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Multiresidue analysis of pesticides in olive oil by gel permeation chromatography followed by gas chromatography–tandem mass-spectrometric determination

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Abstract

A method for the multiresidue analysis of olive oil samples for 26 pesticides is proposed. Residual pesticides are extracted from oil using an *n*-hexane/acetonitrile mixture, extracts being cleaned-up by gel permeation chromatography (GPC) for analysis by gas chromatography–tandem mass spectrometry (GC–MS/MS). Electron ionization and chemical ionization are employed in a single analysis for the determination of pesticides. Pesticide recoveries from virgin and refined olive oil spiked with 10, 100 and 250 μg/kg concentrations of the pesticides ranged from 83.8 to 110.3%. The proposed method features good sensitivity: its limits of quantification are low enough to allow pesticide residues to be determined at concentrations below the maximum residue levels legally accepted. The precision, expressed as relative standard deviation, ranges from 4.93 to 8.11%. Applicability was tested on 40 olive oil samples. Several pesticides were detected in most of the virgin olive oil samples. By contrast, refined olive samples contained few pesticides, and only endosulfan sulphate was detected in all.

Keywords: Pesticides; Olive oil; Gel permeation Chromatography; Gas chromatography; Tandem mass spectrometry

1. Introduction

Several hundreds of pesticides of diverse chemical nature are widely used worldwide for agricultural and non-agricultural purposes. Fortunately, a large fraction of applied pesticides is degraded via chemical, physical or biological mechanisms in soil or water. The residual fraction, however, can persist in the environment and contaminate agricultural products via chemical effects or as a result of too early harvesting. As a consequence, consumers are indirectly exposed to pesticides, usually in minute quantities, in several food groups including meat, dairy products, fruits, vegetables, oil, dried foods, most processed foods and many other household staples [1,2]. In order to protect consumers' health, many countries have restricted the use of pesticides by establishing legal directives on maximum residue levels (MRLs) to control their presence in food [3,4].

Olive oil is a major ingredient of the Mediterranean diet on account of its nutritional and biological properties, but can be contaminated by insecticides and herbicides used in the production of olives. The Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have established MRLs for pesticide residues in olives and olive oil [3]. The European Community has set maximum levels for pesticide residues in and on certain products of plant origin including olives [4].

The low detection levels required by regulatory bodies and the complex nature of olive oil, efficient sample preparation and trace-level detection and identification capabilities are essential with a view to ensuring reliable analyses. The development of effective methods for pesticide multiresidue analysis is made difficult by the need to simultaneously extract and analyse compounds of variable polarity, volatility, solubility and pK_a. Lentza-Rizos and Avramides have reviewed existing methods for this purpose [5]. Most are based on partitioning between hexane or light petroleum and acetonitrile, followed by clean-up and chromatographic determination. Many clean-up procedures employ fractionation of extracts based on polarity as in liquid–liquid

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partitioning [6,7], or adsorption chromatography using either Florisil, neutral alumina or silica gel column [7–9], or gel permeation chromatography (GPC) [10]. Of the three techniques, GPC appears to be the best suited to multiresidue analysis as it affords clean-up of both polar and non-polar pesticides with a single injection on a fully automated system [11,12].

The technique of choice for the determination and quantification of pesticide residues in olive oil is currently gas chromatography (GC). The development of element-selective and specific detectors, and the replacement of packed columns with capillary columns, have turned GC into a highly successful analytical tool for pesticide analysis [5]. The GC detectors most commonly used for this purpose are of the flame ionization (FID) [11], nitrogen–phosphorus (NPD) [6,7,9,11,13], flame photometric (FPD) [7,12], electron capture [14] or mass-spectrometric (MS) type [15]. In recent years, tandem MS (MS/MS) based on bench-top ion-trap systems has become a major choice for pesticide residue analysis with increased selectivity and sensitivity [16–18].

Some pesticides are thermolabile and hence difficult to volatilize without decomposing. High-performance liquid chromatography (HPLC) can be effective for this purpose [19]. Thus, pesticide residues in olive oil have been successfully determined by reversed-phase liquid chromatography with mass-spectrometric detection [15]. Recently, Goto et al. developed a method for the determination of *N*-methyl carbamate pesticides in wine and juice by electrospray ionization liquid chromatography–tandem mass spectrometry [20]. Liquid chromatographic–triple quadrupole mass spectrometry and the liquid chromatograph–ion trap mass spectrometry have also been compared in the determination of pesticides in oranges [21].

The purpose of this work was to develop a multiresidue method for the determination of pesticide residues in olive oil. Sample preparation involved extraction with various organic solvents and clean-up by gel permeation chromatography. We tested a variety of solvents and GPC purification mechanisms in order to ensure the best possible analytical conditions for determining pesticides in such a complex matrix as olive oil. Gas chromatography with mass-selective detection afforded the determination of the pesticides at concentrations below their maximum allowed level. The proposed method was used to analyse various types of olive oil.

2. Experimental

2.1. Materials

Acetonitrile, *n*-hexane, cyclohexane, dichloromethane, diethyl ether, petroleum ether and methanol, all chromatographic-grade, were supplied by Merck (Darmstadt, Germany). All products (particularly dichloromethane, which is toxic) were handled with care, using efficient fume hoods and wearing protective gloves. Analytical-grade anhydrous sodium sulphate from Merck (Darmstadt) was also used. The pesticides and bromophos-methyl (the internal standard) were obtained from Dr. Ehrenstorfer (Ausburg, Germany).

Pesticide stock solutions (50 mg/l) were prepared by dissolving appropriate amounts of the pesticide standards in acetone and stored refrigerated at −20 °C in glass-stopped bottles in the dark. Pesticide working solutions were prepared by dilution of the stock solutions in cyclohexane. No degradation of the pesticides was detected under the storage conditions used at any time during the experiment.

2.2. Apparatus and methods

2.2.1. Solvent extraction

An amount of ca. 2 g of filtered olive oil sample was weighed into a 30 ml all-glass measuring flask with *n*-hexane (2 ml) and acetonitrile (10 ml), and supplied with 3 mg of anhydrous sodium sulphate and 40 μl of 25 μg/ml (1 μg) of internal standard (IS). The resulting mixture was stirred for 30 min and allowed to stand for 30 min. A volume of 10 ml of the extract (light layer) was then dried in a rotary evaporator at a reduced pressure and at temperature of about 45 °C, the residue being collected with 10 ml of dichloromethane for subsequent purification by GPC.

2.2.2. Clean-up by gel permeation chromatography

The GPC system consisted of a Varian Prostar 220 isocratic pump and Varian Prostar 410 auto-sampler attached to two Waters Envirogel GPC clean-up columns: a guard column (150 mm × 19 mm) and a main clean-up column (300 mm × 19 mm) packed with styrene–divinylbenzene copolymer (200–400 mesh). A Prostar 704 fraction collector was used to collect extracts, which were passed through a syringe filter (0.2 μm pore size) attached to a disposable plastic 5 ml syringe. The filtered extracts (5 ml, Section 2.2.1) were injected into the GPC column. The flow-rate was set at 5.0 ml/min and the mobile phase was dichloromethane at ambient temperature. The eluent was collected between 14 and 23 min in fraction collector tubes. A Waters 2487 Dual l UV–vis detector (Mildford, MA, USA) was used to make absorbance measurements of the GPC column eluates at 220 and 254 nm. The volume (45 ml) of extract containing the pesticides was reduced to 500 μl in a rotary evaporator at a low pressure at a temperature below 50 °C. Finally, the extract was diluted to 1 ml with cyclohexane.

2.2.3. GC–MS analysis

A Varian Instrument GC–MS (Sunnyvale, CA, USA) was used, equipped with a Varian 3800 GC and Saturn 2200 ion trap mass spectrometer. The MS was equipped with electron ionization (EI) and chemical ionization (CI) sources. The GC component was equipped with a 1079 PTV temperature programmable injector, electronic flow control (EFC), CP-1177 split/splitless injector and CP-8400 auto-sampler were used with the GC–MS. The glass liner was furnished with a plug of carbofrit (Resteck, Bellefonte, PA, USA). The sample was separated on HP-5 (crosslinked with 5% phenylmethylpolysiloxane) fused-silica column of 30 mm × 0.25 mm i.d., with 0.25 μm thickness. A 2 m × 0.25 mm i.d. column was used as precolumn (deactived silica tube).

2.2.3.1. GC conditions used for the separation. A 99.999% purity of helium carrier gas at a flow-rate of 1.0 ml/min was used. The PTV injector port temperature was programmed as follows: 70 °C (hold 0.5 min), ramp to 300 °C at 150 °C/min (hold 15 min). The oven temperature program was as follows: 70 °C (hold 3.5 min); ramp to 180 °C at 25 °C/min (hold 10 min); ramp to 300 °C at 4 °C/min (hold 10 min). Sample injection was done in the splitless mode, using a CP-8400 auto-sampler (injected volume 7 μl).

2.2.3.2. MS conditions used for the detection. The detector temperature (GC–MS transfer line) was 280 °C and the MS conditions were as follows: solvent delay 7 min; electron impact ionization voltage 70 eV; scan-rate 1.5 scans/s; scanned-mass range *m*/*z* 80–450. The ion-trap mass spectrometer was operated in the EI mode for the most of the pesticides and also in the CI mode for seven. The filament emission current was 30 and 90 μA for CI and EI, respectively. The reagent gas used for chemical ionization was methanol. The MS/MS process was carried out by collision-induced dissociation (CID) with non-resonant excitation for all analytes.

3. Results and discussion

3.1. Optimization of experimental variables

Seven organic solvents (viz. *n*-hexane, cyclohexane, petroleum ether, dichloromethane, diethyl ether, acetonitrile and methanol) were studied as extractants for the pesticides from olive oil. A mixture of 2 ml of *n*-hexane and 10 ml of acetonitrile was found to be the most efficient for extraction of the pesticides from 2 g of refined olive oil containing a 100 μg/g concentration of pesticides. The use of a vibromatic shaker was found to increase the extraction efficiency. A time of 30 min was found to ensure blending of the mixture of organic solvent and sample to an extent resulting in extraction yields close to 100%.

Extracts were dried in the rotary evaporator. The optimum conditions for this purpose were 50 °C, 45 rpm and a reduced pressure (50% vacuum).

In order to optimize the collection window for the pesticide fraction from GPC system, the extracts (5 ml) obtained as described in Section 2.2.1 were injected into the GPC column at 5.0 ml/min. Dichloromethane was found to provide the most efficient mobile phase among the seven organic solvents studied (*n*-hexane, cyclohexane, petroleum ether, dichloromethane, diethyl ether, acetonitrile and methanol). Molecular masses of most synthetic pesticides was between 200 and 400, whereas that of most lipids ranged from 600 to 1500. Hence, the larger lipid molecules that are too long to pass through the pores of polymer beads are not retained, and they are the first to be eluted from the column [10,22]. As can be seen in Fig. 1, the fat fraction was eluted between 9 and 12 min. On the other hand, the pesticides were detected between 15 and 22 min. Therefore, a pesticide collection window between 14 and 23 min was selected (1 min around the emergence of the pesticide fraction) in order to ensure their complete collection (Fig. 1). No perturbation phenol and

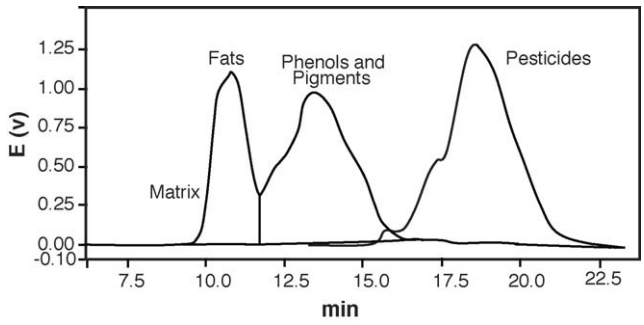

Fig. 1. Fractions obtained upon GPC clean-up of the olive oil extracts as determined by spectrophotometry at 220 and 254 nm.

pigment fraction (between 12 and 17 min) was detected over the chromatographic separation of the pesticides.

3.2. Optimization of the MS/MS parameters

MS/MS processes involve two steps, namely: formation and detection of ions. In the first step, the precursor ion, or an entire cluster of parent ions, are isolated in the trap; in the second, the precursor ion or product ions, are dissociated by collisional activation with an inert gas. Normally, the strongest parent ions are selected; when such ions have a low mass, however, it may be preferable to choose slightly weaker ions of higher mass. Once the ions have been selected, the excitation storage level must be set before the CID step is optimized [23].

The most common way of implementing CID is by applying a radio-frequency (RF) voltage to the end-cap electrodes of the ion trap in order to isolate ions with a selected *m*/*z* value or spanning a short range of *m*/*z* values. After isolation, a non-resonant excitation voltage is applied over the trap and collisions with helium buffer gas lead to the formation of product ions [24]. In order to perform these operations, the instrument's software uses a procedure called "automated method development" (AMD) for each individual pesticide. The principal variables of this process are the excitation amplitude and the excitation storage level. The parameter values used in this work are listed in Table 1. An excitation time of 25 ms was used throughout.

Once the MS/MS conditions were optimized, quantitation ions were selected and MS/MS espectra stored in our customized MS/MS library, for comparison with those of the target analytes. A positive analyte identification required a minimum spectral fit greater than 800 (with 1000 for identical spectra) and a signal-to-noise (S/N) ratio greater than 3. For quantification, the S/N ratio must be higher than 10.

3.3. Analytical performance

A calibration curve was constructed for each individual pesticide in order to determine its linearity range and limit of detection. The curves, in the form of straight lines, were obtained by injecting extracts of the pesticides at variable concentrations in cyclohexane into the gas chromatograph. The injected extracts were prepared from amounts of 2 g of refined olive oil (without pesticides) that were spiked with variable

Table 1
MS/MS conditions for the monitored pesticides

Serial no.	Pesticide	RTW[a] (min)	Range (*m*/*z*)	Precursor ion[b] (*m*/*z*)	CID[c] amplitude (V)	CID RF[d] (*m*/*z*)	Quantifiers (*m*/*z*)	Most abundant ion (*m*/*z*)
IS	Bromophos (IS)[e]	21.57–21.97	150–345	331 EI	75	90	284:286 + 316[f]	286
1	Trichlorfon	7.59–7.99	100–225	221 CI	62	80	141 + 145	141
2	Diuron	8.00–8.30	80–195	187 EI	77	75	124	124
3	Carbaryl	9.87–10.19	100–150	145 CI	51	60	117	117
4	Promecarb	11.80–12.10	80–215	208 CI	20	48	151	151
5	Dimethoate	12.69–12.99	100–240	230 CI	47	100	199	199
6	Simazine	13.01–13.30	100–240	202 CI	72	80	104	104
7	Terbuthylazine	13.71–14.01	135–235	230 CI	55	80	174	174
8	Formothion	15.61–16.21	100–205	199 CI	60	90	171 + 157	171
9	Chlorpyrifos methyl	16.48–17.08	80–300	286 EI	75	85	208 + 241	208
10	Parathion methyl	17.02–17.61	80–300	263 EI	44	80	136 + 246	136
11	Pirimiphos methyl	18.70–19.10	100–300	290 EI	64	85	151	151
12	Terbutryn	19.10–19.60	100–300	241 EI	48	75	170 + 185	170
13	Malathion	19.28–20.08	90–180	173 EI	39	75	99 + 127	99
14	Chlorpyrifos	19.77–20.57	150–325	314 EI	98	172	258	258
15	Parathion ethyl	20.57–20.97	110–300	291 EI	58	110	114 + 235	114
16	Methidathion	24.06–24.66	80–150	145 EI	45	70	85	85
17	Endosulfan α	24.72–25.32	150–345	339 EI	54	125	265:267	267
18	Oxyfluorfen	27.00–27.50	100–310	300 EI	90	90	132 + 223	132
19	Endonsulfan β	28.34–28.94	150–345	339 EI	53	125	265:267	267
20	Endosulfan sulphate	30.37–30.97	200–395	387 EI	36	71	251:255 + 289	289
21	Diflufenican	31.75–32.35	200–400	394 EI	82	150	266 + 374	374
22	Phosmet	33.20–33.50	90–165	160 EI	74	75	133	133
23	Fenoxycarb	33.50–34.0	100–265	255 EI	60	80	186	186
24	ʟ-Cyhalothrin	35.97–36.37	100–190	181 EI	64	60	152	152
25	α-Cypermethrin	40.65–41.15	85–190	181 EI	84	75	152	152
26	Deltamethrin	44.05–44.55	85–260	253 EI	42	70	172:174	172

[a] RTW: retention time window.
[b] EI: electron ionization, CI: chemical ionization.
[c] CID: collision-induced dissociation.
[d] RF: radio-frequency.
[e] IS: internal standard.
[f] Quantifiers: 284 + 285 + 286 + 316.

concentrations of the pesticides and 1 μg of IS, and subjected to the procedure described in Section 2.2. The linear ranges for the spiked refined olive oil samples are listed in Table 2. Linearity was good throughout the concentration range studied, with correlation coefficients between 0.9910 and 0.9995. The limits of detection (LOD) and quantification (LOQ) were determined as the minimum concentrations providing chromatographic signals 3 times and 10 times, respectively, higher than background noise (in our study, blank refined olive oil extracts without pesticides) [25]. The ranges for LOD and LOQ were 0.1–1.6 and 0.3–3.6 μg/kg, respectively. Similarly, the precision of the method (expressed as repeatability, $n = 11$) was checked on standards containing 10 μg/kg pesticide concentrations; the relative standard deviations obtained are shown in Table 2.

3.4. Recovery study

Pesticide recoveries were determined in triplicate at three different concentration levels (viz. 10, 100 and 250 μg/kg) in spiked samples of virgin olive oil and refined olive oil. As can be seen in Table 3, recoveries were satisfactory (>84%), most exceeding 90%. The precision, as determined under repeatability conditions, was good, with the vast majority of relative standard deviation values falling below 6%.

3.5. Application to olive oil samples

The validated method was applied to the analysis of 40 samples of olive oil (25 of virgin oil and 15 of refined oil) from Andalucía (Spain). This allowed us to compare the pesticides present in the samples depending on the manufacturing procedure used. Each sample was analysed in triplicate following the procedure described in Section 2.2.

As can be seen from Table 4, the virgin olive oil samples contained many of the pesticides studied including oxyfluorfen, endosulfan β, endosulfan sulphate, diflufenican, α-cypermethrin, diuron and terbuthylazine. Note the high concentrations of terbutryn in samples V5 and V9, those of terbuthylazine in V16 and V17, and that of simazine in V2. By contrast, the refined olive oil samples contained fewer pesticides (see Table 5). This was probably due to some of the pesticides being removed from the refining oil, but remaining in the natural, untreated oil matrix without altering its composition. In any case, all refined oil samples but one (R2) were found to contain endosulfan sulphate. In addition contain diuron, terbuthylazine

Table 2
Analytical figures of merit of the determination of pesticides

Serial no.	Pesticide	Linear range (μg/kg)	R^2	R.S.D.[a] (%)	Limit of detection (μg/kg)	Limit of quantification (μg/kg)
1	Trichlorfon	5–500	0.9994	6.71	1.6	3.6
2	Diuron	1–500	0.9989	6.14	0.2	0.6
3	Carbaryl	4–500	0.9976	7.35	0.9	2.4
4	Promecarb	2–500	0.9990	6.92	0.4	1.0
5	Dimethoate	2–500	0.9984	6.85	0.3	0.8
6	Simazine	3–500	0.9948	7.80	0.7	1.7
7	Terbuthylazine	1–500	0.9917	5.36	0.2	0.7
8	Formothion	1–500	0.9913	5.49	0.2	0.6
9	Chlorpyrifos methyl	2–500	0.9941	5.85	0.2	0.8
10	Parathion methyl	2–500	0.9933	6.12	0.3	0.8
11	Pirimiphos methyl	1–500	0.9916	4.93	0.1	0.3
12	Terbutryn	5–500	0.9983	7.81	1.4	3.4
13	Malathion	2–500	0.9963	6.72	0.4	1.1
14	Chlorpyrifos	2–500	0.9912	6.80	0.3	0.8
15	Parathion ethyl	3–500	0.9954	6.90	0.7	1.8
16	Methidathion	2–500	0.9911	6.12	0.4	1.2
17	Endosulfan α	3–500	0.9910	6.36	0.8	2.2
18	Oxyfluorfen	2–500	0.9948	7.62	0.7	1.5
19	Endosulfan β	3–500	0.9985	8.11	0.7	1.6
20	Endosulfan sulphate	2–500	0.9918	6.98	0.4	1.0
21	Diflufenican	1–500	0.9944	5.22	0.1	0.4
22	Phosmet	1–500	0.9937	5.75	0.1	0.3
23	Fenoxycarb	5–500	0.9989	7.50	1.1	3.2
24	L-Cyhalothrin	1–500	0.9931	5.19	0.1	0.3
25	α-Cypermethrin	1–500	0.9995	6.28	0.2	0.5
26	Deltamethrin	1–500	0.9994	6.32	0.2	0.6

[a] Relative standard deviation ($n = 11$) for 10 μg/kg.

Table 3
Percent recoveries of pesticides spiked to oil samples

Serial no.	Pesticide	Virgin olive oil			Refined olive oil		
		10 μg/kg[a]	100 μg/kg[a]	250 μg/kg[a]	10 μg/kg[a]	100 μg/kg[a]	250 μg/kg[a]
1	Trichlorfon	97 ± 6	105 ± 3	98 ± 5	88 ± 5	107 ± 4	87 ± 3
2	Diuron	97 ± 4	108 ± 3	105 ± 3	100 ± 4	105 ± 4	94 ± 3
3	Carbaryl	110 ± 5	84 ± 3	103 ± 3	86 ± 4	89 ± 3	88 ± 3
4	Promecarb	86 ± 4	93 ± 3	101 ± 3	87 ± 5	93 ± 4	88 ± 3
5	Dimethoate	109 ± 5	107 ± 3	101 ± 4	84 ± 4	86 ± 3	106 ± 3
6	Simazine	108 ± 4	95 ± 3	100 ± 4	95 ± 4	106 ± 4	106 ± 4
7	Terbuthylazine	102 ± 3	106 ± 3	97 ± 4	92 ± 6	106 ± 3	96 ± 4
8	Formothion	105 ± 5	90 ± 3	108 ± 3	101 ± 4	102 ± 3	90 ± 3
9	Chlorpyrifos methyl	103 ± 4	103 ± 3	98 ± 4	101 ± 3	99 ± 3	101 ± 3
10	Parathion methyl	100 ± 5	104 ± 4	98 ± 3	103 ± 5	94 ± 4	102 ± 3
11	Pirimiphos methyl	94 ± 4	103 ± 3	100 ± 5	101 ± 4	95 ± 3	99 ± 3
12	Terbutryn	96 ± 5	109 ± 5	97 ± 4	106 ± 5	94 ± 5	104 ± 6
13	Malathion	97 ± 5	98 ± 3	96 ± 4	109 ± 6	88 ± 3	106 ± 4
14	Chlorpyrifos	89 ± 3	99 ± 4	101 ± 3	107 ± 3	92 ± 4	91 ± 3
15	Parathion ethyl	90 ± 4	104 ± 4	96 ± 3	109 ± 3	106 ± 4	109 ± 4
16	Methidathion	89 ± 5	106 ± 3	96 ± 3	90 ± 4	108 ± 3	110 ± 4
17	Endosulfan α	97 ± 3	95 ± 4	101 ± 3	103 ± 3	93 ± 4	88 ± 3
18	Oxyfluorfen	95 ± 3	101 ± 4	96 ± 4	103 ± 4	109 ± 4	104 ± 3
19	Endosulfan β	109 ± 3	104 ± 4	104 ± 3	97 ± 4	106 ± 4	97 ± 3
20	Endosulfan sulphate	96 ± 4	105 ± 3	98 ± 4	109 ± 4	107 ± 4	101 ± 3
21	Diflufenican	95 ± 4	108 ± 3	94 ± 4	101 ± 5	99 ± 3	103 ± 4
22	Phosmet	101 ± 4	107 ± 3	96 ± 3	107 ± 4	98 ± 4	108 ± 3
23	Fenoxycarb	87 ± 5	85 ± 5	106 ± 3	110 ± 3	109 ± 4	92 ± 5
24	L-Cyhalothrin	86 ± 3	90 ± 4	103 ± 3	101 ± 3	96 ± 4	89 ± 3
25	α-Cypermethrin	89 ± 5	105 ± 4	100 ± 4	104 ± 4	97 ± 3	92 ± 3
26	Deltamethrin	90 ± 4	89 ± 3	93 ± 4	105 ± 3	87 ± 4	103 ± 3

Percent recovery ± standard deviation ($n = 3$).
[a] Concentration added.

Table 4
Results (μg/kg) obtained in the analysis of virgin olive oil by GPC–GC–MS/MS

Serial no.	Pesticide	V1[a]	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25
1	Trichlorfon	–[b]	–	–	–	11.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	Diuron	48.0	33.4	46.2	34.2	31.2	26.5	33.7	22.2	46.3	44.0	97.3	42.6	27.6	39.2	81.4	96.2	98.1	107.2	91.4	110	56.3	10.4	21.4	23.6	–
3	Carbaryl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	Promecarb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	Dimethoate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6	Simazine	–	120.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	Terbuthylazine	96.3	51.2	52.4	66.0	–	55.5	62.6	88.8	76.4	64.6	58.3	81.4	65.1	55.4	190.4	–	130.0	166.1	176.0	33.3	34.2	42.5	55.6	5.6	44.4
8	Formothion	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	Chlorpyrifos methyl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	Parathion methyl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	Pirimiphos methyl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	Terbutryn	–	–	–	–	130.0	–	–	–	126.0	–	–	–	–	–	–	111.1	80.0	–	–	–	–	–	–	–	–
13	Malathion	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	Chlorpyrifos	–	–	–	–	–	–	–	–	–	–	23.5	–	–	–	–	–	–	–	–	–	24.7	–	–	–	–
15	Parathion ethyl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	Methidathion	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	Endosulfan α	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18	Oxyfluorfen	66.3	–	–	–	32.3	46.1	38.8	15.4	29.4	41.2	12.9	–	–	20.2	14.1	19.8	28.4	31.4	31.5	29.4	–	18.4	31.2	29.4	21.0
19	Endosulfan β	39.5	–	–	–	11.4	–	9.4	–	11.1	–	20.2	–	–	–	–	–	–	–	–	–	12.6	–	–	–	–
20	Endosulfan sulphate	52.7	56.0	48.2	42.6	58.1	44.2	46.5	42.6	33.6	30.2	91.7	18.7	22.6	24.5	33.8	31.0	24.3	36.3	21.7	35.0	109.1	31.7	36.7	44.4	28.6
21	Diflufenican	34.3	22.2	10.9	–	12.7	26.8	19.3	9.9	–	–	–	–	–	–	–	–	–	–	–	–	–	11.4	12.0	98.0	–
22	Phosmet	15.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
23	Fenoxycarb	–	–	–	–	13.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24	L-Cyhalothrin	10.8	–	–	–	12.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
25	α-Cypermethrin	76.4	–	–	18.4	26.7	21.3	34.5	17.2	19.2	18.8	27.2	19.0	26.2	20.2	18.4	–	–	–	–	–	–	–	–	–	–
26	Deltamethrin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	19.3	21.1	22.2	29.7	16.5	10.2	17.7	9.9	10.6

[a] V: virgin olive oil.
[b] Not detectable.

Table 5
Results (μg/kg) obtained in the analysis of refined olive oil by GPC–GC–MS/MS

Serial no.	Pesticide	R1[a]	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	Trichlorfon	–[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	Diuron	–	–	–	24.2	–	–	–	–	18.4	9.8	–	31.6	24.6	18.5	20.1
3	Carbaryl	–	–	–	–	–	–	–	–	–	9.4	–	–	–	–	–
4	Promecarb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	Dimethoate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6	Simazine	–	–	–	10.6	–	–	–	–	–	–	–	–	–	–	–
7	Terbuthylazine	–	–	–	11.6	–	–	–	9.6	–	11.2	10.9	–	9.6	18.2	9.2
8	Formothion	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	Chlorpyrifos methyl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	Parathion methyl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	Pirimiphos methyl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	Terbutryn	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	Malathion	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	Chlorpyrifos	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	Parathion ethyl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	Methidathion	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	Endosulfan α	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18	Oxyfluorfen	–	–	–	–	–	–	–	–	11.4	–	–	–	–	–	–
19	Endosulfan β	9.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
20	Endosulfan sulphate	9.8	–	18.6	9.5	9.7	10.4	11.1	10.2	20.2	31.6	21.6	22.8	19.3	18.9	21.6
21	Diflufenican	–	–	–	10.3	–	–	–	–	22.5	–	–	11.6	12.4	–	24.8
22	Phosmet	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
23	Fenoxycarb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24	L-Cyhalothrin	61.4	11.4	–	–	–	–	–	–	–	–	–	–	–	–	–
25	α-Cypermethrin	–	9.9	–	–	–	–	–	–	–	19.4	–	–	–	–	–
26	Deltamethrin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] R: refined olive oil.
[b] Not detectable.

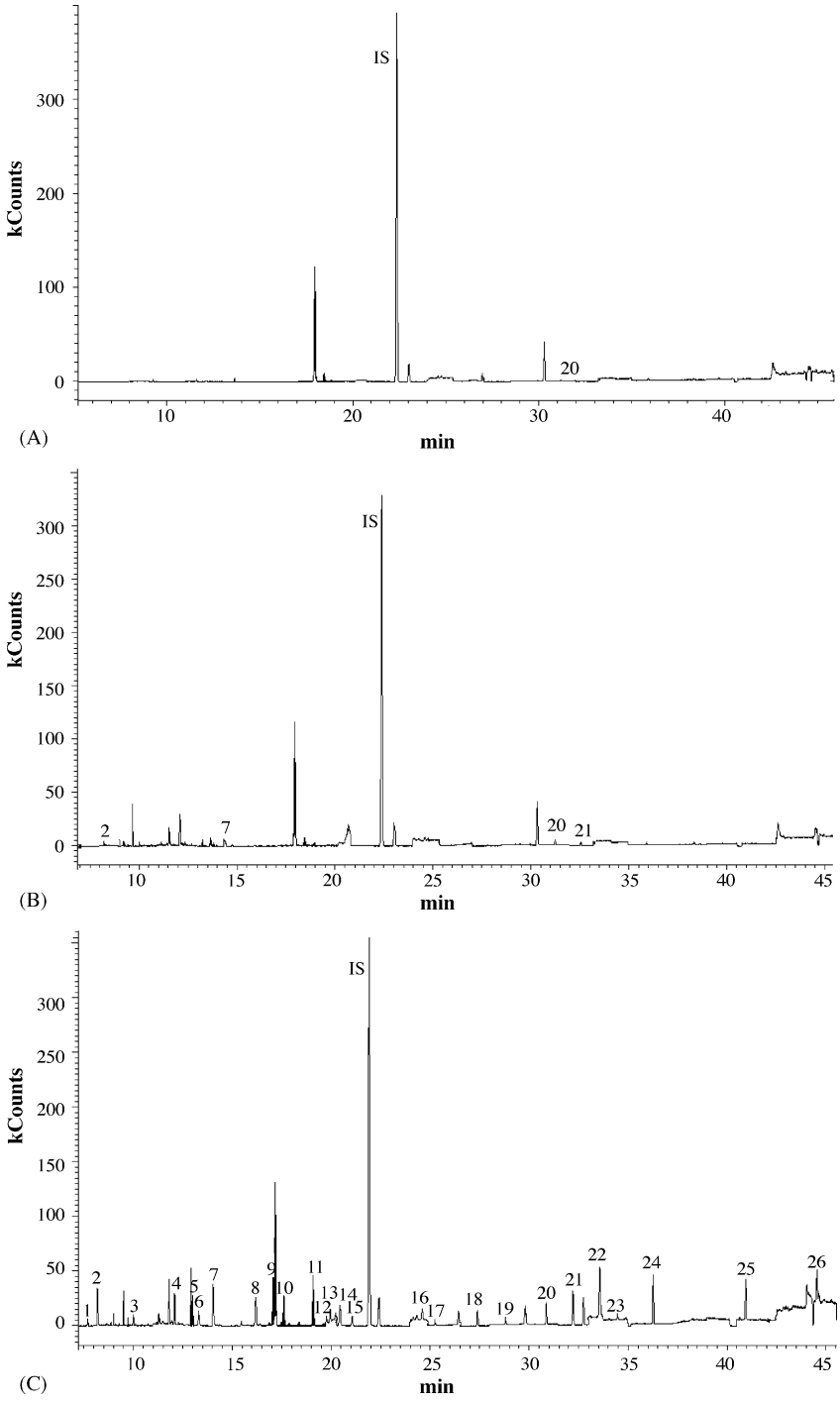

Fig. 2. Gas chromatograms for a sample of refined olive oil (A), virgin olive oil (B) and virgin olive oil spiked with a 50 μg/kg concentration of 26 pesticides (C). IS, internal standard; 1, trichlorfon; 2, diuron; 3, carbaryl; 4, promecarb; 5, dimethoate; 6, simazine; 7, terbuthylazine; 8, formothion; 9, chlorpyrifos methyl; 10, parathion methyl; 11, pirimiphos methyl; 12, terbutryn; 13, malathion; 14, chlorpyrifos; 15, parathion ethyl; 16, methidathion; 17, endosulfan α; 18, oxyfluorfen; 19, endosulfan β; 20, endosulfan sulphate; 21, diflufenican; 22, phosmet; 23, fenoxycarb; 24, L-cyhalothrine; 25, α-cypermethrin; 26, deltamethrin. For other conditions, see Section 2.2.

and diflufenican. Fig. 2 shows the chromatograms obtained in the analysis of refined olive oil (A), virgin olive oil (B) and virgin olive oil spiked with 50 μg/kg (C). As can be seen in Fig. 2A, only one pesticide (endosulfan sulphate) was detected in the refined olive oil. On the other hand, diuron, terbuthylazine, endosulfan sulphate and diflufenican were all detected in virgin olive oil (Fig. 2B).

4. Conclusions

A method for the GPC clean-up of olive oil extracts and determination of 26 pesticides by GC–MS/MS was developed. The GPC technique was found to substantially simplify the removal of fat matter relative to other sample treatments. In addition, the use of tandem MS (MS/MS) with a bench-top ion-trap system

increased the sensitivity of the determination of the pesticides in the oil samples. The accuracy and precision of the method allows its use in routine quality control analyses of olive oil.

The analysis of real revealed the presence of substantial differences in number of pesticides and concentrations between virgin olive oil and refined olive oil. Therefore, refining reduces the amount of residual pesticides in olive oil.

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