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Analysis of Pesticides in Honey by Solid-Phase Extraction and Gas Chromatography−Mass Spectrometry

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An analytical method for the simultaneous determination of 51 pesticides in commercial honeys was developed. Honey (10 g) was dissolved in water/methanol (70:30; 10 mL) and transferred to a C_{18} column (1 g) preconditioned with acetonitrile and water. Pesticides were subsequently eluted with a hexane/ethyl acetate mixture (50:50) and determined by gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode (GC-MS-SIM). Spiked blank samples were used as standards to counteract the matrix effect observed in the chromatographic determination. Pesticides were confirmed by their retention times, their qualifier and target ions, and their qualifier/target abundance ratios. Recovery studies were performed at 0.1, 0.05, and 0.025 μg/g fortification levels for each pesticide, and the recoveries obtained were >86% with relative standard deviations of <10%. Good resolution of the pesticide mixture was achieved in ~41 min. The detection limits of the method ranged from 0.1 to 6.1 μg/kg for the different pesticides studied. The developed method is linear over the range assayed, 25−200 μg/L, with determination coefficients of >0.996. The proposed method was applied to the analysis of pesticides in honey samples, and low levels of a few pesticides (dichlofluanid, ethalfluralin, and triallate) were detected in some samples.

KEYWORDS: Multiresidue; honey; pesticides; gas chromatography−mass spectrometry; SPE

INTRODUCTION

Pesticides are widely used in present agricultural practices, causing the contamination of the environment and foodstuffs. Pesticide residues in honey can originate from the contamination of plants where bees collect pollen and nectar or from the treatment of beehives to control some pests and diseases they suffer.

Various methods have been reported for the determination of pesticides in honey. Analysis of honey is difficult due to its complex composition and, particularly, the presence of waxes and pigments. The classical extraction technique used in the determination of pesticide residues in honey has been partitioning with organic solvents, often followed by subsequent cleanup procedures before gas chromatographic determination (*1*−*4*). The drawbacks of the traditional extraction methods, such as the use of large amounts of solvents and glassware and the high time consumption, can be reduced by using other extraction techniques developed recently. Supercritical fluid extraction (SFE) (*5*), solid-phase extraction (SPE) with the stationary phase packed in a cartridge or in disks (*6*−*10*), solid-phase microextraction (SPME) (*11*−*13*), and matrix solid-phase dispersion (MSPD) (*14*, *15*) are different techniques that have been used with that aim in the analysis of pesticides. Analysis of extracts is generally performed by gas chromatography with different selective detectors such as electron capture (ECD) for pyrethroids and organohalogen compounds (*1*, *15*−*17*) or nitrogen−phosphorus (NPD) (*14*, *18*) for organonitrogen and organophosphate pesticides. The determination of pesticide residues, particularly those thermally unstable, can be also carried out by high-performance liquid chromatography (HPLC) (*10*, *19*), but generally the sensitivity achieved in this case is somewhat lower. Mass spectrometry coupled to gas (GC-MS) or liquid (HPLC-MS) chromatography is more often used at present for pesticide analysis in honey (*5*, *13*, *20*, *21*), due to the possibility of confirming the identity of pesticide residues.

Multiresidue methods have been reported for the determination of pesticides in food (*22*, *23*). Methods for multiresidue analysis of pesticides in honey are scarce in the scientific literature, and there is a need to develop analytical procedures allowing the reliable and rapid quantification and confirmation of as many pesticides as possible in a single determination in a cost-effective manner. Analytical methods published for the determination of pesticides in honey generally determine only compounds belonging to different chemical classes, mainly organochlorine (*1*, *8*), organophosphorus (*2*, *20*), or acaricide (*24*). To our knowledge, no multiresidue method has been reported until now for the analysis of more than 50 pesticides in honey in a single determination using GC-MS-SIM.

In previous studies, a MSPD method for the extraction of different pesticide classes (*14*, *15*) or organic pollutants (*25*) from honey samples was used in our laboratory. Nevertheless, this method suffered from not obtaining good detection limits

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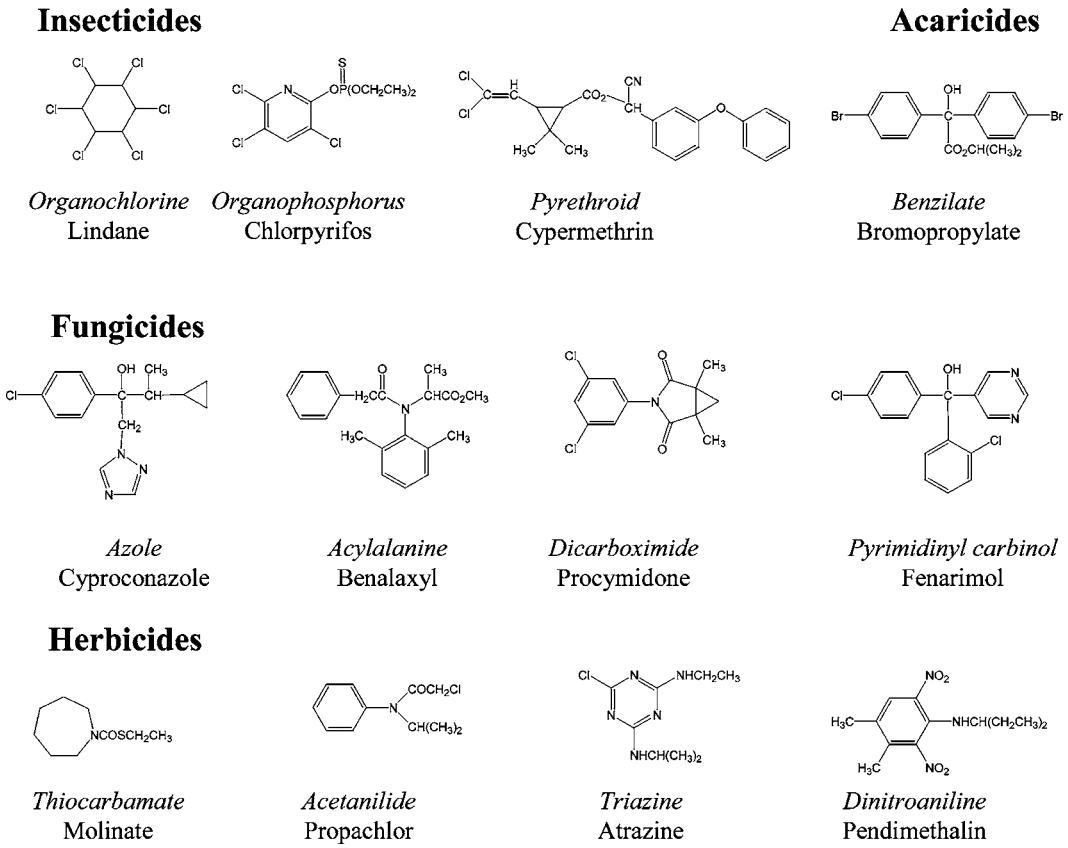

Figure 1. Chemical structures of representative compounds of the main pesticide groups studied.

Table 1. Retention Times (t_R), Molecular Weights (MW), Target (T) and Qualifier Ions (Q_1, Q_2,), and Abundance Ratios of Qualifier Ion/Target Ion (Q_1/T, Q_2/T)[a]

	pesticide	t_R (min)	MW	T	Q_1	Q_2	Q_1/T (%)	Q_2/T (%)		pesticide	t_R (min)	MW	T	Q_1	Q_2	Q_1/T (%)	Q_2/T (%)
1	EPTC	7.93	189.3	128	189	86	24.2	65.8	28	butralin	22.85	295.3	266	267	295	100	58.9
2	molinate	10.81	187.3	126	187	83	21.5	14.5	29	pendimethalin	23.54	281.3	252	281	220	13.0	16.0
3	propachlor	12.30	211.7	120	176	93	38.2	31.0	30	chlorfenvinphos	23.97	359.6	267	323	295	54.1	19.8
4	ethalfluralin	13.33	333.3	276	316	292	76.2	46.3	31	procymidone	24.31	284.1	283	96	285	118.8	170
5	trifluralin	13.73	335.5	306	264	290	74.8	13.4	32	methidathion	24.60	302.3	145	85	125	83.8	16.4
6	simazine	15.19	201.7	201	186	173	73.3	29.4	33	endosulfan i	24.94	406.9	241	195	239	94.5	33.0
7	atrazine	15.46	215.7	200	215	173	57.4	22.4	34	profenophos	25.85	373. 6	208	339	139	75.6	80.2
8	lindane	15.87	290.8	183	219	147	82.6	38.4	35	oxadiazon	26.21	345.2	175	258	344	51.9	18.5
9	terbuthylazine	16.18	229.7	214	229	173	28.2	37.6	36	cyproconazole	26.71	291.8	222	139		51.3	
10	diazinon	16.87	304.3	179	137	304	103.2	48.3	37	endosulfan II	27.00	406.9	195	237	241	83.7	36.4
11	chlorothalonil	17.35	265.9	266	264	270	100	10.8	38	ethion	27.59	384.5	231	153	384	67.5	11.7
12	triallate	17.46	304.7	86	268	128	39.6	21.2	39	ofurace	28.11	281.7	132	160	281	79.4	34.5
13	metribuzin	18.84	214.3	198	144	182	14.3	8.4	40	benalaxyl	28.26	325.4	148	206	91	25.9	
14	parathion-methyl	19.22	263.2	263	109	125	92.8	79.4	41	endosulfan sulfate	28.37	423.0	272	229	387	63.6	52.9
15	tolclofos- methyl	19.46	301.1	265	125	250	23.5	10.8	42	hexazinone	28.83	252.3	171	128	83	14.9	12.2
16	alachlor	19.66	269.8	160	188	146	88.4	91.2	43	nuarimol	28.92	314.7	235	203	314	78.1	53.5
17	prometryn	19.96	241.4	241	184	226	73.1	55.4	44	bromopropylate	29.95	428.1	341	183	343	42.4	49.4
18	terbutryn	20.63	241. 4	226	241	185	48.7	73.8	45	tetradifon	30.66	356.1	159	229	111	58.2	50.3
19	fenitrothion	20.76	277.2	277	125	260	113.8	39.9	46	amitraz	31.25	293.4	121	162	293	78.8	73.2
20	pirimiphos-methyl	20.95	333.4	290	276	305	85.9	67.7	47	cyhalothrin	31.47	449.9	181	197	208	71.1	52.7
21	dichlofluanid	21.12	333.2	123	224	167	47.0	39.3	48	fenarimol	31.61	331.2	139	219	251	76.2	31.9
22	aldrin	21.34	364.9	263	293	221	38.1	19.8	49	acrinathrin	31.72	541.4	181	208	289	70.8	35.1
23	malathion	21.45	330.4	173	127	93	104.3	79.3	50	coumaphos	32.81	362.8	362	226	109	62.0	102.4
24	metolachlor	21.64	283.8	162	238	146	57.0	14.2	51	cypermethrin	34.25	416.3	181	163	209	98.9	63.9
25	fenthion	21.83	278.3	278	169	109	24.1	26.6	52	fluvalinate tau-I	36.27	502.9	250	181	252	19.4	33.8
26	chlorpyrifos	21.95	350.6	197	314	97	67.4	69.2	53	fluvalinate tau-II	36.42	502.9	250	181	252	20.1	33.1
27	triadimefon	22.12	293.8	208	181	128	27.6	51.6									

[a] Q/T (%) are the results of abundance values of the qualifier ion (Q1, Q2) divided by the abundance of the target ion (T) × 100.

when a large number of compounds were determined. With the aim of overcoming these problems, an alternative method was developed. This paper presents a rapid and sensitive method for the simultaneous quantification and confirmation of 51 pesticides in honey, based on solid-phase extraction and subsequent determination by GC-MS in the SIM mode. **Figure 1** depicts the chemical structures of representative compounds of the main pesticide groups studied. The developed method

Table 2. SIM Program Used To Analyze and Confirm Pesticides in Honey

group	time (min)	pesticide	*m/z*	dwell time (ms)	scan rate (cycles/s)
1	5.00	EPTC, molinate	128, 189, 86, 126, 187, 83	100	2.15
2	11.70	propachlor	120, 176, 93	100	4.26
3	12.70	ethalfluralin, trifluralin	276, 316, 292, 264, 306, 290	100	2.15
4	14.40	simazine, atrazine	201, 186, 173, 200, 215	100	2.15
5	15.70	lindane, terbuthylazine	183, 219, 147, 214, 229, 173	100	2.15
6	16.60	diazinon	179, 137, 304	100	2.86
7	17.15	chlorothalonil, triallate	266, 264, 270, 86, 268, 128	100	1.72
8	17.90	metribuzin	198, 144, 182	100	4.26
9	19.00	parathion methyl, tolclofos-methyl	263, 109, 125, 265, 250	100	2.15
10	19.59	alachlor, prometryn	160, 188, 146, 241, 184, 226	100	2.15
11	20.40	terbutryn, fenitrothion, pirimiphos-methyl, dichlofluanid	226, 241, 185, 277, 125, 260, 290, 276, 305, 123, 224, 167	50	1.90
12	21.26	aldrin, malathion	263, 293, 221, 127, 173, 93	100	2.15
13	21.59	metolachlor, fenthion, chlorpyrifos, triadimefon	162, 238, 146, 278, 169, 109, 197, 314, 97, 208, 181, 128	50	1.90
14	22.50	butralin, pendimethalin	266, 267, 295, 252, 281, 220	100	2.15
15	23.85	chlorfenvinphos, procymidone	267, 323, 295, 96, 283, 285	100	1.72
16	24.45	methidathion, endosulfan I	145, 85, 125, 195, 241, 239	100	2.15
17	25.40	profenophos, oxadiazon	208, 339, 139, 175, 258, 344	100	1.72
18	26.40	cyproconazole, endosulfan II	222, 139, 195, 237, 241	100	1.72
19	27.30	ethion	231, 153, 384	100	8.33
20	27.90	ofurace, benalaxyl, endosulfan sulfate	132, 160, 281, 91, 148, 206, 229, 272, 387	50	2.17
21	28.60	hexazinone (IS),[a] nuarimol	171, 128, 83, 203, 235, 314	100	1.72
22	29.50	bromopropylate, tetradifon	341,183, 343, 111, 159, 229	100	1.72
23	31.10	amitraz, cyhalothrin, fenarimol, acrinathrin	121, 162, 293, 181, 197, 208, 139, 219, 251, 181, 289	50	2.17
24	32.50	coumaphos, cypermethrin	362, 226, 109, 163, 181, 209	100	2.15
25	36.00	fluvalinate tau-I, fluvalinate tau- II	250, 181, 252	100	4.26

[a] Internal standard.

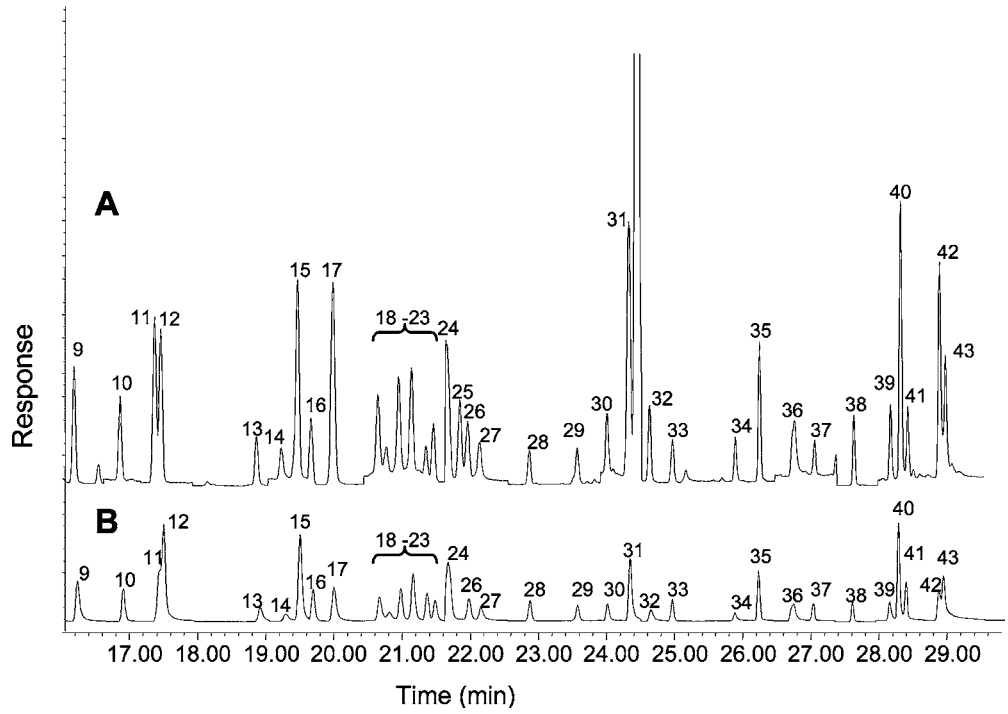

Figure 2. Matrix effect on the GC analysis of pesticides: (**A**) blank rosemary honey sample fortified at 0.05 μg/mL and (**B**) standard mixture solution in ethyl acetate at 0.05 μg/mL. See **Table 1** for peak identification.

was applied to the analysis of pesticide residues in various types of Spanish honeys.

MATERIALS AND METHODS

Materials and Standards. Pesticide standards were obtained from Reidel-de Haën (Seelze, Germany), and all compounds were of 99% purity. Ethyl acetate, hexane, methanol, acetonitrile, and dichloromethane, of residue analysis grade, were purchased from Scharlab (Barcelona, Spain). A Milli-Q water purification system from Millipore (Bedford, MA) was used to provide ultrapure water. Silica Bondesil-C_{18}, particle diameter of 40 μm, was acquired from Scharlab, and anhydrous sodium sulfate, of reagent grade, was obtained from Merck (Darmstadt, Germany).

Stock solutions (500 μg/mL) of each pesticide standard were prepared by dissolving 0.050 g of the pesticide in 100 mL of ethyl acetate. A

Table 3. Limits of Detection (LOD) and Quantification (LOQ), Calibration Data, and Repeatability of the Studied Pesticides

pesticide	LOD (μg/kg)	LOQ (μg/kg)	calibration data		repeatability (RSD,%)[a]	
			equation	r^2	peak area	t_R (min)
EPTC	0.5	1.7	$y = 3.31 \times 10^{-1}x - 2.36 \times 10^{-2}$	1.000	3.1	0.06
molinate	0.3	1.0	$y = 5.76 \times 10^{-1}x - 4.80 \times 10^{-2}$	1.000	4.6	0.06
propachlor	0.1	0.3	$y = 5.61 \times 10^{-1}x - 2.88 \times 10^{-2}$	1.000	2.8	0.05
ethalfluralin	0.3	1.0	$y = 7.12 \times 10^{-2}x - 5.55 \times 10^{-3}$	0.999	4.6	0.06
trifluralin	0.1	0.3	$y = 3.14 \times 10^{-1}x - 3.05 \times 10^{-2}$	0.999	5.1	0.05
simazine	0.3	1.0	$y = 2.43 \times 10^{-1}x - 2.03 \times 10^{-2}$	0.999	5.1	0.06
atrazine	0.5	1.7	$y = 3.40 \times 10^{-1}x - 2.88 \times 10^{-2}$	1.000	4.8	0.06
lindane	1.1	3.6	$y = 6.69 \times 10^{-1}x - 4.90 \times 10^{-3}$	1.000	4.2	0.003
terbuthylazine	0.3	1.0	$y = 5.58 \times 10^{-1}x - 5.42 \times 10^{-2}$	0.999	4.9	0.05
diazinon	0.1	0.3	$y = 2.39 \times 10^{-1}x - 9.65 \times 10^{-3}$	1.000	5.1	0.04
chlorothalonil	0.3	1.0	$y = 5.82 \times 10^{-1}x - 5.41 \times 10^{-2}$	0.999	5.3	0.04
triallate	0.5	1.7	$y = 5.26 \times 10^{-1}x - 4.12 \times 10^{-2}$	0.999	5.2	0.04
metribuzin	0.3	1.0	$y = 4.90 \times 10^{-1}x - 5.39 \times 10^{-2}$	0.999	6.1	0.04
oarathion-methyl	0.1	0.3	$y = 9.50 \times 10^{-2}x - 8.93 \times 10^{-3}$	0.999	6.5	0.03
tolclofos-methyl	0.3	0.3	$y = 1.06x - 9.40 \times 10^{-2}$	0.999	5.8	0.03
alachlor	0.4	1.3	$y = 2.59 \times 10^{-1}x - 3.27 \times 10^{-2}$	1.000	4.8	0.04
prometryn	5.0	16.5	$y = 6.36 \times 10^{-1}x + 3.28 \times 10^{-1}$	1.000	6.0	0.03
terbutryn	0.3	1.0	$y = 4.60 \times 10^{-1}x - 4.43 \times 10^{-2}$	0.999	5.1	0.03
fenitrothion	0.2	0.7	$y = 1.02 \times 10^{-1}x - 9.99 \times 10^{-3}$	0.999	7.0	0.03
pirimiphos-methyl	1.5	5.0	$y = 3.02 \times 10^{-1}x - 2.91 \times 10^{-2}$	0.999	6.2	0.03
dichlofluanid	0.2	0.7	$y = 4.62 \times 10^{-1}x - 4.23 \times 10^{-2}$	0.999	6.3	0.03
aldrin	3.0	9.9	$y = 6.04 \times 10^{-1}x + 2.33 \times 10^{-1}$	1.000	5.2	0.02
malathion	0.3	1.0	$y = 3.15 \times 10^{-1}x - 2.89 \times 10^{-2}$	0.999	6.4	0.03
metolachlor	0.1	0.3	$y = 6.33 \times 10^{-1}x - 9.13 \times 10^{-2}$	1.000	5.9	0.01
fenthion	0.5	1.7	$y = 6.00 \times 10^{-1}x - 6.93 \times 10^{-2}$	0.999	5.4	0.03
chlorpyrifos	1.1	3.6	$y = 2.18 \times 10^{-1}x - 1.78 \times 10^{-2}$	0.999	6.3	0.02
triadimefon	0.1	0.3	$y = 2.55 \times 10^{-1}x - 2.16 \times 10^{-2}$	0.999	5.4	0.03
butralin	0.1	0.3	$y = 3.40 \times 10^{-1}x - 4.17 \times 10^{-2}$	0.998	7.0	0.02
pendimethalin	1.1	3.6	$y = 2.76 \times 10^{-1}x - 3.12 \times 10^{-2}$	0.998	7.3	0.02
chlorfenvinphos	1.8	5.9	$y = 3.38 \times 10^{-1}x - 2.83 \times 10^{-2}$	1.000	5.2	0.02
procymidone	5.4	17.8	$y = 3.07 \times 10^{-1}x - 2.54 \times 10^{-2}$	1.000	5.5	0.04
methidathion	1.4	4.6	$y = 6.42 \times 10^{-1}x - 4.81 \times 10^{-2}$	1.000	6.5	0.01
endosulfan I	6.1	20.1	$y = 5.94 \times 10^{-1}x + 3.90 \times 10^{-2}$	0.996	8.0	0.03
profenophos	0.2	0.7	$y = 1.54 \times 10^{-1}x - 1.22 \times 10^{-2}$	1.000	6.4	0.01
oxadiazon	0.1	0.3	$y = 3.53 \times 10^{-1}x - 2.76 \times 10^{-2}$	1.000	5.2	0.01
cyproconazole	0.2	0.7	$y = 5.69 \times 10^{-1}x - 5.08 \times 10^{-2}$	1.000	6.7	0.01
endosulfan II	1.2	4.0	$y = 6.33 \times 10^{-2}x - 3.43 \times 10^{-3}$	0.999	4.8	0.01
ethion	0.1	0.3	$y = 5.61 \times 10^{-1}x - 4.59 \times 10^{-2}$	0.999	6.0	0.01
ofurace	0.6	2.0	$y = 1.94 \times 10^{-1}x - 9.26 \times 10^{-3}$	0.999	8.0	0.01
benalaxyl	0.3	1.0	$y = 9.24 \times 10^{-1}x - 7.58 \times 10^{-2}$	1.000	5.6	0.01
endosulfan sulfate	0.4	1.3	$y = 1.49 \times 10^{-1}x - 1.19 \times 10^{-2}$	1.000	5.6	0.01
nuarimol	1.5	5.0	$y = 2.24 \times 10^{-1}x - 2.30 \times 10^{-2}$	0.999	5.5	0.01
bromopropylate	0.2	0.7	$y = 4.27 \times 10^{-1}x + 4.05 \times 10^{-2}$	0.999	6.2	0.01
tetradifon	1.6	5.3	$y = 2.15 \times 10^{-1}x - 1.96 \times 10^{-2}$	0.999	6.0	0.005
amitraz	3.3	10.9	$y = 3.05 \times 10^{-2}x + 4.01 \times 10^{-2}$	1.000	4.8	0.005
cyhalothrin	0.1	0.3	$y = 3.45 \times 10^{-1}x - 3.77 \times 10^{-2}$	0.999	7.0	0.004
fenarimol	0.9	3.0	$y = 2.17 \times 10^{-1}x - 1.75 \times 10^{-2}$	1.000	6.1	0.003
acrinathrin	0.3	1.0	$y = 2.59 \times 10^{-1}x - 3.27 \times 10^{-2}$	0.999	7.5	0.004
coumaphos	0.1	0.3	$y = 2.18 \times 10^{-1}x - 2.38 \times 10^{-2}$	0.999	6.0	0.01
cypermethrin	0.2	0.7	$y = 2.67 \times 10^{-1}x - 2.85 \times 10^{-2}$	0.999	5.4	0.01
fluvalinate tau-I	0.2	0.7	$y = 3.26 \times 10^{-1}x - 1.39 \times 10^{-2}$	0.999	6.7	0.01
fluvalinate tau-II	0.2	0.7	$y = 3.45 \times 10^{-1}x - 4.00 \times 10^{-2}$	0.999	6.5	0.01

[a] Relative standard deviations of retention times and peak areas ($n = 10$).

pesticide intermediate standard solution (5 μg/mL) was prepared by transferring 1 mL from each pesticide to a 100 mL volumetric flask and diluting to volume with ethyl acetate to obtain a concentration of 5 μg/L. A set of calibration standard solutions of 4.0, 2.0, 1.0, and 0.5 μg/mL was prepared by dilution. The solutions containing 2.0, 1.0, and 0.5 μg/mL of each pesticide were used to fortify honey samples. The internal standard was prepared by dissolving hexazinone in ethyl acetate to make a 500 μg/ mL solution. Stock standard and working solutions were stored at 4 °C and used for no longer than 3 months and 1 week, respectively.

Apparatus. *Extraction Equipment.* Polypropylene columns (5 mL) of 6 cm × 12 mm i.d. (Becton-Dickinson) with Teflon frits of 1 cm diameter and 20 μm pore size (Varian) were used in the extraction step.

A 12-port vacuum manifold (Supelco Visiprep, Madrid, Spain) was employed to filter the extraction solvent.

GC-MS Analysis. GC-MS analysis was performed with an Agilent 6890 (Waldbronn, Germany) gas chromatograph equipped with an automatic split−splitless injector model HP 7683 and a mass spectrometric detector (MSD) model HP 5973. A fused silica capillary column (ZB-5MS), 5% phenyl polysiloxane as nonpolar stationary phase (30 m × 0.25 mm i.d.) and 0.25 μm film thickness, supplied by Phenomenex (Torrance, CA), was employed. Operating conditions were as follows: injector port temperature, 280 °C; helium as carrier gas at a flow rate of 1.0 mL/min; pulsed splitless mode (pulsed pressure = 310 kPa for 1.5 min). The column temperature was maintained at 70 °C for 2 min, then programmed at 25 °C/min to 150 °C, increased to 200 °C at a rate of 3 °C/min, followed by a final ramp to 280 °C at a rate of 8 °C/min, and held for 10 min. The total analysis time was 41.87 min and the equilibration time 2 min. A 2 μL volume was injected splitless, with the split valve closed for 1 min.

Table 4. Recovery of the Studied Pesticides from Honey Samples[a] (Mean ± RSD, %)

compound	fortification levels 0.1 μg/g	0.05 μg/g	0.025 μg/g	compound	fortification levels 0.1 μg/g	0.05 μg/g	0.025 μg/g
EPTC	98.0 ± 2.7	98.1 ± 4.3	96.6 ± 4.3	triadimefon	96.4 ± 2.3	97.5 ± 3.3	98.7 ± 6.6
molinate	98.3 ± 2.4	99.1 ± 3.8	97.8 ± 4.5	butralin	95.8 ± 3.5	96.9 ± 4.7	97.4 ± 6.5
propachlor	99.0 ± 2.3	97.8 ± 5.4	98.9 ± 5.5	pendimethalin	95.3 ± 3.3	97.3 ± 3.8	96.4 ± 6.3
ethalfluralin	97.0 ± 2.1	97.9 ± 2.9	98.0 ± 5.4	chlorfenvinphos	95.0 ± 3.8	99.7 ± 8.6	98.9 ± 6.4
trifluralin	96.6 ± 2.8	96.9 ± 3.2	97.9 ± 5.0	procymidone	97.0 ± 2.8	99.8 ± 5.4	97.7 ± 3.8
simazine	93.2 ± 2.0	90.7 ± 3.2	89.4 ± 3.3	methidathion	97.3 ± 3.1	99.5 ± 4.5	97.6 ± 3.7
atrazine	98.1 ± 1.6	98.6 ± 2.9	97.4 ± 2.4	endosulfan I	100.5 ± 2.6	97.1 ± 8.6	100.9 ± 5.5
lindane	97.8 ± 2.3	98.5 ± 3.9	97.8 ± 3.4	profenophos	96.7 ± 4.1	98.5 ± 4.9	96.7 ± 4.9
terbuthylazine	97.3 ± 2.0	98.7 ± 2.5	98.2 ± 2.4	oxadiazon	96.8 ± 3.5	98.3 ± 4.4	96.5 ± 4.7
diazinon	97.1 ± 2.0	98.0 ± 2.6	98.1 ± 2.8	cyproconazole	96.0 ± 4.0	98.0 ± 6.0	97.3 ± 3.5
chlorothalonil	97.6 ± 1.9	99.0 ± 3.2	98.2 ± 3.6	endosulfan II	97.2 ± 3.5	99.4 ± 5.9	98.9 ± 9.0
triallate	96.5 ± 4.4	98.7 ± 4.4	97.9 ± 4.6	ethion	96.7 ± 3.2	98.3 ± 5.5	97.9 ± 3.9
metribuzin	89.9 ± 2.4	87.2 ± 5.6	86.4 ± 4.2	ofurace	91.9 ± 4.3	89.2 ± 6.6	89.7 ± 9.2
parathion-methyl	97.5 ± 2.5	98.9 ± 4.8	100.2 ± 8.3	benalaxyl	96.7 ± 3.9	98.3 ± 4.9	96.2 ± 3.4
tolclofos-methyl	97.1 ± 2.9	98.3 ± 2.9	98.2 ± 3.0	endosulfan sulfate	96.9 ± 3.4	98.6 ± 5.3	97.4 ± 4.5
alachlor	95.7 ± 4.4	98.4 ± 3.6	96.8 ± 3.9	nuarimol	97.4 ± 4.4	97.8 ± 2.4	98.3 ± 2.7
prometryn	98.2 ± 4.6	97.3 ± 6.6	100.6 ± 5.6	bromopropylate	98.1 ± 5.1	98.1 ± 2.3	99.4 ± 3.7
terbutryn	90.3 ± 2.2	92.2 ± 3.4	90.6 ± 2.9	tetradifon	97.6 ± 4.3	100.1 ± 3.3	100.2 ± 6.9
fenitrothion	96.3 ± 3.0	98.3 ± 4.5	98.8 ± 7.2	amitraz	88.8 ± 5.1	96.8 ± 3.6	89.4 ± 5.8
pirimiphos-methyl	97.2 ± 2.8	96.7 ± 3.0	97.2 ± 4.0	cyhalothrin	94.4 ± 3.4	95.8 ± 3.7	97.5 ± 4.6
dichlofluanid	96.4 ± 3.7	98.1 ± 3.8	95.8 ± 4.5	fenarimol	96.9 ± 3.7	99.0 ± 3.6	96.3 ± 4.8
aldrin	96.4 ± 5.3	96.4 ± 4.2	95.9 ± 4.4	acrinathrin	93.7 ± 4.3	96.6 ± 4.3	95.2 ± 3.5
malathion	97.2 ± 2.2	97.8 ± 3.0	98.4 ± 3.5	coumaphos	98.8 ± 4.8	100.7 ± 4.7	99.8 ± 3.9
metolachlor	95.9 ± 2.7	98.2 ± 4.5	95.7 ± 5.2	cypermethrin	96.9 ± 3.8	98.4 ± 3.7	96.3 ± 3.2
fenthion	98.7 ± 2.8	100.8 ± 3.7	100.4 ± 5.5	fluvalinate tau-I	98.3 ± 3.7	98.2 ± 5.9	98.8 ± 5.9
chlorpyrifos	96.5 ± 1.6	95.1 ± 5.7	98.0 ± 6.4	fluvalinate tau-II	97.1 ± 4.9	98.0 ± 5.7	96.7 ± 4.0

[a] Results are the mean of three different honeys (orange, rosemary, and multifloral) (four replicates of each honey at each fortification level).

The mass spectrometric detector (MSD) was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from *m*/*z* 60 to 500 at 3.62 s per scan. The ion source temperature was 230 °C and the quadrupole temperature 150 °C. The electron multiplier voltage (EM voltage) was maintained 1000 V above autotune, and a solvent delay of 5 min was employed.

Analysis was performed with selected ion monitoring (SIM) using one target and two qualifier ions. The target and qualifier abundances were determined by injection of individual pesticide standards under the same chromatographic conditions using full scan with the mass/charge ratio ranging from *m*/*z* 60 to 500. Quantification was based on the peak area ratio of the target ion divided by the peak area of the internal standard in samples versus those found in the calibration standard. Standards were prepared in blank matrix extracts, to counteract the matrix effect. **Table 1** lists the pesticides along with their retention times, the target and qualifier ions, and their qualifier to target abundance ratios. The SIM program used to determine and confirm pesticides in honey is indicated in **Table 2**. Pesticides were confirmed by their retention times, the identification of target and qualifier ions, and the determination of qualifier to target ratios. Retention times had to be within ±0.2 min of the expected time, and qualifier to target ratios had to be within a 20% range for positive confirmation.

Sample Preparation. *Honey Samples.* Various Spanish commercial honeys were purchased: five unifloral (orange, rosemary, lavender, eucalyptus, and thyme) and one multifloral. In addition, several citrus honeys were collected directly from the producers in Valencia. The honey samples were stored a 4 °C until analysis.

Extraction Procedure. A 10 g amount of honey was dissolved in 10 mL of water/methanol (70:30) in a Sovirell tube and fortified with 0.5 mL of the mixture of the different pesticides in ethyl acetate, to give final concentrations in the range of 0.025−0.1 μg/g. Half a milliliter of ethyl acetate was added instead to unfortified samples. One Teflon frit and 1 g of C_{18} were placed at the end of each column and preconditioned by washing first with 3 mL of acetonitrile and then with 5 mL of water. The honey solutions were transferred to the column, and the Sovirell tube was washed with 5 mL of water/methanol (70:30), which was also transferred to the column. Pesticides retained in the solid phase were eluted twice with 5 mL of hexane/ethyl acetate (50:50). Columns were placed on a 12-port vacuum manifold, and solvent was filtered and collected in 10 mL graduated tubes. A 1.0 mL volume of the internal standard solution (hexazinone, 1 μg/mL) was added to each sample, which was previously concentrated with a gentle stream of air to allow a final volume of 10 mL for higher recovery levels or 2 mL for the lowest recovery assay and real samples. A small amount of anhydrous sodium sulfate was added to dry the concentrated extracts, which were stored at 4 °C until analyzed by GC-MS. Chromatographic standards were prepared using blank sample extracts. These blank extracts were fortified with 0.5 mL of the pesticide standard solution and 1.0 mL of the internal standard solution (1 μg/mL).

RESULTS AND DISCUSSION

Gas Chromatographic Determination. Pesticide residue levels were determined by GC-MS-SIM. When standards were prepared by spiking blank honey samples with known amounts of pesticides, higher peak areas were obtained for the same pesticide concentration. This can be explained by a matrix effect that improves transfer of analytes from the injection port to the column and enhances the chromatographic response of pesticides. **Figure 2** shows the chromatographic response enhancement when blank honey samples are fortified with standard solutions. This effect is clearly observed in triazine and organophosphorus pesticides, with a response increase from 2- to 7-fold. Fenthion only appears when fortified blank samples are injected. Thiocarbamate, dinitroaniline, and organochlorine pesticides are the compounds presenting a lower matrix effect, with an increase of ∼80%. A matrix effect in the determination of pesticides in honey and other foodstuffs has been previously reported by other authors (*5*, *22*, *26*). Therefore, the quantification of pesticide residues was carried out using fortified blank samples with the addition of an internal standard. The chromatographic program used allows a good resolution of the pesticide mixture in ∼41 min. The developed method provides adequately clean blank extracts for the determination of pesticide residues by the chromatographic method used.

Method Validation. *Linearity*. The linearity of the chromatographic method was determined using blank honey samples

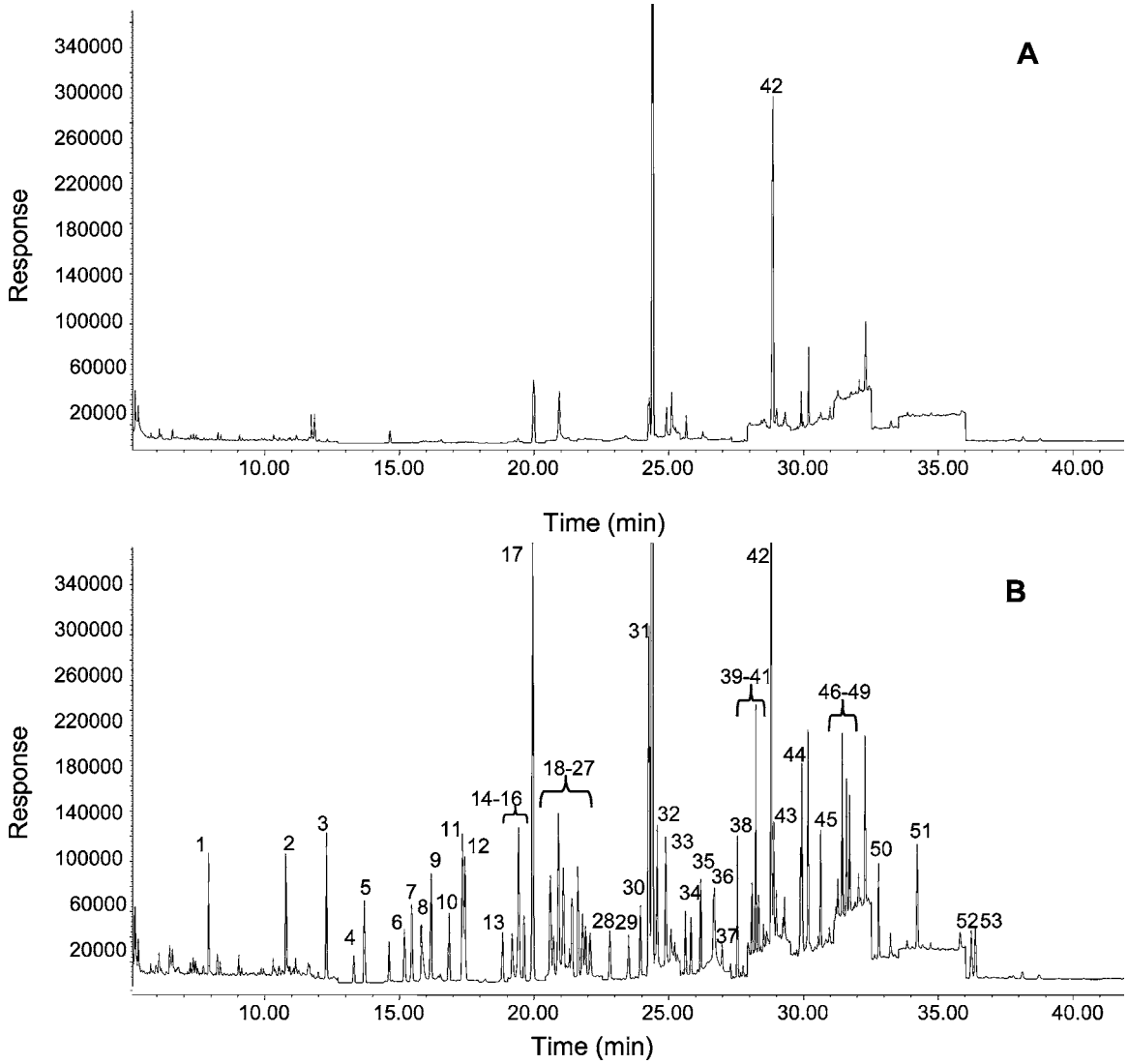

Figure 3. GC-MS-SIM chromatograms of a (**A**) blank multifloral honey sample and (**B**) multifloral honey sample fortified at 0.05 μg/g. See **Table 1** for peak identification.

fortified at levels of 25, 50, 100, and 200 μg/L containing 100 μg/L of the internal standard. The MS response for all pesticides was linear in the concentration range assayed with determination coefficients of >0.996 for all compounds. **Table 3** summarizes the calibration data for the studied pesticides.

Repeatability. The repeatability of the chromatographic method was determined by performing the analysis of a sample spiked at 50 μg/L. The sample was injected 10 times with automatic injection, and the relative standard deviation (RSD) values obtained for the retention times ranged from 0.06 to 0.003%, whereas for relative peak areas the values ranged from 2.8 to 8.0% (**Table 3**). Therefore, the repeatability achieved in these chromatographic conditions is very good. The repeatability of the complete analytical method was also determined by replicate analysis of a fortified sample during different days. The repeatability of the method, expressed as RSD, was <11% for all compounds.

Recovery. Pesticides were extracted from honey by SPE using hexanee/ethyl acetate (50:50) as elution solvent. This elution mixture showed the best results in comparison with other assayed solvents, such as hexane or hexane/dichloromethane (data not shown). **Table 4** shows the pesticide recovery results obtained with hexane/ethyl acetate (50:50). Honey was fortified at 0.1, 0.05, and 0.025 μg/g before extraction by adding 0.5 mL of the appropriate working standard solution and 1 mL of the internal standard (1 μg/mL), prior to the analysis by GC-MS-SIM. Four sample replicates spiked at each fortification level were assayed. **Figure 3** shows representative chromatograms of a blank and a fortified honey sample.

Similar results were observed for the various kinds of honeys analyzed and, therefore, the average values obtained for the different honeys at each fortification level are summarized in **Table 4**. The recovery obtained for all pesticides ranged from 86 to 101%. The precision of the method, expressed as the RSDs

Table 5. Pesticide Levels (Micrograms per Kilogram) Found in Honey Samples[a]

honey sample	dichlofluanid	ethalfluralin	triallate
raw citrus 1	7.5	ND[b]	ND
raw citrus 2	9.2	ND	ND
raw citrus 3	10.8	ND	ND
raw citrus 4	5.8	ND	ND
raw citrus 5	6.6	ND	4.4
thyme	ND	ND	4.3
lavender	ND	NQ[c]	ND

[a] A total of 11 honey samples were analyzed, and 7 samples (64%) were found to contain at least one of the pesticides determined. [b] Not detected. [c] Detected but not quantified (lower than LOQ).

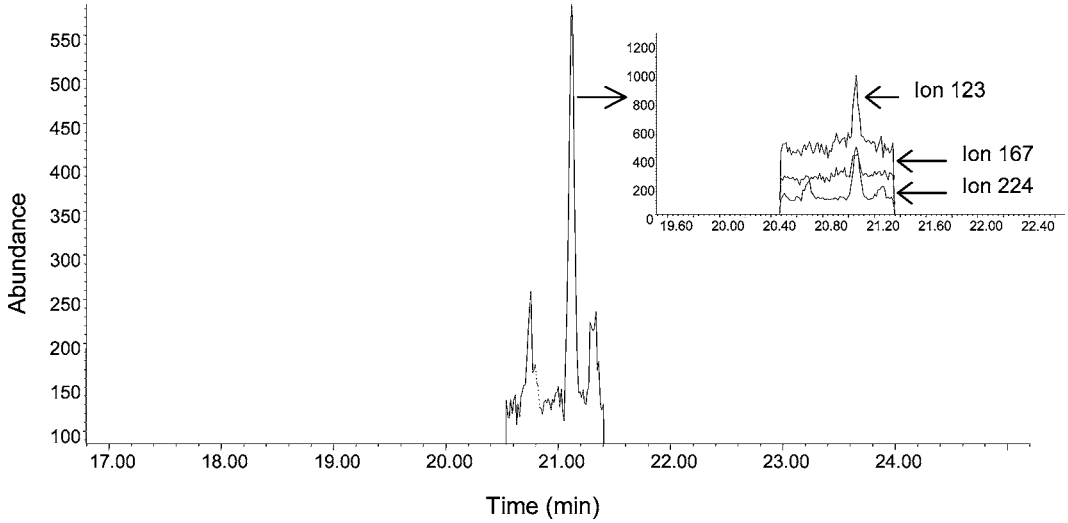

Figure 4. GC-MS-SIM chromatogram of a raw citrus honey. Dichlofluanid was determined at 9.2 μg/kg and confirmed by the selected ions (123, 167, and 224).

of analyte recoveries, is good, <10%. The ions used for quantification are shown in **Table 1**. The obtained values are similar to the recoveries reported by other authors using SPE (*8*, *26*) or SFE (*5*) for the analysis of pesticides in honey.

Detection and Quantification Limits. Honey blank samples were used to determine the detection and quantification limits. The limits of detection (LOD) and the limits of quantification (LOQ) were established by considering values 3 and 10 times the background noise of the blank samples, respectively. **Table 3** summarizes the LODs and LOQs obtained for the individual pesticides in honey. Prometryn, endosulfan I, and procymidone presented higher LODs due to the higher background noise around their retention times. The range of LODs achieved is, in general, lower than those obtained by other authors (*4*, *8*, *18*, *24*).

Application of the SPE Procedure to Real Samples. The developed method was applied to the analysis of 11 different honeys, 6 of them being commercial honey samples of different botanical origin (lavender, orange, thyme, rosemary, eucalyptus, and multifloral) and 5 being raw citrus honey samples obtained directly from beekeepers. Seven samples (64%) contained at least one of the pesticides determined. The pesticides found were dichlofluanid, triallate, and ethalfluralin. **Table 5** summarizes the pesticide levels encountered in the honey samples analyzed, and **Figure 4** depicts the chromatogram of a raw citrus honey sample containing dichlofluanid.

A few works on the monitoring of pesticide residue levels in honey have been previously published. In former studies with Spanish honeys from the northwestern region a few acaricides, amitraz, coumaphos, and fluvalinate (*27*), and some organophosphorus pesticides, azinphos-methyl, diazinon, ethion, methamidophos, and phosalone (*28*), were found at low microgram per kilogram levels.

In a more recent study on Portuguese and Spanish honeys (*21*), organochlorine pesticides were the compounds most frequently detected at concentrations from 0.01 to 4.3 μg/g, mainly in Portuguese honeys, with lindane presenting the highest levels. Some acaricides and organophosphorus pesticides were also detected, but the concentrations found were lower than those observed for organochlorines.

In comparison with these studies, the pesticide residue concentrations found in our work are in the lower end. Contamination of the area surrounding bee colonies as well as pesticide use for treatment of beehives has a marked influence on the kind and concentration of contaminants found in honey. This may explain the different types and levels of pesticides encountered in the diverse honey production areas studied.

Conclusions. A rapid and sensitive method was developed for the determination in honey of more than 50 compounds belonging to various classes of pesticides in a single analysis. The proposed method involves SPE and direct GC-MS analysis without a further cleanup step. The main advantages of this method are that a small volume of organic solvents is required and a large number of pesticides can be simultaneously determined and confirmed in a single step with good reproducibility and low detection limits. The developed method was applied to the determination of the studied pesticides in various Spanish honeys, and dichlofluanid, triallate, and ethalfluralin were the pesticides found. Although at least one pesticide was detected in 64% of the samples, the levels found were very low.

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