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Application of Gas Chromatography Coupled to Triple Quadrupole Mass Spectrometry for the Multiresidue Analysis of Pesticides in Olive Oil

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A new multiresidue method has been developed and validated for the simultaneous determination of 100 pesticide residues in olive oil. The determination of pesticide residues was carried out in only 19 min by gas chromatography coupled to tandem mass spectrometry using a triple quadrupole mass analyzer. The mass spectrometer was operated in electron ionization and the selection reaction monitoring mode was used, acquiring two or three fragmentation reactions per compound. Two extraction processes were studied, and an evaluation of the stability and sensitivity of the chromatographic system has been performed for the tested extraction procedures. The final proposed methodology was based on a liquid–liquid partition with an *n*-hexane/acetonitrile mixture followed by a gel permeation chromatography cleanup step. An adequate lineal relation was obtained in the studied concentration range (10–200 μg kg^{-1}); the recovery values were in the range 70–110% for the two levels of concentration studied: 12 and 50 μg kg^{-1}. Precision values, expressed as relative standard deviation, were lower than 18% at the aforementioned spiking levels; detection limits, confirmation limits, and quantitation limits were below or equal to 1.9, 2.6, and 3.6 μg kg^{-1}, respectively. The developed methodology was applied to the analysis of pesticide residues in real samples of olive oil from the south of Spain.

KEYWORDS: Olive oil; pesticides; gel permeation chromatography; tandem quadrupole mass spectrometry

INTRODUCTION

Olive oil is a natural juice directly obtained from olives (*Olea europaea*), a traditional crop of the Mediterranean Basin, by mechanical or physical procedures, without any chemical step, preserving the vitamins, aroma, and properties of the fruit. Nowadays, this vegetable oil is considered an essential foodstuff in the so-called Mediterranean diet due to its nutritional and sensory properties as well as for its healthy effects, provided by its high antioxidant and monounsaturated fatty acids content (*1, 2*). The aforementioned positive characteristics have increased the demand for the product throughout the world. In fact, the European Union (EU) is the leading olive oil world producer and main consumer, although interest is also increasing in other countries (*3*). To meet consumer demands, agricultural production requires the application of pesticides to olive trees in order to control pests and diseases and to increase crop yields. However, the use of phytosanitary products involves a risk of contaminating olive oil with pesticide residues, its metabolites or degradation products, which can remain in the crop after it is harvested. There is also a concern related to the pesticide concentrations in olive oil, since a liter of oil requires four to five times more olives. Therefore, in order to protect consumer's health, the EU and the Codex Alimentarius Commission of the Food and Agriculture Organization have established maximum pesticide residue limits (MRLs) for olive oil (*4*).

The determination of pesticides in a complex matrix such as olive oil requires both an adequate extraction method and effective cleanup process to remove total or partially the lipidic components coextracted together with the target compounds. This allows an increase in selectivity that reduces the maintenance of the chromatographic system and improves the reliability of the analytical results. The sample pretreatment of olive oil normally consists of the use of different sorbents such as alumina, silica gel, florisil, or C_{18}, to perform a solid phase extraction (*5–10*). Among the variety of methodologies available, the use of gel permeation chromatography (GPC) followed by a liquid–liquid partition stage (LLP) (*11–15*) has been also used in the extraction of fatty samples. Other approaches such as matrix solid phase dispersion or headspace solid phase microextraction have been also proposed to determine pesticide residues in vegetable oils (*16, 17*). However, GPC is the most suitable methodology to isolate multiclass pesticides from

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different kinds of matrices in routine analysis (*11–15, 18*), and especially from samples with high content in fat.

On the other hand, sample throughput is one of the main objectives in the development of new multiresidue analysis methods. In this way, gas chromatography coupled to tandem mass spectrometry (GC–MS/MS) is particularly useful for qualitative and quantitative purposes. The ion trap mass analyzer has been previously proposed for the determination of pesticide residues in fat vegetable matrix in a reasonable time (*18*), but the number of analytes that can be simultaneously monitorized in MS/MS mode is limited. This paper shows a new approach in the use of a triple quadrupole analyzer (QqQ) (working in selection reaction monitoring mode, SRM) to the determination of pesticides in a fatty matrix. The QqQ analyzer allows reducing the analysis time and increasing the number of simultaneously determined compounds, due to its high acquisition speed together with its extraordinary selectivity and sensibility. It has been recently shown as a suitable methodology to determine pesticide residues in fatty and nonfatty samples (*15, 19–21*).

In this study, a new multiresidue method for determining 100 multiclass pesticide residues is developed and validated in olive oil. The sample treatment is based on a fast liquid–liquid partition and a GPC cleanup step and determination by GC–QqQ–MS/MS This paper represents the first application of the QqQ analyzer coupled to GC to determine multiclass pesticide residues in olive oil. The methodology was applied to the analysis of real olive oil samples from Andalusia (Spain).

EXPERIMENTAL PROCEDURES

Standards and Reagents. Pesticide standards and the internal standard (IS), caffeine, were obtained from Riedel-de Haën (Seelze-Hannover, Germany); purity was always >99%. Pesticide-quality solvents (cyclohexane, acetonitrile, *n*-hexane, ethyl acetate, and acetone) were supplied by Panreac (Barcelona, Spain). Stock standard solutions (between 75 and 550 mg L^{-1}), prepared by exact weighting and dissolution in acetone, were stored in a freezer (−30 °C). Working standard solutions (2 mg L^{-1} concentration of each compound) were prepared by appropriate dilution with acetone and stored under refrigeration (4 °C).

Chromatography and Apparatus. The ProStar GPC system used in the cleanup stage was provided by Varian (Walnut Creek, CA). The GPC apparatus consisted of a 410 autosampler with a 24-vial (10 mL) tray, a 230 solvent delivery module, a 325 UV–vis detector which operated at a wavelength of 254 nm, a 704 fraction collector, and two online connected Envirogel GPC cleanup columns from Waters (Milford, MA) packed with polystyrene–divinylbenzene (150 × 19 mm and 300 × 19 mm, respectively).

Final extract analyses were performed with a 3800 gas chromatograph from Varian equipped with electronic flow control. Samples were injected with a CombiPAL autosampler (CTC Analytics AG, Zwingen, Swizerland) into a SPI/1079 split/splitless injector. The glass liner was equipped with a plug of Carbofrit (Resteck, Bellefonte, PA). A fused-silica untreated capillary column (2 m × 0.25 mm) from Supelco (Bellefonte, PA) was used as retention gap connected to a FactorFour VF-5ms capillary column (30 m × 0.25 mm × 0.25 μm film thickness) from Varian. Helium (99.9999%) at a flow rate of 1 mL min^{-1} was used as carrier gas. The gas chromatograph was interfaced to a Varian 1200L triple quadrupole mass analyzer using an electron ionization (EI) source. Argon (99.999%) was used as collision gas at 2.0 mTorr. The mass spectrometer was calibrated weekly with perfluorotributylamine. The computer controlling the system held an EI-MS/MS self-made library specifically created under our experimental conditions.

An analytical balance AB204-S from Mettler Toledo (Greifensee, Switzerland) and a rotary evaporator R-114 (Büchi, Flawil, Switzerland) were available for processing samples.

Sample Pretreatment. Two different sample pretreatments were evaluated before the cleanup step:

Procedure 1. An aliquot of 4 g of sample was weighed into a 100 mL glass tube with top from a homogenized sample of 1 L of olive oil at room temperature. The sample was dissolved with 20 mL of *n*-hexane by shaking for 30 s with a minishaker. Twenty milliliters of acetonitrile saturated with *n*-hexane was added to the glass tube, and the volume was shaken again with the minishaker during 1 min. After that, the mixture was transferred to a separation funnel. The acetonitrile phase was collected and the *n*-hexane phase was transferred to the glass tube to be submitted to a second partition. The acetonitrile extracts were combined in a 100 mL spherical flask. Evaporation of the solvent to a small volume (1–2 mL) was done in a rotary evaporator (50 °C), and then the extract was taken to nearly dryness under a soft nitrogen stream. The residue was dissolved in 5 mL of an ethyl acetate–cyclohexane mixture (1/1, v/v). The redissolved extract was transferred to a 10 mL vial.

Procedure 2. An aliquot of 1 g of sample was weighed into a 10 mL glass vial from a homogenized sample of 1 L of olive oil at room temperature. The sample was dissolved with 8 mL of GPC mobile phase (ethyl acetate–cyclohexane, 1:1, v/v) by shaking for 15 s with a minishaker.

Cleanup Procedure. A pretreated sample volume of 2.5 mL was injected in the GPC system. The mobile phase was ethyl acetate–cyclohexane (1:1, v/v) at a flow of 5 mL min^{-1}. The representative fraction containing the target pesticides was collected from 15 to 22 min (approximately 35 mL). The collected fraction was evaporated to a residual volume in a rotary evaporator (50 °C) and then taken to dryness under a nitrogen stream. The residue was finally dissolved in 1 mL of cyclohexane containing 0.5 μg L^{-1} of the IS.

GC–QqQ–MS/MS Analysis. Aliquots of 10 μL of the above residue (sample eluent) were injected into the gas chromatograph. Large-volume injection (LVI) was used, with a split/splitless programmed-temperature injection (PTV). The initial injector temperature was set at 70 °C during the injection and then held for 0.5 min, and then the temperature was increased at a rate of 100 °C min^{-1} to 300 °C and then held for 7.1 min. The injector split ratio was set initially at 20:1. At 0.5 min the splitless mode was switched on until 3.5 min. After that, the split ratio was programmed at 100:1, and 6.5 min later the split ratio was reduced to 20:1. After the injection, the column oven program was as follows: the initial temperature was set at 70 °C, with a 3.5 min hold, then increased to 180 °C at a rate of 50 °C min^{-1}, and finally increased at a rate of 25 °C min^{-1} to 300 °C which was held for 10 min.

The mass spectrometer was operated in EI generating electrons with a kinetic energy of 70 eV and SRM acquisition mode. The temperatures of the transfer line, ionization source, and manifold were set at 280, 250, and 40 °C, respectively. The scan time was set at 0.25 s. A multiplier voltage 200 V higher than the fixed one set by the autotune and a filament current of 100 μA were established. The analysis was performed with a filament-multiplier delay of 4.5 min in order to prevent instrument damage. The specific MS/MS parameters used are shown in **Table 1**.

RESULTS AND DISCUSSION

Optimization of the GC–QqQ–MS Parameters. Considering that LVI was applied, a PTV was used in order to improve sensitivity and achieve lower limits of detection (LODs). The initial temperature (70 °C) was selected according to the boiling point of the injection solvent. It was held for 0.5 min with a low split rate (20:1) eliminating the solvent which allows the enrichment of the analytes. Then, the splitless mode was activated and a fast increase up to 300 °C of the injector temperature was carried out in order to transfer the analytes from the injector to the column. After 3.5 min, a split ratio of 100:1 was set to clean the injector, keeping the temperature during 7.1 min.

For the optimization of the MS method, all compounds were monitored in full scan mode in the range *m/z* 50–550, using EI mode. Then, the precursor ion was selected with the aim of achieving a compromise between both selectivity (the highest *m/z* ion is preferred) and sensitivity (the highest abundance ion). Next,

Table 1. GC–QqQ–MS/MS Parameters

pesticide	segment	parent ion, *m/z*	product ions, *m/z* (collision energy, v)	pesticide	segment	parent ion, *m/z*	product ions, *m/z* (collision energy, v)
Acephate	1	136	94 (−20), 70−140(−20)	Kresoxim *methyl*	6	206	131 (−20), 89 (−40)
Acrinathrin	7	181	152 (−40), 126 (−50)	Lindane	3	219	183 (−10), 147 (−40), 109 (−50)
Anilazine	5	239	178 (−20), 142 (−50), 116 (−50)	Malathion	4	173	99 (−20), 127 (−10), 145 (−10)
Atrazine	3	217	202 (−10), 160 (−20), 174 (−20)	Metalaxyl	4	206	132 (−20), 104 (−40), 117 (−50)
Azinphos *mehyl*	8	160	132 (−10), 105 (−20)	Methamidophos	1	141	94 (−10), 79 (−30)
Azoxystrobin	8	344	329 (−30), 172 (−50), 156 (−40)	Methidathion	6	302	145 (−10), 85 (−30)
Benalaxyl	7	148	118 (−30), 105 (−30)	Methoxichlor	7	227	169 (−40), 140 (−50), 115 (−50)
Bifenthrin	7	181	165 (−40), 141 (−20), 153 (−20)	Mevinphos	2	192	164 (−10), 127 (−30)
Bromopropylate	7	341	183 (−30), 155 (−50)	Myclobutanil	6	206	179 (−10), 150 (−40)
Bupimirate	6	273	193 (−10), 108 (−20), 150 (−10)	Molinate	2	187	126 (−10), 83 (−30), 98 (−30)
Buprofezin	6	172	131 (−10), 115 (−20)	Norflurazon	7	303	145 (−20), 173 (−10)
caffeine	4	194	109 (−20), 120 (−50), 137 (−30)	Omethoate	2	156	79 (−30), 110 (−10)
Carbophenothion	7	342	157 (−10), 97 (−50), 143 (−50)	Oxadixyl	7	163	132 (−20), 105 (−40), 117 (−40)
Cyfluthrin	8	206	150 (−50), 176 (−40)	Oxyfluorfen	6	361	300 (−20), 252 (−40), 317 (−10)
Cyhalothrin	7	181	152 (−30), 126 (−50)	*p,p′*-DDD	7	235	165 (−30), 199 (−20)
Cypermethrin	8	163	127 (−10), 91 (−20)	*p,p′*-DDT	7	235	165 (−40), 199 (−20)
Cyproconazole	7	222	125 (−30), 82 (−10)	Parathion *ethyl*	5	291	137 (−10), 81 (−30), 109 (−10)
Cyromazine	3	151	109 (−20), 82 (−30)	Parathion *methyl*	4	263	246 (−1), 109 (−10), 153 (−1)
Chlorfenvinphos	5	324	267 (−20), 159 (−50), 296 (−10)	Penconazole	5	248	192 (−20), 157 (−50), 206 (−20)
Chlorpyriphos *ethyl*	5	314	258 (−20), 286 (−20)	Pendimethalin	5	252	162 (−20), 118 (−50), 206 (−50)
Chlorpyriphos *methyl*	4	286	208 (−10), 112 (−40), 127 (−20)	Permethrin	8	183	153 (−30), 127 (−40), 115 (−50)
Chlorthalonil	3	266	133 (−50), 160 (−30), 231 (−40)	Phorate	2	260	75 (−10), 129 (−50)
Chlozolinate	5	259	188 (−20), 145 (−50), 153 (−40)	Phosalone	8	182	102 (−30), 111 (−20), 138 (−10)
Diazinon	3	304	179 (−20), 137 (−50)	Phosmet	7	160	133 (−10), 104 (−20)
Dichlorvos	1	185	109 (−50), 145 (−30)	Pirimicarb	3	166	96 (−10). 80−170(−10)
Difeconazole	8	323	265 (−20), 201 (−50)	Pirimiphos *methyl*	4	305	290 (−20), 125 (−50), 180 (−10)
Dimethoate	3	125	79 (−30), 93 (−20)	Pyriproxyfen	8	136	96 (−10), 70−140 (−10)
Disulfoton	3	274	88 (−10), 97 (−50)	Procymidone	6	283	185 (−50), 145 (−50), 96 (−20)
Endosulfan lactone	6	321	267 (−10), 159 (−50), 296 (−10)	Profenofos	6	338	267 (−20), 249 (−40)
Endosulfan β	6	241	241 (−20), 133 (−40), 170 (−40)	Propiconazole	7	259	173 (−20), 145 (−50), 191 (−20)
Endosulfan ether	4	241	170 (−40), 207(−50)	Propoxur	2	152	110 (−10), 90−160(−10)
Endosulfan sulfate	7	272	237 (−10), 165 (−50)	Pyrimethanil	3	198	183 (−20), 118 (−50)
Endosulfan α	6	241	241 (−20), 133 (−40), 170 (−40)	Pyridaben	8	309	147 (−20), 117 (−50), 132 (−50)
Endrin	7	281	211 (−30), 245 (−20), 208 (−40)	Pyrifenox	6	263	228 (−20), 116 (−40), 201 (−30)
Ethion	7	231	175 (−20), 129 (−30), 185 (−10)	Quinalphos	6	146	118 (−20), 90 (−40)
Etoprophos	2	199	129 (−20), 97 (−40)	Quinomethionate	6	234	206 (−10), 116 (−40), 148 (−40)
Etrimphos	3	292	281 (−10), 153 (−30), 125 (−50)	Quintozene	3	297	267 (−10), 239 (−20)
Famphur	7	218	109 (−30), 93 (−10)	Simazine	3	201	173 (−10), 158 (−20)
Fenamimphos	6	303	195 (−10), 260 (−20), 153 (−40)	Sulfotep	2	322	202 (−20), 146 (−30), 174 (−20)
Fenitrothion	4	277	260 (−10), 109 (−20), 125 (−20)	Tebuconazole	7	250	125 (−30), 153 (−10), 163 (−10)
Fenoxycarb	7	186	157 (−20), 109 (−30), 77 (−40)	Terbuthylazine	3	214	104 (−30), 83 (−30), 132 (−50)
Fenpropathrin	7	265	210 (−20), 89 (−40), 181 (−50)	Terbutryn	4	241	185 (−10), 111 (−50), 170 (−20)
Fenthion	5	278	109 (−20), 125 (−40), 169 (−40)	Tetradifon	8	227	199 (−20), 143 (−40), 164 (−30)
Feranimol	8	330	139 (−20), 111 (−50)	Tetramethrin	7	164	135 (−10), 107 (−30), 93 (−10)
Fludioxonil	6	248	183 (−20), 127 (−50), 154 (−20)	Thionazin	2	248	140 (−10), 106 (−20)
Formothion	4	224	155 (−10), 109 (−50), 125 (−20)	Tolyfluanid	6	181	137 (−30), 91 (−50)
Furathiocarb	7	194	161 (−10), 151 (−30), 105 (−40)	Triadimefon	5	209	182 (−10), 112 (−40), 127 (−20)
Feptenophos	2	215	109 (−20), 89 (−30)	Triadimenol	5	168	168 (−10), 81 (−20)
Fepthachlor	4	272	237 (−20), 165 (−50), 141 (−50)	Trichlorfon	1	185	93 (−20), 108 (−30)
Iprodione	7	187	124 (−40), 159 (−30)	Vinclozoline	4	285	212 (−10), 145 (−40), 198 (−30)
Isofenphos	5	213	185 (−5), 121 (−10)				

the selected precursor ion was submitted to collision-induced dissociation with argon gas at collision energies ranging 10–50 V. A minimum of two MS/MS transitions were selected for each compound.

The QqQ analyzer is characterized by a higher data collecting speed than MS/MS in time instruments such as ion traps. The mass scanning speed of the analyzer is a critical parameter which determines chromatographic peak shape (point across a peak) and sensitivity. The scan time was previously evaluated, and a value of 0.25 s was shown as the best for quantitation purposes (*19*, *20*). The mass spectrometer was time programmed for acquiring the MS/MS specific parameters of the compounds defining eight segments (**Table 1**). A compromise between the number of monitored transitions per segment and the chromatographic separation was reached in order to maintain the optimum peak shape. At least from six to eight points per peak were collected in these conditions, which have been suitable for quantitation purposes (*22*). In **Figure 1** a chromatographic peak of α-Endosulfan with a six-point peak is shown. The number of transitions simultaneously determined per segment ranged from 8 (4 compounds) in the first segment to 60 (24 compounds) in the seventh segment. **Figure 2** shows the segment 4 with 12 coeluting compounds.

Development of the Extraction and Cleanup Method. A comparison between the two proposed extraction methods was carried out. Both options included the GPC stage. In this sense an evaluation of the GPC separation profile was performed in order to collect the most suitable fraction with a high content of pesticides and a low content of matrix compounds. Consequently, a solution of the target analytes at 500 μg kg^{-1} made up in pure solvent was injected into the GPC obtaining the elution range of pesticides. Eluted fractions of 1 min were collected and injected in the GC–QqQ–MS/MS to evaluate the fraction with the highest percentage of pesticides. The fraction containing pesticides was eluted from 14 to 22 min (with

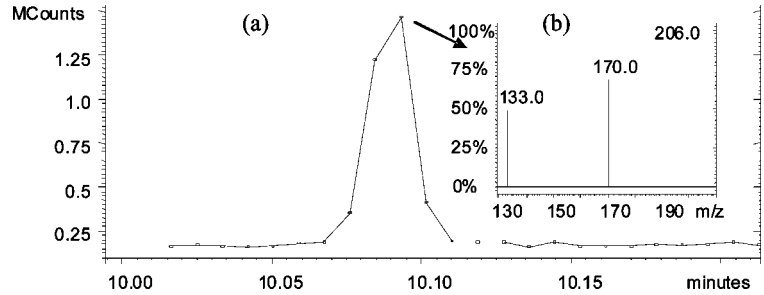

Figure 1. (**a**) Chromatogram and (**b**) MS/MS spectra of a positive real sample of α-Endosulfan in olive oil (concentration determined, 30 μg kg^{-1}).

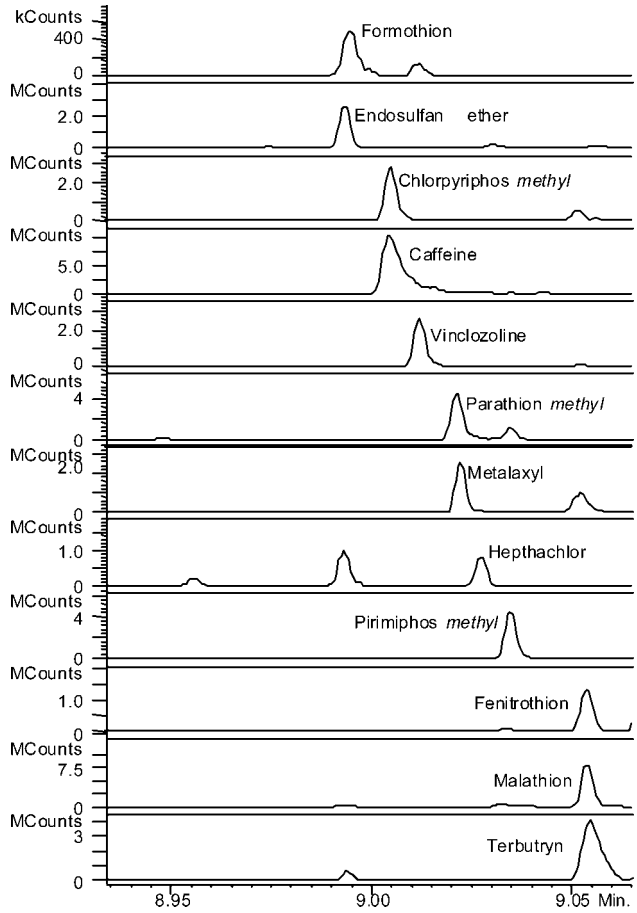

Figure 2. Snapshot of a gas chromatogram obtained for a spiked blank sample at 200 μg kg^{-1}. Twelve compounds of segment 4 are monitored.

Acrinathrin and Fenthion as the respective bordering compounds). Next, 2.5 mL aliquots of blank eluent of olive oil obtained with both extraction procedures were injected into the GPC system. The elution profiles showed that the matrix components eluted between 9 and 20 min. In this optimization, an intermediate solution was set in order to achieve adequate isolation of the target pesticides and minimum coextraction of matrix components. For that, the GCP fraction from 15 to 22 min was collected.

The GPC-treated sample eluents from procedure 1 contained fewer matrix interferents than those from extraction procedure 2. This aspect is very important because GPC-treated sample eluents are later injected into the gas chromatographic system. In consequence and in order to monitor the chromatographic system drift, a follow up of the IS response was performed by plotting the stability of the IS area versus the number of consecutive injections (**Figure 3**) of sample eluents. This extraction procedure was not suitable in this type of matrix since the stability of the IS response decreased with the number of

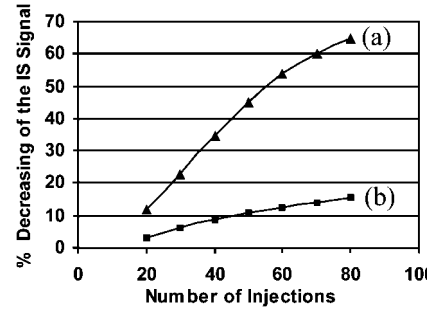

Figure 3. Stability of the IS signal of repeated injections into the GC–QqQ–MS/MS system of sample extracts using after (**a**) direct injection into GPC and (**b**) LLP previous to the GPC cleanup stage.

injections. In fact, a reduction of about a 70% was observed in the area after 80 injections. This means that the effectiveness of the GPC cleanup was not strong enough in combination with this nonselective extraction to maintain a certain stability of the instrumental response.

Therefore, it was mandatory to include a previous cleanup step as carried out by a LLP with acetonitrile saturated in *n*-hexane before the GPC stage, which is used in other applications (*11–15*). In order to increase sample throughput, mechanical agitation with a minishaker was carried out instead of the tedious manual shaking. The combination of these two cleanup steps permitted an improved sensitivity of the chromatographic system, since the reduction of the IS area was not significant after repeated injections (**Figure 3**). A loss of only about 15% was observed after 80 injections; therefore, this second strategy was set as the most appropriate sample treatment to remove the lipidic interferences from the olive oil extract.

Validation of the Methodology. The proposed methodology was validated according to the European SANCO guidelines to provide evidence that the method was fit for the purpose to be used (*23, 24*).

Identification Criteria. The identification of the target compounds was based on the relative retention time (RRT), which is the ratio of the chromatographic retention time of the analyte to that of the IS. The RRT of the analyte in the sample must match that of the matrix matched calibration standard at 50 μg kg^{-1} with a tolerance of ±0.5%. The average retention times of the pesticides in olive oil extracts ($n = 10$) are shown in **Table 2**. Precision of RRT was always lower than 0.5% (expressed as relative standard deviation, RSD, in %).

Confirmation Criteria. The verification of the identity of a compound previously identified by its RRT was based on the relative intensities of the diagnostic ions (precursor/product ion pairs). A system of identification points has been used to interpret the MS/MS data obtained. For the confirmation of substances like pesticides, a minimum of three identification points are required (*25*). Such identification points can be earned by MS techniques monitoring two different low-resolution MS/

Table 2. Validation Data: Average Relative Retention Time (RRT), Determination Coefficients (R^2), Precision (RSD %), Recovery at 50 and 12 μg kg^{-1} Spiking Levels, LOD, LOQ, and LOC Values

			50 μg kg^{-1}		12 μg kg^{-1}				
pesticide	RRT	R^2	RSD (%)	R (%)	RSD (%)	R (%)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	LOC (μg kg^{-1})
Acephate	0.83	0.9999	10	89	17	79	1.3	1.9	2.3
Acrinathrin	1.23	0.9995	6	96	9	102	0.3	0.8	1.5
Anilazine	1.05	0.9953	17	106	18	100	1.2	1.8	1.5
Atrazine	0.92	0.9920	7	78	14	82	1.3	1.7	1.6
Azinphos *methyl*	1.24	0.9907	7	96	10	85	0.8	1.2	1.2
Azoxystrobin	1.59	0.9914	12	105	14	82	0.5	0.8	1.1
Benalaxyl	1.19	0.9986	5	100	8	79	0.9	1.2	1.4
Bifenthrin	1.19	0.9935	6	85	8	95	0.2	0.6	0.5
Bromopropylate	1.22	0.9970	13	79	12	87	0.3	0.8	0.8
Bupimirate	1.10	1.0000	9	83	11	84	0.9	1.2	1.0
Buprofezin	1.11	0.9979	9	79	9	79	0.2	0.7	0.6
Caffeine	1.00								
Carbophenothion	1.19	0.9969	10	75	11	98	0.6	2.0	1.8
Cyfluthrin	1.34	0.9962	6	94	6	99	0.7	1.0	0.8
Cyhalothrin	1.23	1.0000	3	85	8	103	0.9	2.1	2.5
Cypermethrin	1.35	0.9958	7	99	11	84	0.9	1.6	1.3
Cyproconazole	1.17	0.9923	5	84	9	77	0.1	0.4	0.4
Cyromazine	0.94	0.9999	4	92	6	76	0.4	0.8	1.1
Chlorfenvinphos	1.05	0.9919	14	101	12	105	0.5	0.9	0.7
Chlorpyriphos *ethyl*	1.02	0.9982	15	85	12	73	0.2	0.5	0.5
Chlorpyriphos *methyl*	0.99	0.9987	9	80	9	73	0.1	0.3	0.3
Chlorthalonil	0.95	0.9979	8	86	11	110	1.9	3.5	2.8
Chlozolinate	1.05	0.9984	5	89	10	75	0.8	2.6	2.3
Diazinon	0.93	1.0000	5	90	8	97	0.6	0.9	1.3
Dichlorvos	0.72	0.9993	4	86	6	80	1.3	2.3	2.0
Difeconazole	1.54	0.9997	17	81	15	78	0.6	0.9	1.5
Dimethoate	0.93	1.0000	8	81	10	92	0.3	0.6	0.5
Disulfoton	0.94	0.9995	6	70	8	89	1.0	1.4	1.9
Endosufan lactone	1.06	0.9966	8	77	15	105	0.7	1.0	1.8
Endosufan β	1.15	0.9969	8	83	10	72	0.1	0.5	0.3
Endosulfan ether	0.99	0.9984	7	73	9	95	0.5	0.8	0.8
Endosulfan sulfate	1.17	0.9934	14	109	12	99	0.4	0.7	0.6
Endosulfan α	1.12	0.9967	4	95	5	101	0.1	0.3	0.2
Endrin	1.17	0.9992	7	78	13	89	0.5	0.9	0.7
Ethion	1.17	0.9982	7	99	8	87	0.7	1.1	0.9
Etoprophos	0.87	1.0000	6	80	13	102	0.5	1.2	0.9
Etrimphos	0.94	1.0000	9	80	12	85	0.2	0.6	0.5
Famphur	1.19	1.0000	7	101	7	94	0.2	0.8	1.4
Fenamimphos	1.09	0.9993	7	90	12	102	0.4	0.7	0.6
Fenitrothion	1.00	0.9991	7	90	11	85	0.3	0.9	0.7
Fenoxycarb	1.21	0.9997	8	82	10	101	0.8	1.5	0.9
Fenpropathrin	1.21	1.0000	7	72	14	108	0.3	0.5	0.4
Fenthion	1.02	0.9999	13	84	7	85	0.2	0.4	0.3
Feranimol	1.25	0.9950	9	73	7	83	0.2	0.6	0.9
Fludioxonil	1.10	0.9974	3	77	5	79	0.3	0.8	0.8
Formothion	1.00	0.9995	6	84	8	104	1.3	1.9	1.5
Furathiocarb	1.22	0.9989	6	97	6	76	1.4	2.5	1.8
Heptenophos	0.85	0.9996	10	92	11	84	0.6	0.9	0.7
Hepthachlor	1.00	0.9996	10	109	12	80	0.8	1.3	1.5
Iprodione	1.19	0.9983	7	99	10	100	0.7	1.6	2.0
Isofenphos	1.05	0.9999	7	88	8	85	0.8	1.1	1.0
Kresoxim *methyl*	1.10	0.9961	4	85	7	103	1.2	2.1	2.6
Lindane	0.94	0.9989	4	101	10	89	0.2	0.8	0.7
Malathion	1.00	0.9998	8	88	12	87	0.3	0.7	0.6
Metalaxyl	0.99	0.9990	7	87	7	84	1.2	1.7	2.1
Methamidophos	0.74	0.9919	9	77	7	109	1.8	3.5	2.9
Methidathion	1.07	0.9915	9	91	10	87	0.3	0.9	1.3
Methoxichlor	1.19	0.9935	6	93	8	81	0.2	0.8	0.6
Mevinphos	0.85	0.9939	7	89	7	103	0.4	0.7	0.7
Myclobutanil	1.11	0.9918	3	90	5	110	0.2	0.7	0.5
Molinate	0.84	0.9972	5	74	6	84	0.2	0.6	1.1
Norflurazon	1.19	0.9906	6	75	10	72	0.4	0.9	0.8
Omethoate	0.88	0.9907	10	87	7	108	1.9	3.6	3.3
Oxadixyl	1.18	1.0000	8	84	9	71	0.4	0.8	1.2
Oxyfluorfen	1.10	0.9991	9	85	13	97	0.3	0.7	0.6
p,p′-DDD	1.18	0.9978	8	83	11	82	0.2	0.8	0.5
p,p′-DDT	1.18	0.9983	8	83	12	84	0.2	0.7	0.6
Parathion *ethyl*	1.02	0.9903	10	85	14	77	0.7	1.2	1.1
Parathion *methyl*	0.98	0.9993	8	73	12	104	0.6	1.1	1.3
Penconazole	1.05	0.9985	8	88	15	84	0.7	1.8	1.2
Pendimethalin	1.04	0.9988	7	72	8	75	0.4	0.9	1.4
Permethrin	1.31	0.9988	8	86	10	100	0.2	0.7	0.5

Table 2. Continued

			50 μg kg^{-1}		12 μg kg^{-1}				
pesticide	RRT	R^2	RSD (%)	R (%)	RSD (%)	R (%)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	LOC (μg kg^{-1})
Phorate	0.89	0.9995	7	83	7	84	0.7	1.2	1.6
Phosalone	1.23	0.9980	5	100	7	84	0.6	1.0	0.9
Phosmet	1.21	0.9942	6	88	12	100	1.2	2.1	1.8
Pirimicarb	0.96	0.9985	6	101	9	76	0.4	1.0	0.8
Pirimiphos *methyl*	0.99	0.9999	10	95	9	79	0.3	0.8	0.6
Pyriproxyfen	1.23	0.9986	6	86	8	96	0.5	0.8	0.6
Procymidone	1.06	0.9997	11	72	13	76	0.6	1.0	1.2
Profenofos	1.10	0.9960	6	95	13	86	0.4	0.8	0.9
Propiconazole	1.19	0.9987	11	77	8	101	0.1	0.3	0.2
Propoxur	0.86	1.0000	5	95	9	81	0.3	0.8	1.1
Pyrimethanil	0.94	0.9999	9	99	10	93	0.2	1.0	0.9
Pyridaben	1.33	0.9992	10	105	14	94	0.5	1.1	0.8
Pyrifenox	1.08	0.9923	8	99	9	105	0.3	0.7	0.7
Quinalphos	1.10	1.0000	5	93	15	73	0.5	1.0	0.6
Quinomethionate	1.09	0.995	7	85	10	106	0.3	0.7	1.0
Quintozene	0.93	0.9983	10	72	10	91	0.1	0.6	0.4
Simazine	0.92	0.9980	12	88	11	80	0.7	1.3	1.1
Sulfotep	0.88	1.0000	6	91	11	78	0.7	1.4	0.9
Tebuconazole	1.19	0.9978	16	107	13	84	0.5	1.2	1.4
Terbuthylazine	0.93	0.9949	14	87	14	89	0.1	0.5	0.4
Terbutryn	1.01	0.9949	5	87	12	84	1.0	2.6	2.3
Tetradifon	1.23	1.0000	11	73	6	88	0.9	1.6	1.7
Tetramethrin	1.19	0.9987	5	86	13	93	0.4	1.1	0.8
Thionazin	0.86	0.9947	7	84	15	92	0.3	0.7	0.5
Tolyfluanid	1.11	0.9967	6	106	7	94	0.5	1.2	1.0
Triadimefon	1.03	0.9984	9	108	10	101	0.2	0.6	0.6
Triadimenol	1.05	0.9931	8	101	13	74	0.3	1.0	0.7
Trichlorfon	0.72	0.9998	7	85	10	93	0.9	2.0	2.2
Vinclozoline	1.00	0.9999	9	90	8	95	0.9	1.8	1.2

MS transition products. However, in order to increase the reliability of the confirmation, four identification points were obtained for each compound (one precursor and two product ions). For a positive confirmation the relative intensities of the detected ions must correspond to those of the matrix-matched calibration standard of 50 μg kg^{-1}, measured under the same experimental conditions, within the following tolerances: ±20% for *m*/*z* of relative intensity higher than 50%; ±25% for *m*/*z* of relative intensity between 20 and 50%; ±30% for *m*/*z* of relative intensity between 10 and 20%; and ±50% for *m*/*z* of relative intensity lower than or equal to 10%.

Calibration. A multistandard calibration approach with three levels was used because it is the most practical calibration scheme when the analyte amount in the samples is unknown. In order to avoid matrix effects, calibration was done using matrix-matched standards. The standards were prepared extracting aliquots of blank olive oil samples which were spiked with concentrations of the target pesticides at 10, 50, and 200 μg kg^{-1}. These calibration points were submitted to the GPC purification in order to compensate for the quantitative but no qualitative loss of target pesticides by limitation of the collected fraction. Linear calibration graphs were constructed by least-squares regression of concentration versus peak area ratio (analyte/IS) of the calibration standards. The calibration curves included the origin but were not forced through it. Reasonable linearity was found in the studied concentration range, with determination coefficients always 0.991 or higher (**Table 2**).

Lower Limits. Detection limits (LOD) and quantitation limits (LOQ) were calculated by injecting spiked blank samples, at the lowest concentration giving a response of 3 (LOD) or 10 (LOQ) times the average of the baseline noise (five injections). LOD values ranged between 0.1 and 1.9 μg kg^{-1}, while LOQ ranged 0.3–3.6 μg kg^{-1} (**Table 2**). Confirmation limits (LOC) were also calculated as the concentration where the weakest diagnostic ion no longer appears at a signal-to-noise (*S*/*N*) ratio of 3 (*26–28*). LOC values are shown in **Table 2**, showing values equal or below the LOQ. LOC calculated ranged between 0.2 μg kg^{-1} (Endosulfan α and Propiconazole) and 2.6 μg kg^{-1} (Kresoxim-methyl). Therefore, all LOC were clearly lower than the lowest MRL stated by the EU (10 μg kg^{-1}). However, 30 compounds presented LOC slightly higher than LOQ. For them, their LOC were also considered as LOQ.

Trueness and Precision. Trueness was evaluated in terms of recovery by spiking blank samples with the corresponding volume of the multicompound working standard solution. It was evaluated through the application of the extraction method at a low spiked level, 12 μg kg^{-1}, and a medium concentration level (50 μg kg^{-1}) (five extractions in both cases). Recovery rates ranged between 70 and 110% with RSD values between 3 and 18% (**Table 2**).

Application to Real Samples. The proposed method was applied to the analysis of eight real samples. Only one positive sample was found, containing α-Endosulfan at 30 μg kg^{-1} (**Figure 1**), while no pesticides were detected in the other samples.

In order to ensure the quality of the results when the proposed method was applied to routine analysis, various internal quality criteria have been established. The set of samples analyzed each day was processed together with: (i) a blank extract which eliminated possible false positives by contamination in the extraction process, instrument, or chemicals used; (ii) a blank extract spiked at the concentration of the second calibration level in order to asses the extraction efficiency (recovery rates between 60 and 120% were accepted); and (iii) calibration curves prepared daily to check both slopes and intercepts, as well as linearity in the working range of concentrations which avoided quantitation mistakes caused by possible matrix effects or instrumental fluctuations ($R^2 > 0.98$ was requested).

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