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Multiresidue determination of pesticides in juice by solid-phase extraction and gas chromatography–mass spectrometry

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Abstract

A multiresidue method based on solid-phase extraction was developed for the simultaneous determination of 50 pesticides in commercial juices. The extraction procedure was carried out in C_{18} columns preconditioned with acetonitrile and water. The subsequent elution of pesticides was performed with a mixture of hexane-ethyl acetate (1:1, v/v) prior to the determination by gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode (GC–MS–SIM), using one target and two qualifier ions. Standards were prepared spiking blank juice samples to counteract the observed matrix effect. Average recoveries for all the pesticides studied were higher than 91% with relative standard deviations lower than 9% in the concentration range of 0.02–0.1 μg/mL and the detection limits achieved ranged from 0.1 to 4.6 μg/L. The proposed method was applied to the analysis of these compounds in commercial juices and diazinon, ethion and procymidone were the pesticides encountered, although the levels found were very low.

Keywords: Multiresidue; Juice; Pesticides; Gas chromatography–mass spectrometry; SPE

1. Introduction

A large number of pesticides are often employed to control pest and diseases that damage fruits. Residues left after pesticide treatment may penetrate plant tissues and appear in the pulp and juice of fruit and vegetables, although their concentrations are, in general, lower than those observed in whole fruit [1]. The presence of pesticide residues in food is one important concern for consumers, due to their possible long adverse health effects, especially for children as they consume a higher proportion of fruits and vegetables in relation to their body weight and are more susceptible to chemicals since they are in early development stages. Government agencies and international organisations limit the amount of pesticides in food establishing maximum residue limits (MRLs), with the aim of protecting the consumers' health [2].

Multiresidue methods have been developed for the analysis of pesticide residues in fruits and vegetables [3–6] but few methods are available in the scientific literature for their determination in juice. Since only trace amounts of pesticides are usually found in juice samples, preconcentration and purification steps are required. The type of matrix has an important influence on the particular sample preparation applied and in the case of fruit juices, solid-phase extraction (SPE) [7,8] and matrix solid-phase dispersion (MSPD) [9–12] have been used with good results. In these cases, a simultaneous extraction and cleanup of extracts occurs, which often allows the direct analysis. Solid-phase microextraction (SPME), a solventless and highly sensitive procedure, has been also applied for multiclass pesticide analysis in juice [13–15].

The most frequently used technique for analysis of pesticides in juices is gas chromatography with different selective detectors as electron-capture (ECD) [16,17], nitrogen-phosphorus (NPD) [18], and flame photometry (FPD) [19]. The confirmation of residue identity is usually performed

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by mass spectrometry (MS) using selected ion monitoring [20]. Among the analytical methods used in residue analysis, liquid chromatography (LC) with diode-array [6,8] is effective in thermally labile and nonvolatile compounds determination. Liquid chromatography coupled with mass spectrometry (LC-MS) or with tandem mass spectrometry (LC-MS-MS) has lately become a powerful analytical technique for the identification and quantification of residues in juices [12,21].

The frequent application of diverse types of pesticides makes necessary the determination of as many compounds as possible in a single analysis. In addition, the use of reliable miniaturised methods is also desirable to carry out the analysis of samples with a low solvent consumption. MSPD was initially employed in our laboratory for the analysis of various pesticide classes in fruit juices [9,22]. Nevertheless, due to some limitations of the method, particularly the small input of sample allowed, this technique presented the drawback of not achieving the required detection limits when a wide number of pesticides were determined, mainly for those compounds showing a low detector response. To overcome these difficulties, the use of SPE as an alternative sample preparation procedure was studied.

This paper presents a rapid multiresidue method based on solid-phase extraction to simultaneously determine and confirm 50 pesticides in fruit and vegetable juices by gas chromatography–mass spectrometry. The method was applied to the analysis of these compounds in different juice samples taken from the supermarket.

2. Experimental

2.1. Apparatus

2.1.1. GC–MS

An Agilent 6890 (Waldbronn, Germany) gas chromatograph equipped with an automatic split-splitless injector Model HP 7683 and a 5973 series mass-selective detector was used for the analysis of the pesticides studied. 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 used, with helium as carrier gas at a constant flow of 1 mL/min. The temperature programme was the following: initial temperature, 70 °C held for 2 min, then at rate of 25 °C/min to 150 °C, 3 °C/min to 200 °C, and 8 °C/min to 280 °C maintaining this temperature 10 min. The total analysis time was 41.87 min and the equilibration time 2 min. The temperature of the injection port was 280 °C and a 2 μL volume was injected in pulsed splitless mode (pulsed pressure 310 kPa for 1.5 min).

Mass spectrometric parameters: electron impact ionisation mode with an ionising energy of 70 eV, ion source temperature 230 °C, MS Quad temperature 150 °C, electron multiplier voltage 1000 V above autotune when performing selected ion monitoring, scanning from *m*/*z* 60 to 500 at 3.62 s per scan; solvent delay, 5 min.

Analysis was performed in the selected ion monitoring mode (SIM) based on the use of one target and two qualifier ions. Pesticides were identified according to the retention times, the target and qualifier ions, and the qualifier to target abundance ratios. Target and qualifier abundances were determined by injection of individual pesticide standards under the same chromatographic conditions in full-scan mode. Quantification was based on peak area ratio of the target ion divided by the internal standard. Spiked blank samples were used as standards to counteract possible matrix effects. Table 1 summarises the pesticides studied with their retention times, the target and qualifier ions, and the qualifier to target abundance ratios. Table 2 indicates the SIM programme used to analyse pesticides in juice.

2.1.2. Extraction equipment

The extraction was carried out in polypropylene columns (5 mL, 6 cm × 12 mm i.d.) supplied by Becton-Dickinson (Madrid, Spain). Teflon frits of 1 cm diameter and 20 μm pore size (Supelco, Bellefonte, PA, USA), placed at the end of the column, were used. A 12-port vacuum manifold (Supelco Visiprep, Madrid, Spain) was employed to filter the samples.

2.2. Materials

2.2.1. Chemicals and reagents

Pesticide standards, 99% purity, were purchased from Riedel-de Häen (Seelze, Germany). Ethyl acetate, hexane, methanol, dichloromethane, and acetonitrile, residue analysis grade, were obtained from Scharlab (Barcelona, Spain). Water, HPLC grade, was purchased from Scharlab (Barcelona, Spain). Silica Bondesil-C_{18}, particle diameter of 40 μm, was obtained from Scharlab (Barcelona, Spain) and anhydrous sodium sulfate, reagent grade, was from Merck (Darmstadt, Germany).

2.2.2. Pesticide solutions

Stock solutions (500 μg/mL) of each pesticide were prepared by dissolving 50 mg of the pesticide in 100 mL ethyl acetate. An intermediate stock solution containing all the pesticides at 5 μg/mL was prepared transferring 1 mL of each stock solution to a 100 mL volumetric flask and diluting to volume with ethyl acetate. Three working solutions containing 2.0, 1.0, and 0.4 μg/mL of each pesticide in ethyl acetate were used to fortify juice samples. Stock and working solutions were stored at 4 °C and used for no longer than 3 months and 1 week, respectively.

2.2.3. Internal standard solution

The internal standard solution was prepared by dissolving 50 mg hexazinone in 100 mL ethyl acetate. A working internal standard solution of 1 μg/mL in ethyl acetate was prepared.

Table 1
Retention times (t_R, min), target ion (T), qualifier ions (Q_1, Q_2), and qualifier to target abundance ratios (Q_1/T, Q_2/T)[a] of the pesticides studied

	Pesticide	t_R	T	Q_1	Q_2	Q_1/T (%)	Q_2/T (%)
1	EPTC	7.91	128	189	86	24.2	65.8
2	Molinate	10.77	126	187	83	21.5	14.5
3	Propachlor	12.27	120	176	93	38.2	31.0
4	Ethalfluralin	13.29	276	316	292	76.2	46.3
5	Trifluralin	13.68	306	264	290	74.8	13.4
6	Simazine	15.18	201	186	173	73.3	29.4
7	Atrazine	15.44	200	215	173	57.4	22.4
8	Lindane	15.88	183	219	147	82.6	38.4
9	Terbuthylazine	16.14	214	229	173	28.2	37.6
10	Diazinon	16.81	179	137	304	103.2	48.3
11	Chlorothalonil	17.33	266	264	270	100.0	10.8
12	Triallate	17.41	86	268	128	39.6	21.2
13	Metribuzin	18.81	198	144	182	14.3	8.4
14	Parathion-methyl	19.18	263	109	125	92.8	79.4
15	Tolclofos-methyl	19.41	265	125	250	23.5	10.8
16	Alachlor	19.66	160	188	146	88.4	91.2
17	Prometryn	19.91	241	184	226	73.1	55.4
18	Terbutryn	20.58	226	241	185	48.7	73.8
19	Fenitrothion	20.71	277	125	260	113.8	39.9
20	Pirimiphos-methyl	20.95	290	276	305	85.9	67.7
21	Dichlofluanid	21.06	123	224	167	47.0	39.3
22	Aldrin	21.34	263	293	221	38.1	19.8
23	Malathion	21.38	173	127	93	104.3	79.3
24	Metolachlor	21.64	162	238	146	57.0	14.2
25	Fenthion	21.77	278	169	109	24.1	26.6
26	Chlorpyrifos	21.89	197	314	97	67.4	69.2
27	Triadimefon	22.06	208	181	128	27.6	51.6
28	Butralin	22.79	266	267	295	100.0	58.9
29	Pendimethalin	23.49	252	281	220	13.0	16.0
30	Phenthoate	24.05	274	246	125	23.4	28.4
31	Procymidone	24.26	283	96	285	118.8	70.0
32	Methidathion	24.56	145	85	125	83.8	16.4
33	Endosulfan I	24.89	241	195	239	94.5	33.0
34	Profenophos	25.80	208	339	139	75.6	80.2
35	Oxadiazon	26.16	175	258	334	51.9	18.5
36	Cyproconazole	26.68	222	139		51.3	
37	Endosulfan II	26.96	195	237	241	83.7	36.4
38	Ethion	27.54	231	153	384	67.5	11.7
39	Ofurace	28.08	132	160	281	79.4	34.5
40	Benalaxyl	28.22	148	206	91	25.9	40.6
41	Endosulfan sulfate	28.33	272	229	387	63.6	52.9
42	Hexazinone	28.80	171	128	83	14.9	12.2
43	Nuarimol	28.88	235	203	314	78.1	53.5
44	Bromopropylate	29.92	341	183	343	42.4	49.4
45	Tetradifon	30.62	159	229	111	58.2	50.3
46	λ-Cyhalothrin	31.43	181	197	208	71.1	52.7
47	Fenarimol	31.58	139	219	330	76.2	31.9
48	Pyrazophos	31.74	221	373	181	17.5	20.6
49	Coumaphos	32.78	362	226	109	62.0	102.4
50	α-Cypermethrin	34.20	181	163	209	98.9	63.9
51	Fluvalinate tau-I	36.21	250	181	252	19.4	33.8
52	Fluvalinate tau-II	36.35	250	181	252	20.1	33.1

[a] Q/T (%) are the results of abundance values of the qualifier ion (Q_1, Q_2) divided by the abundance of the target ion (T) × 100.

2.3. Juice samples

Various commercial brands of carrot, peach, grape, orange, pineapple, and apple juices were purchased from supermarkets in Madrid. Juice samples were analysed following the procedure described below and those samples showing the absence of target analytes were used as blank juice samples in the preparation of standards and in the recovery study.

2.4. Procedure

A 10 mL volume of juice was placed in a Sovirell tube and 3 mL of methanol was added. For recovery studies, samples were previously fortified adding 0.5 mL of the pesticide

Table 2
SIM program used to analyse and confirm pesticides in juice

Group	Time (min)	Pesticide	*m/z*	Dwell time (ms)	Scan rate (cycles/s)
1	5.00	EPTC, molinate	83, 86, 126, 128, 187, 189	100	1.44
2	11.70	Propachlor	93, 120, 176	100	2.86
3	12.70	Ethalfluralin, trifluralin	264, 276, 290, 292, 306, 316	100	1.44
4	14.40	Simazine, atrazine	173, 186, 200, 201, 215	100	1.72
5	15.70	Lindane, terbuthylazine	147, 173, 183, 214, 219, 229	100	1.44
6	16.60	Diazinon	137, 179, 304	100	2.86
7	17.15	Chlorothalonil, triallate	86, 128, 264, 266, 268, 270	100	1.44
8	17.90	Metribuzin	144, 182, 198	100	2.86
9	19.00	Parathion-methyl, tolclofos-methyl	109, 125, 250, 263, 265	100	1.72
10	19.59	Alachlor, prometryn	146, 160, 184, 188, 226, 241	100	1.44
11	20.40	Terbutryn, fenitrothion, pirimiphos-methyl, dichlofluanid	123, 125, 167, 185, 224, 226, 241, 260, 276, 277, 290, 305	50	1.27
12	21.26	Aldrin, malathion	93, 127, 173, 221, 263, 293	100	1.44
13	21.59	Metolachlor, fenthion, chlorpyrifos, triadimefon	97, 109, 128, 146, 162, 181, 197, 208, 238, 278, 279, 314	50	1.27
14	22.50	Butralin, pendimethalin	220, 252, 266, 267, 281, 295	100	1.44
15	23.85	Phenthoate, procymidone	96, 125, 246, 274, 283, 285	100	1.44
16	24.45	Methidathion, endosulfan I	85, 125, 145, 195, 239, 241	100	1.44
17	25.40	Profenophos, oxadiazon	139, 175, 208, 258, 339, 344	100	1.44
18	26.40	Cyproconazole, endosulfan II	139, 195, 222, 237, 241	100	1.72
19	27.30	Ethion	153, 231, 384	100	2.86
20	27.90	Ofurace, benalaxyl, endosulfan sulfate	91, 132, 148, 160, 206, 229, 272, 281, 387	50	1.69
21	28.60	Hexazinone (IS)[a], nuarimol	83, 128, 171, 203, 235, 314	100	1.44
22	29.50	Bromopropylate, tetradifon	111, 159, 183, 229, 341, 343	100	1.44
23	31.10	λ-Cyhalothrin, fenarimol, pyrazophos	139, 181, 197, 208, 219, 221, 330, 373	50	2.17
24	32.50	Coumaphos	109, 226, 362	100	2.86
25	33.50	α-Cypermethrin	163, 181, 209	100	2.86
26	36.00	Fluvalinate tau-I, fluvalinate tau-II	181, 250, 252	100	2.86

[a] IS: internal standard.

mixture in ethyl acetate, to give final concentrations in the range of 0.02–0.1 μg/mL. The extraction columns, with a Teflon frit and 1 g of C_{18} placed at the end, were preconditioned with 3 mL of acetonitrile and 5 mL of water using a 12-port vacuum manifold to filter the solvents. The juice solution was transferred to the column and the Sovirell tube was washed with 5 mL of water that were also transferred to the column. The elution of the pesticides retained in the solid-phase was carried out twice with 5 mL of hexane-ethyl acetate (1:1, v/v) and the combined eluates were collected in 10 mL graduated tubes. The eluates were concentrated under a gentle stream of air to allow the addition of 1 mL of the internal standard. The final volume of the higher fortification levels was 10 and 2 mL for the lowest fortification level and real samples. Anhydrous sodium sulfate was added to guarantee the dryness of the extracts that were then stored at 4 °C until the chromatographic analysis.

3. Results and discussion

3.1. Analysis by gas chromatography–mass spectrometry

In previous works, an enhancement of the chromatographic response was observed when blank juice samples were spiked with known amounts of pesticides, due to the matrix effect [9,22]. Fig. 1 shows the different responses obtained when standards are prepared with blank juice samples or with ethyl acetate, and a blank juice sample chromatogram showing the absence of interferences at the retention time of the target analytes. Many compounds presented an increase in their chromatographic response, some of them from two- to five-fold, although organochlorine pesticides were the compounds that presented the lowest matrix effect. Sample components may compete for the active sites of the glass liner decreasing the interaction between the active sites and the analyte and thus a larger amount of analyte is transferred to the chromatographic column. This effect has been reported by other authors in the determination of pesticides in food commodities [18,23]. Therefore, quantification was carried out preparing standards with blank juice samples to counteract this matrix effect.

Pesticide levels were quantified and its identity confirmed with GC–MS–SIM as this chromatographic technique provides the necessary sensitivity required for trace analysis in juice samples. The use of target and qualifier ions, together with the qualifier to target abundance ratios and the retention times, allowed to confirm positively the identity of the pesticides studied. The possible variations in retention times and peak areas were diminished by the addition of an internal standard. A good resolution of all the pesticides stud-

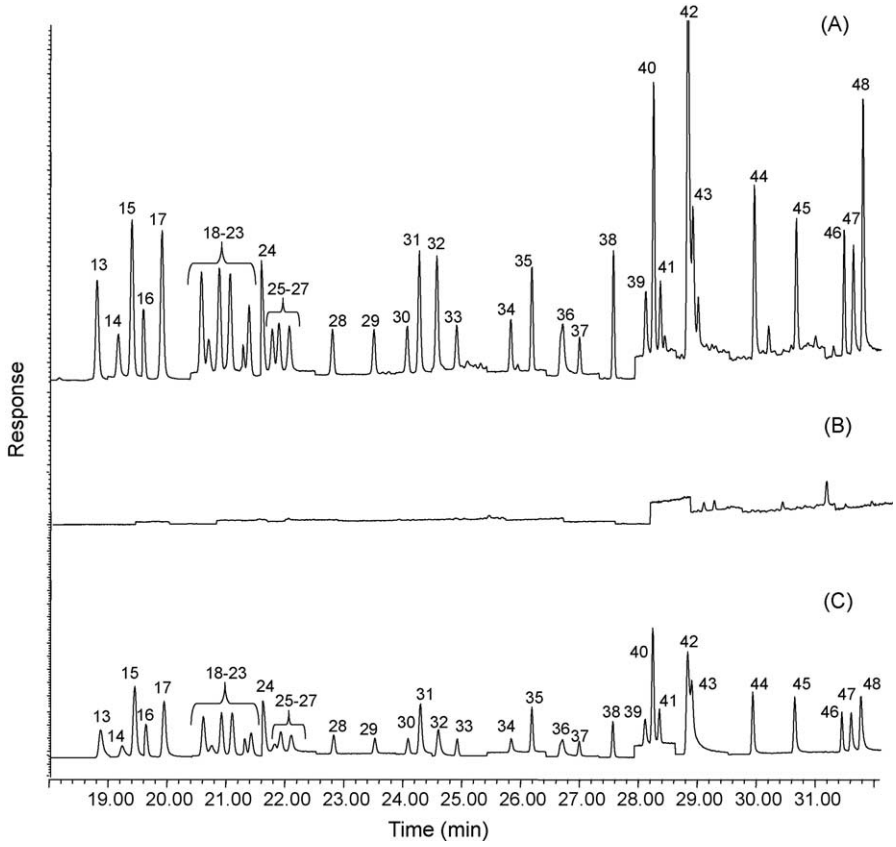

Fig. 1. Matrix effect observed in the GC–MS–SIM analysis of pesticides: (A) blank grape juice sample spiked at 0.05 μg/mL, (B) blank grape juice sample and (C) standard mixture solution in ethyl acetate at 0.05 μg/mL. See Table 1 for peak identification.

ied was achieved with the proposed chromatographic programme, Fig. 2.

3.2. Validation of the analytical method

3.2.1. Linearity

The linearity of the method was assayed by analysing blank juice samples fortified in the range from 0.02 to 0.2 μg/mL with the internal standard at 0.1 μg/mL. The detector response was linear in the range of concentrations studied with determination coefficients ≥0.996 for all the compounds. Table 3 summarises the calibration data for the pesticides studied.

3.2.2. Repeatability

The repeatability of the chromatographic method was established performing the analysis of a juice sample fortified

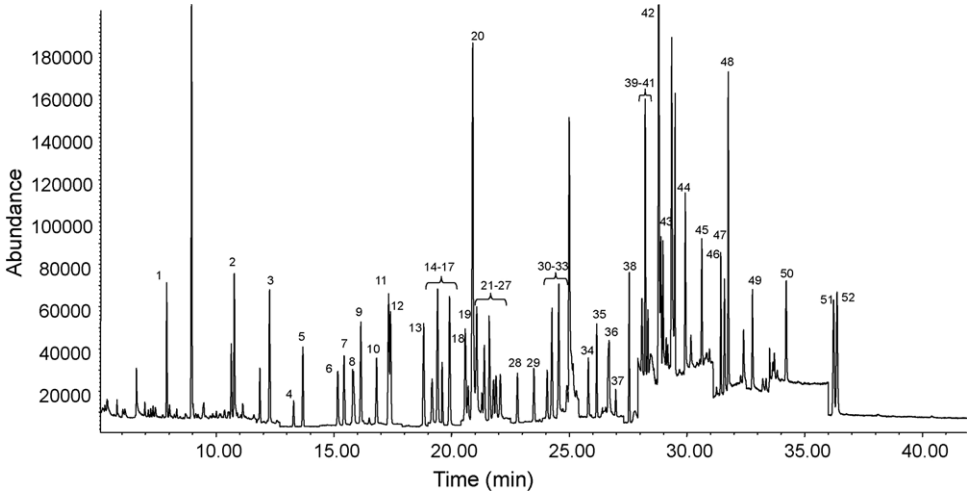

Fig. 2. GC–MS–SIM chromatogram of a carrot juice fortified at 0.05 μg/mL. See Table 1 for peak identification.

Table 3
Calibration data, limits of detection (LOD, μg/L), limits of quantification (LOQ, μg/L), and repeatability of the studied pesticides

Pesticide	Calibration data		LOD	LOQ	Repeatability (RSD, %)[a]	
	Equation	Determination coefficient			Peak area	t_R
EPTC	$y = 3.50 \times 10^{-1}x + 5.81 \times 10^{-3}$	1.000	4.6	15.2	3.8	0.018
Molinate	$y = 5.77 \times 10^{-1}x - 1.10 \times 10^{-3}$	1.000	0.1	0.3	4.7	0.009
Propachlor	$y = 4.77 \times 10^{-1}x + 1.76 \times 10^{-3}$	1.000	0.1	0.3	5.8	0.007
Ethalfluralin	$y = 6.98 \times 10^{-2}x - 4.87 \times 10^{-3}$	0.999	0.6	2.0	5.5	0.009
Trifluralin	$y = 3.45 \times 10^{-1}x - 5.81 \times 10^{-2}$	0.999	0.1	0.3	4.9	0.009
Simazine	$y = 1.95 \times 10^{-1}x - 5.47 \times 10^{-3}$	0.999	0.3	1.2	6.7	0.010
Atrazine	$y = 2.73 \times 10^{-1}x - 6.21 \times 10^{-3}$	1.000	0.1	0.3	5.7	0.008
Lindane	$y = 2.24 \times 10^{-1}x - 5.63 \times 10^{-3}$	0.999	1.3	4.3	5.2	0.007
Terbuthylazine	$y = 4.59 \times 10^{-1}x - 9.86 \times 10^{-4}$	1.000	1.4	4.7	6.5	0.007
Diazinon	$y = 2.12 \times 10^{-1}x + 4.35 \times 10^{-3}$	0.999	0.1	0.3	5.5	0.006
Chlorothalonil	$y = 4.48 \times 10^{-1}x + 1.18 \times 10^{-4}$	1.000	1.5	4.9	6.8	0.004
Triallate	$y = 4.00 \times 10^{-1}x - 4.24 \times 10^{-3}$	1.000	0.2	0.7	6.4	0.005
Metribuzin	$y = 8.18 \times 10^{-1}x - 7.35 \times 10^{-2}$	0.998	0.1	0.3	7.4	0.002
Parathion-methyl	$y = 1.26 \times 10^{-1}x - 8.67 \times 10^{-3}$	0.998	0.4	1.3	7.6	0.004
Tolclofos-methyl	$y = 8.85 \times 10^{-1}x - 7.04 \times 10^{-3}$	1.000	0.3	1.0	6.2	0.005
Alachlor	$y = 1.51 \times 10^{-1}x - 3.81 \times 10^{-3}$	1.000	0.5	1.6	7.0	0.003
Prometryn	$y = 5.17 \times 10^{-1}x - 2.11 \times 10^{-2}$	0.999	0.4	1.3	6.8	0.005
Terbutryn	$y = 3.51 \times 10^{-1}x - 1.49 \times 10^{-2}$	0.999	0.1	0.3	7.0	0.004
Fenitrothion	$y = 1.07 \times 10^{-1}x - 7.73 \times 10^{-3}$	1.000	0.6	2.0	8.1	0.005
Pirimiphos-methyl	$y = 2.54 \times 10^{-1}x - 1.47 \times 10^{-2}$	0.999	3.8	12.5	6.6	0.003
Dichlofluanid	$y = 3.53 \times 10^{-1}x - 1.21 \times 10^{-2}$	0.999	1.2	4.0	7.3	0.003
Aldrin	$y = 6.96 \times 10^{-2}x - 3.08 \times 10^{-4}$	1.000	1.7	5.6	7.3	0.002
Malathion	$y = 2.43 \times 10^{-1}x - 1.48 \times 10^{-2}$	0.998	0.1	0.3	7.4	0.004
Metolachlor	$y = 2.86 \times 10^{-1}x - 2.73 \times 10^{-3}$	1.000	4.2	13.9	8.2	0.000
Fenthion	$y = 5.37 \times 10^{-1}x - 6.31 \times 10^{-2}$	0.996	0.4	1.3	7.9	0.005
Chlorpyrifos	$y = 1.28 \times 10^{-1}x - 8.21 \times 10^{-3}$	0.999	0.4	1.3	7.0	0.003
Triadimefon	$y = 2.14 \times 10^{-1}x - 1.04 \times 10^{-2}$	1.000	0.2	0.7	7.4	0.004
Butralin	$y = 3.38 \times 10^{-1}x - 6.39 \times 10^{-2}$	0.999	0.6	2.0	7.7	0.002
Pendimethalin	$y = 2.88 \times 10^{-1}x - 5.27 \times 10^{-2}$	1.000	0.5	1.6	6.4	0.004
Phenthoate	$y = 2.51 \times 10^{-1}x - 1.27 \times 10^{-2}$	0.999	0.3	1.0	7.3	0.002
Procymidone	$y = 2.70 \times 10^{-1}x + 5.92 \times 10^{-3}$	1.000	0.1	0.3	6.4	0.005
Methidathion	$y = 4.67 \times 10^{-1}x - 1.75 \times 10^{-2}$	0.999	0.1	0.3	8.0	0.003
Endosulfan I	$y = 5.44 \times 10^{-2}x + 5.23 \times 10^{-4}$	1.000	0.2	0.7	6.0	0.004
Profenophos	$y = 1.10 \times 10^{-1}x - 1.26 \times 10^{-3}$	1.000	0.3	1.0	8.7	0.001
Oxadiazon	$y = 2.75 \times 10^{-1}x + 1.89 \times 10^{-3}$	1.000	1.1	3.6	7.0	0.002
Cyproconazole	$y = 4.27 \times 10^{-1}x - 3.13 \times 10^{-2}$	0.998	0.1	0.3	6.4	0.002
Endosulfan II	$y = 5.65 \times 10^{-2}x - 1.27 \times 10^{-4}$	0.999	0.8	2.6	7.3	0.003
Ethion	$y = 4.13 \times 10^{-1}x - 3.96 \times 10^{-2}$	0.998	0.1	0.3	7.7	0.003
Ofurace	$y = 1.50 \times 10^{-1}x - 8.87 \times 10^{-5}$	0.999	0.2	0.7	7.2	0.002
Benalaxyl	$y = 7.58 \times 10^{-1}x - 1.68 \times 10^{-2}$	1.000	0.1	0.3	5.7	0.002
Endosulfan sulfate	$y = 1.39 \times 10^{-1}x - 3.14 \times 10^{-3}$	0.999	0.6	2.0	5.5	0.002
Nuarimol	$y = 2.48 \times 10^{-1}x - 2.31 \times 10^{-3}$	1.000	0.2	0.7	5.5	0.003
Bromopropylate	$y = 4.61 \times 10^{-1}x - 1.45 \times 10^{-2}$	1.000	0.1	0.3	5.3	0.001
Tetradifon	$y = 2.50 \times 10^{-1}x + 8.81 \times 10^{-3}$	1.000	0.5	1.6	4.3	0.002
λ-Cyhalothrin	$y = 3.15 \times 10^{-1}x - 4.18 \times 10^{-2}$	0.999	1.2	3.9	5.2	0.002
Fenarimol	$y = 2.31 \times 10^{-1}x - 6.43 \times 10^{-3}$	1.000	0.8	2.6	4.0	0.003
Pyrazophos	$y = 1{,}13 \times 10^{0}x - 8.27 \times 10^{-2}$	0.999	2.9	9.6	4.4	0.001
Coumaphos	$y = 2.35 \times 10^{-1}x - 2.23 \times 10^{-2}$	0.998	1.1	3.6	5.2	0.002
α-Cypermethrin	$y = 2.34 \times 10^{-1}x - 1.56 \times 10^{-2}$	0.999	1.5	4.9	5.4	0.003
Fluvalinate tau-I	$y = 3.95 \times 10^{-1}x - 1.72 \times 10^{-2}$	1.000	2.4	7.9	7.7	0.002
Fluvalinate tau-II	$y = 6.56 \times 10^{-1}x - 6.41 \times 10^{-2}$	0.999	0.4	1.3	5.0	0.004

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

at 0.05 μg/mL. The sample was injected 10 times with an automatic injector. A good repeatability, expressed as relative standard deviations (RSDs), was obtained for retention times and peak areas with values lower than 0.02 and 9%, respectively (Table 3). Moreover, the repeatability of the complete analytical method, expressed as RSD, was in the range from 4.0 to 8.9% for all the studied compounds and it was determined performing replicate analysis of a fortified sample during different days.

3.2.3. Recovery

Juice samples, previously analysed to verify the lack of the pesticides studied, were fortified at 0.1, 0.05, and 0.02 μg/mL before extraction and 1 mL of the internal standard (1 μg/mL)

Table 4
Recovery of the studied pesticides from juice samples (mean ± RSD, %)[a]

Compound	Fortification levels (μg/mL)			Compound	Fortification levels (μg/mL)		
	0.1	0.05	0.02		0.1	0.05	0.02
EPTC	97.1 ± 3.5	98.2 ± 3.5	100.5 ± 4.4	Triadimefon	99.7 ± 2.7	97.7 ± 6.1	100.6 ± 4.6
Molinate	98.0 ± 1.9	97.6 ± 3.2	99.0 ± 3.6	Butralin	99.9 ± 3.8	96.1 ± 7.6	98.8 ± 5.5
Propachlor	98.6 ± 1.6	96.1 ± 5.6	98.9 ± 2.5	Pendimethalin	100.0 ± 4.4	95.9 ± 6.9	100.0 ± 8.7
Ethalfluralin	99.3 ± 2.8	97.1 ± 4.3	98.6 ± 4.5	Phenthoate	101.1 ± 5.0	97.0 ± 7.5	96.5 ± 5.8
Trifluralin	99.7 ± 2.8	96.3 ± 4.0	99.1 ± 4.6	Procymidone	100.9 ± 3.7	98.3 ± 7.8	96.3 ± 4.7
Simazine	97.7 ± 1.3	91.5 ± 6.1	95.6 ± 3.2	Methidathion	99.5 ± 4.6	97.1 ± 6.5	98.7 ± 5.7
Atrazine	99.9 ± 1.8	95.7 ± 4.4	99.1 ± 2.8	Endosulfan I	98.2 ± 4.5	95.5 ± 8.5	97.8 ± 5.1
Lindane	98.3 ± 2.7	92.4 ± 4.1	96.7 ± 3.4	Profenophos	98.7 ± 4.0	97.2 ± 6.9	100.3 ± 6.2
Terbuthylazine	100.3 ± 1.8	97.2 ± 2.5	99.4 ± 2.1	Oxadiazon	98.1 ± 3.4	97.3 ± 6.6	97.3 ± 3.2
Diazinon	99.7 ± 1.5	97.3 ± 2.0	94.9 ± 6.5	Cyproconazole	98.5 ± 4.0	96.1 ± 6.9	97.7 ± 4.6
Chlorothalonil	100.1 ± 2.1	96.7 ± 2.9	100.1 ± 4.5	Endosulfan II	98.9 ± 3.1	94.5 ± 8.9	101.2 ± 5.1
Triallate	98.0 ± 2.7	96.8 ± 3.2	100.1 ± 7.6	Ethion	99.0 ± 4.5	96.6 ± 7.8	98.9 ± 5.5
Metribuzin	98.9 ± 2.8	91.4 ± 4.9	98.0 ± 4.5	Ofurace	98.8 ± 4.1	97.2 ± 5.5	99.1 ± 6.0
Parathion-methyl	98.8 ± 2.8	96.2 ± 4.9	97.5 ± 5.6	Benalaxyl	99.0 ± 3.7	98.6 ± 6.1	99.3 ± 3.4
Tolclofos-methyl	100.3 ± 2.3	97.9 ± 5.4	98.6 ± 2.9	Endosulfan sulfate	99.6 ± 3.8	97.6 ± 7.3	98.7 ± 4.2
Alachlor	99.7 ± 3.1	96.7 ± 4.9	98.3 ± 3.2	Nuarimol	101.5 ± 3.5	98.2 ± 3.8	101.8 ± 4.0
Prometryn	98.6 ± 2.4	95.7 ± 5.8	97.3 ± 3.8	Bromopropylate	101.5 ± 3.1	98.0 ± 5.3	101.0 ± 3.1
Terbutryn	98.3 ± 2.8	95.3 ± 6.0	95.9 ± 4.5	Tetradifon	99.9 ± 2.1	98.0 ± 4.2	101.1 ± 5.0
Fenitrothion	99.1 ± 4.1	97.3 ± 6.9	98.3 ± 4.5	λ-Cyhalothrin	96.8 ± 4.1	95.4 ± 6.4	96.4 ± 4.6
Pirimiphos-methyl	99.5 ± 2.8	96.8 ± 6.2	98.8 ± 3.8	Fenarimol	100.0 ± 3.2	98.3 ± 2.4	101.8 ± 6.8
Dichlofluanid	98.8 ± 2.7	96.4 ± 7.0	98.4 ± 4.1	Pyrazophos	101.2 ± 2.9	98.4 ± 3.3	99.9 ± 5.0
Aldrin	99.2 ± 3.2	95.2 ± 6.4	98.1 ± 4.9	Coumaphos	100.2 ± 4.6	97.8 ± 5.0	100.5 ± 3.9
Malathion	99.0 ± 3.1	97.0 ± 5.8	98.2 ± 7.0	α-Cypermethrin	97.9 ± 4.0	97.0 ± 6.8	98.5 ± 5.1
Metolachlor	100.8 ± 4.6	95.2 ± 6.4	98.1 ± 4.7	Fluvalinate tau-I	96.5 ± 4.5	94.9 ± 7.9	99.1 ± 4.0
Fenthion	101.0 ± 4.0	97.5 ± 6.3	101.8 ± 4.9	Fluvalinate tau-II	96.6 ± 4.7	96.4 ± 7.3	97.6 ± 2.7
Chlorpyrifos	100.1 ± 2.9	97.1 ± 7.2	99.1 ± 3.9				

[a] Results are the mean of three different juices (orange, grape, and carrot) (four replicates of each juice at each fortification level).

was added prior to the chromatographic analysis. The average recoveries achieved following the proposed method are shown in Table 4. The recoveries obtained for all pesticides were >91% with RSDs <9%.

3.2.4. Detection and quantification limits

Blank juice samples were used to determine the detection and quantification limits for each pesticide. The limits of detection (LODs) were established by considering a value three times the background noise of the blank sample at the retention time of each pesticide, and the limits of quantification (LOQs) were calculated by considering a value 10 times that background noise. Table 3 shows the LODs and LOQs obtained for each pesticide. The LODs achieved with the proposed method are similar to those previously obtained by other authors for pesticides in fruit juices [8,14].

3.2.5. Application to real samples

The developed SPE method was applied to the determination of pesticides in commercial juices. Table 5 summarises

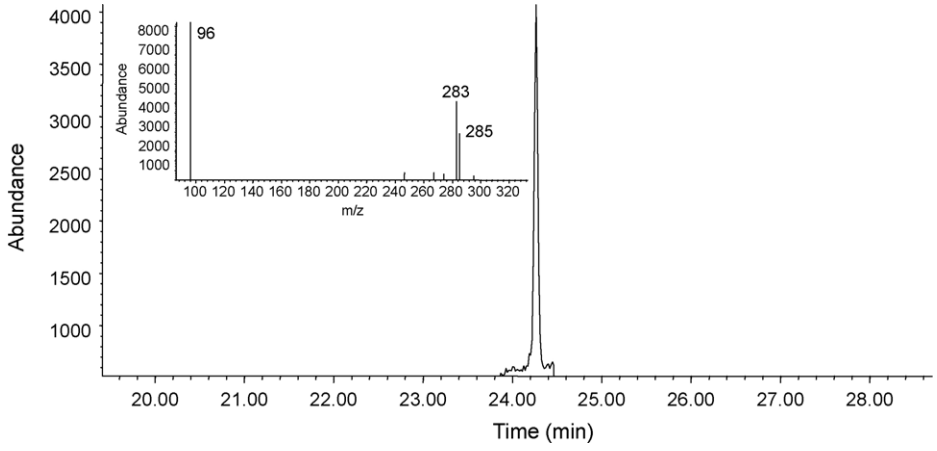

Fig. 3. GC–MS–SIM chromatogram of a commercial peach juice sample that contained procymidone at 3.1 μg/L.

Table 5
Pesticide levels (μg/L) found in juice samples[a]

Sample	Diazinon	Ethion	Procymidone
Orange			
1	ND[b]	0.3	ND
Pineapple			
1	0.5	ND	ND
2	0.5	ND	ND
Apple			
1	0.3	ND	ND
2	0.3	ND	ND
Peach			
1	ND	ND	3.1

[a] A total of 12 juice samples were analysed and six samples (50%) were found to contain at least one of the pesticides studied.
[b] ND: not detected.

the pesticide levels found in 12 juices commercialised in Spain (two different brands of each kind of juice: apple, peach, pineapple, orange, grape and carrot). Six samples (50%) contained at least one of the pesticides studied. The pesticides found were diazinon, ethion, and procymidone. Fig. 3 shows the chromatogram of a commercial peach juice sample that contained 3.1 μg/L of procymidone.

Data on pesticide residues found in fruit juices is rather scarce in the scientific literature. A few insecticides and fungicides have been detected in fruit juices by other authors [12,24] and the levels found are of the same order of those encountered in our study. However, the detected levels are much lower than the MRLs established for these pesticides in fruits and vegetables.

4. Conclusions

A simple and rapid method was developed to determine residues of 50 pesticides in various commercial fruit and vegetable juices. This method involves solid-phase extraction and direct GC analysis without a further clean up step. The GC–MS–SIM analysis showed a high sensitivity and confirmatory power necessary for the determination of pesticide residues at the levels found in juices. The proposed method allowed the simultaneous determination and confirmation of a large number of pesticides with good reproducibility and low detection limits. The developed method was applied to the determination of the studied pesticides in various Spanish juices and diazinon, ethion, and procymidone were the pesticides found, with 50% of the samples containing at least one pesticide.

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