991 in PI mode and from 0.9931 to 0.9993 in NI mode. The intraday precision (repeatability) of the method was evaluated with five replicate determinations of a standard mixture of 1 μg mL^{-1} on the same day; the relative standard deviations (RSD) were within the range of 3−8%. The interday precision (i.e., reproducibility) was evaluated by analyzing fortified extracts at the same concentration over 5 days, and the RSD ranged from 7 to 13%.

The limits of detection (LOD) were calculated from $3s_b$/slope of the calibration curve, where s_b is the standard deviation of a black measurement. Table 5 shows the LODs obtained by SIM mode detection; LODs ranged from 1 μg kg^{-1} of pirimiphos ethyl to 90 μg kg^{-1} of methidathion in PI and from 1 μg kg^{-1} of pyrazophos and parathion-ethyl to 30 μg kg^{-1} of heptenophos in NI. The limits of quantitation (LOQ) were determined as

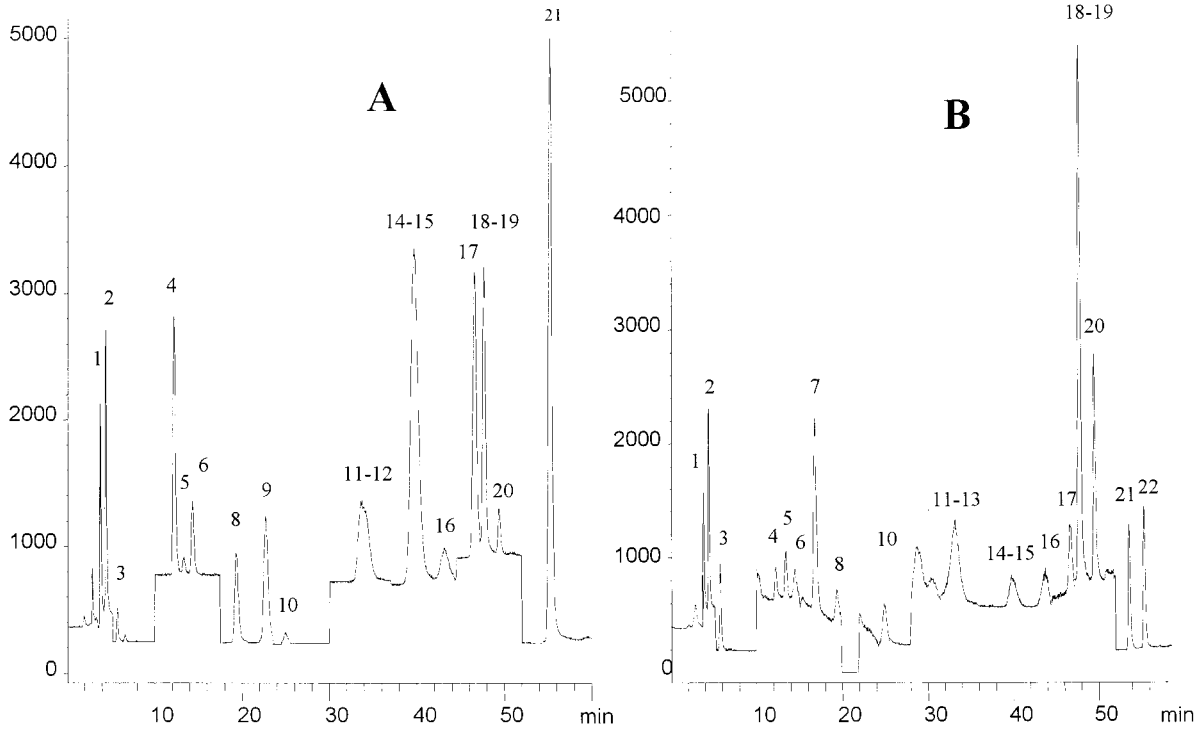


Figure 3. SIM chromatograms of a honeybee sample spiked at 1.5 mg kg^{-1} of each pesticide in PI mode (A) and NI mode (B). Peaks: 1, vamidothion; 2, dimethoate; 3, paraoxon; 4, heptenophos; 5, methidathion; 6, phosmet; 7, parathion-methyl; 8, malathion; 9, triazophos; 10, azinphos-ethyl; 11, phenthoate; 12, quinalphos; 13, parathion-ethyl; 14, diazinon; 15, fonofos; 16, coumaphos; 17, pirimiphos-methyl; 18, phosalone; 19, pyrazophos; 20, chlorpyriphos-methyl; 21, pirimiphos-ethyl; 22, bromophos.

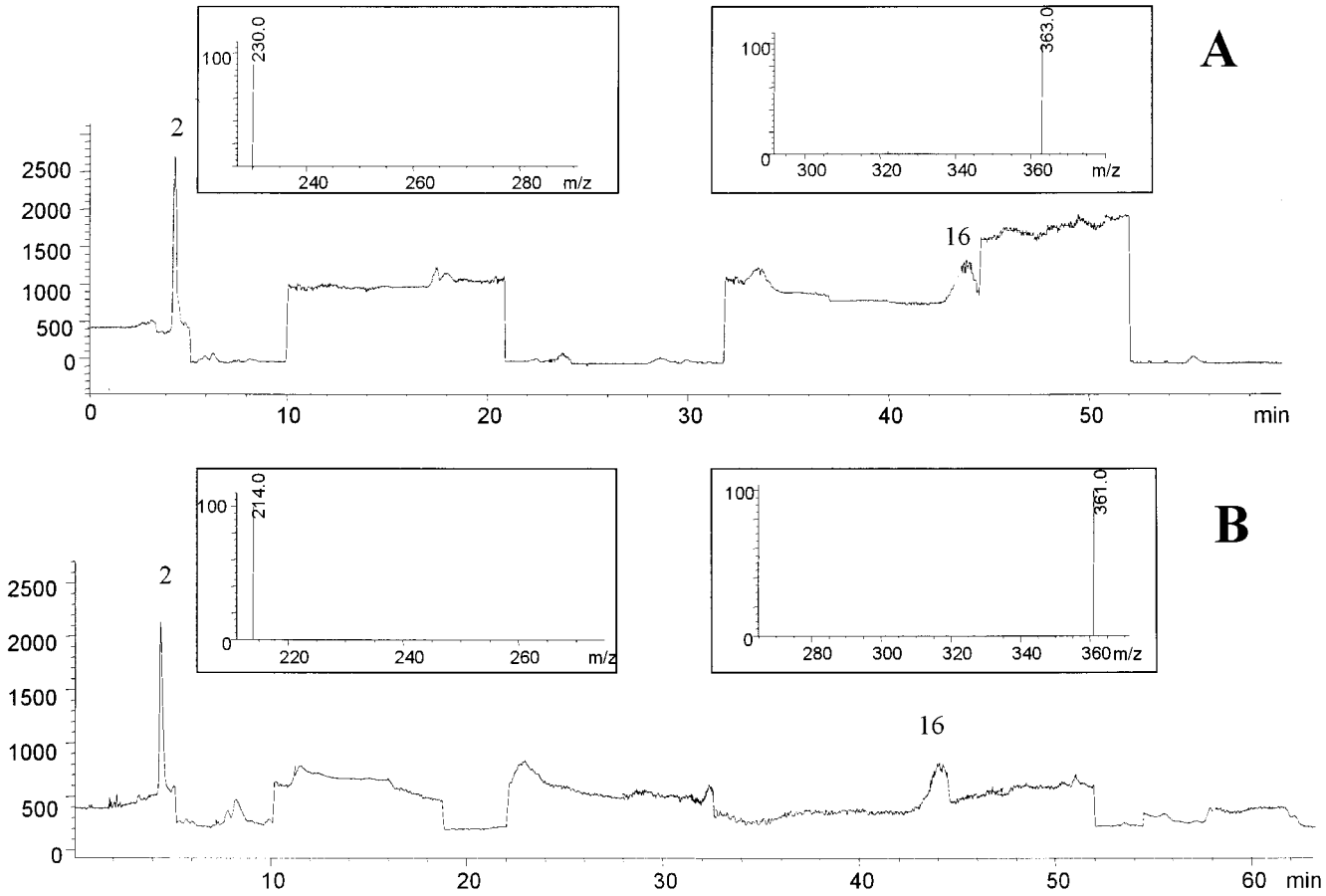


Figure 4. Chromatograms of real honeybee samples by (A) PI mode and (B) NI mode in which were found coumaphos and dimethoate.

the lowest concentration of pesticide that gives a response that could be quantified with an interassay RSD of <26% and ranged between 4 and 200 μg kg^{-1}. Both the LOD and LOQ are dependent on the sensitivity

Table 6. Mean Recoveries[a] of Honeybee Samples Spiked at Three Different Concentrations and Relative Standard Deviations (RSD) ($n = 5$)

compound	recovery ± RSD at 0.5 mg kg^{-1}	1 mg kg^{-1}	5 mg kg^{-1}
azinphos-ethyl	86 ± 11	87 ± 8	91 ± 8
bromophos	81 ± 12	83 ± 10	85 ± 8
chlorpyriphos-methyl	68 ± 7	71 ± 6	73 ± 7
coumaphos	90 ± 13	95 ± 7	91 ± 7
diazinon	85 ± 6	86 ± 9	87 ± 4
dimethoate	71 ± 7	72 ± 6	79 ± 5
fonofos	68 ± 9	69 ± 11	71 ± 12
heptenophos	77 ± 15	74 ± 9	79 ± 11
malathion	69 ± 7	73 ± 7	78 ± 6
methidathion	89 ± 12	90 ± 10	94 ± 8
paraoxon	76 ± 7	80 ± 8	82 ± 9
parathion-ethyl	91 ± 12	96 ± 11	103 ± 11
parathion-methyl	76 ± 13	79 ± 9	81 ± 7
phenthoate	65 ± 10	68 ± 12	73 ± 13
phosalone	95 ± 7	82 ± 7	89 ± 6
pyrazophos	80 ± 7	83 ± 5	90 ± 8
phosmet	81 ± 12	87 ± 8	86 ± 9
pirimiphos-ethyl	87 ± 10	81 ± 12	89 ± 11
pirimiphos-methyl	91 ± 13	88 ± 9	85 ± 7
quinalphos	102 ± 12	98 ± 10	97 ± 9
triazophos	67 ± 6	69 ± 5	73 ± 4
vamidothion	78 ± 12	78 ± 7	81 ± 8

[a] Recoveries were calculated in the more adequate ionization mode according to Table 1.

for the analyte and the baseline noise at the time of analysis. Table 6 shows the mean recoveries obtained from spiked samples at three concentration levels. The recovery rates were satisfactory, ranging from 65 to 103% with an RSD below 15%. Figure 3 shows the SIM chromatogram obtained in both PI and NI modes from a spiked honeybee sample. Although some compounds are not resolved, they can be easily identified and quantified on individual ion chromatograms. Seven samples taken from hives of a Valencian community were analyzed following the proposed method. Positive findings of only coumaphos and methidation were detected at 1.1 and 0.73 mg kg^{-1}, respectively. The SIM chromatograms obtained are shown in Figure 4.

Conclusion. The LLE method proposed provides good recovery and reproducibility, and only a few coextractive compounds from the honeybee matrix were detected. The suitability of LC-MS using an APCI interface for multiresidue analysis of pesticides from the honeybee samples has been demonstrated. The combination of PI and NI modes provides a rapid screening procedure for a wide range of pesticides with different polarities and constitutes an important confirmatory tool. Using an MS detector in SIM mode, the analytes are quantitated with very high specificity of their unique masses or fragments of the analyte, which solves the problem of coeluting pesticides or the presence of interference as fatty compounds.

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