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Determination of pesticide residues in olives and olive oil by matrix solid-phase dispersion followed by gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry

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Abstract

A novel analytical approach has been developed and evaluated for the quantitative analysis of a selected group of widely used pesticides (dimethoate, simazine, atrazine, diuron, terbuthylazine, methyl-parathion, methyl-pirimiphos, endosulfan I, endosulfan II, endosulfan sulphate, cypermethrin and deltamethrin), which can be found at trace levels in olive oil and olives. The proposed methodology is based on matrix solid-phase dispersion (MSPD), (with a preliminary liquid–liquid extraction in olive oil samples) using aminopropyl as sorbent material with a clean-up performed in the elution step with Florisil, followed by mass spectrometric identification and quantitation of the selected pesticides using both gas chromatography–mass spectrometry (GC–MS) in selected ion monitoring (SIM) mode and liquid chromatography tandem mass spectrometry (LC–MS–MS) in positive ionization mode. The recoveries obtained (with mean values between 85 and 115% (obtained at different fortification levels) with RSD values below 10% in most cases, confirm the usefulness of the proposed methodology for the analyses of these kind of complex samples with a high fat content. Moreover, the obtained detection limits, which were below 5 $\mu g\,kg^{-1}$ by LC–MS analyses and ranged from 10 to 60 $\mu g\,kg^{-1}$ by GC–MS meet the requirements established by the olive oil pesticide regulatory programs. The method was satisfactorily applied to different olives and olive oil samples.
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1. Introduction

Olive oil represents an important commodity in terms of health and economy in Europe. Thus, this product has a great importance in the sustainable economy of important regions in Spain, Greece and Italy. "Virgin" olive oil is obtained from the fruit of the olive tree (*Olea Europaea*) exclusively by mechanical and/or physics means without any further treatment, mainly under thermal conditions which do not alter the olive oil quality. The positive effects of olive oil on health have prompted a demand for this product worldwide.

The most extensively applied agrochemicals in olive plantations of Mediterranean countries are by far herbicides and insecticides. These pesticide residues can persist to the harvest stage, making possible the contamination of the olives used to produce the olive oil. This can cause the presence of trace amounts of these pesticides in olive oil samples. Consequently, both the European Union and the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations (FAO) have established maximum pesticide residue limits in olives and olive oil. Currently, various Olive Oil Pesticide Residue Regulatory Programs are being

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carried out to up-date and establish new and more stringent regulations concerning the maximum residue levels in these commodities [1].

Analytical problems associated with the analysis of pesticides in these fatty foods are well known, especially when common GC analysis is applied. Therefore sample preparation is a crucial step in the analytical procedure since even small amount of lipids can harm columns, detectors or cause signal suppression. Many multi-residue procedures employing different clean-up techniques and a variety of detection methods have been reported for the determination of pesticide residues in olive oil. The most commonly used methodology is based on GC [2–5] after a comprehensive clean-up step, in most cases based on liquid–liquid partitioning [6,7] or gel permeation chromatography (GPC) [8,9] to separate the low molecular mass pesticides from the higher molecular mass fat constituents of the oil, such as triglycerides. The preparation of oil samples for the determination of pesticides by GC requires the complete removal of the high-molecular-mass fat from the sample to maintain the chromatographic system in working order. Most methods currently applied are based on GPC clean up, which represents much analysis time and is typically bottleneck of the analytical procedure furthermore large amount of organic wastes are produced that require safe disposal. Other alternatives could include the use of various solid-phase extraction (SPE) based procedures by using adsorbents such as florisil, alumina, silica gel, etc. Afterwards more sophisticated instrumentation such as supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) are also tested. However, the practical needs for an appropriate pesticide control are mainly focused in simple and fast sample treatment methods that may be easily implemented in routine laboratories. In this sense, matrix solid-phase dispersion (MSPD) [10–12] based methods can represent a method of choice taking into consideration these requirements. A second point is the fact that very few studies have evaluated the application of methods for this kind of samples, based on liquid chromatography–mass spectrometry, even when in many cases herbicides are clear examples of LC amenable compounds [13].

As can be seen in Fig. 1, MSPD is a SPE based strategy in which a fine dispersion of the matrix is mixed with a sorbent material (silica, alumina, C_{18}, etc.) with a mortar and a pestle. After blending, this material is packed into a mini-column, where the analytes are eluted by a relatively small volume of a suitable eluting solvent. This step can be accomplished together with a "co-column" clean-up, to achieve a further degree of matrix removal. The co-column material (florisil or silica, in example) is packed into the bottom of the same column of the sorbent, cleaning the sample as it elutes from the MSPD sorbent-matrix mixture. Therefore, MSPD enables the development of straightforward extraction and clean up steps, reducing the use of large amount of toxic solvents and speeding up the sample treatment process.

This work is focused on the development and evaluation of a simple sample preparation strategy based on matrix solid-phase dispersion using aminopropyl as sorbent material and acetonitrile as eluting solvent, with a clean-up performed in the elution step using florisil, followed by mass spectrometric identification and quantitation of the selected group of insecticides and herbicides, typically found in olive oil, using both gas chromatography–mass spectrometry (GC–MS) in selected ion monitoring (SIM) mode and liquid chromatography tandem mass spectrometry (LC–MS–MS) in positive ionization mode. To the best of our knowledge, this is the first LC–MS–MS method applied to determine pesticides in both olives and olive oil.

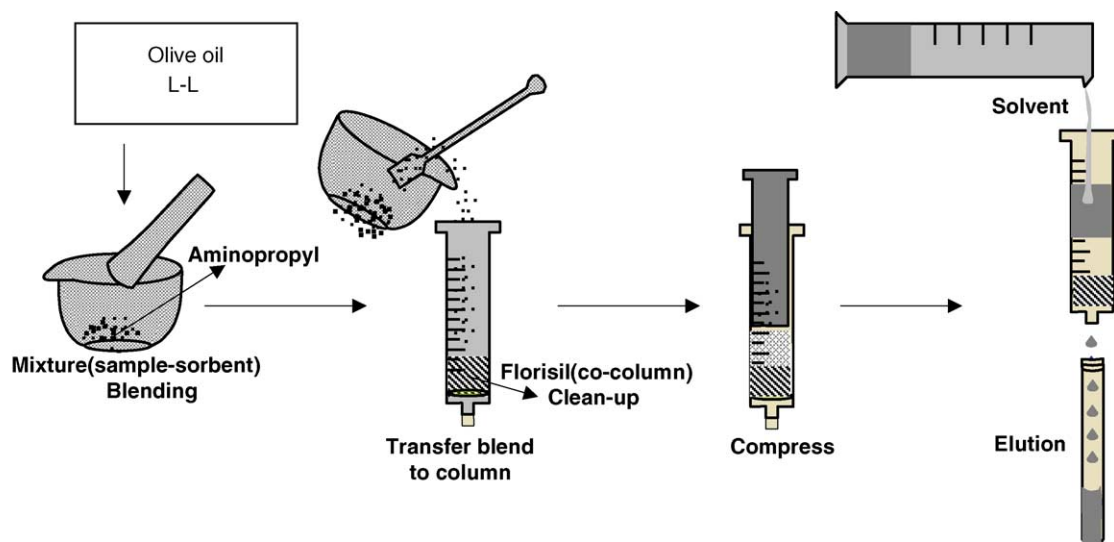

Fig. 1. Schematic representation of the MSPD extraction procedure.

2. Experimental

2.1. Reagents and solutions

Pesticide analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany) and Riedel-de-Haën (Seize, Germany). Individual pesticide stock solution (200–300 $\mu g\, ml^{-1}$) were prepared in pure methanol or ethyl acetate and stored at −18 °C. HPLC grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). Petroleum ether was purchased from Panreac (Barcelona, Spain). Petroleum ether saturated with acetonitrile was prepared by adding 100 ml of acetonitrile to 500 ml of petroleum ether. Acetonitrile saturated with petroleum ether was prepared by adding 100 ml of petroleum ether to 500 ml of acetonitrile. A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used during the analyses.

2.2. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were run on a HP 6890 Series gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) interfaced to a HP 5973 mass-selective detector. Data acquisition and processing, and instrumental control were performed by the HP mass-selective detector Chem-Station software. Analytes were separated in a ZB-5MS capillary column (5% diphenyl/95% dimethylsiloxane), 30 m × 0.25 mm i.d., 0.25 μm film thickness (Phenomenex, Torrance, CA, USA). A split/splitless injector was used in pulse splitless mode. An empty liner was filled with 0.5 cm Carbofrit (Restek, Bellefonte, USA) placed at 3.6 cm from the upper part of the liner. The injector operating conditions were as follows: injection volume 10 μl; injector temperature 250 °C; initial pulse pressure 30 psi (1.5 min). The helium carrier gas flow was maintained at 1 ml/min. The oven temperature programme was 4.0 min at 105 °C, 20 °C/min to 180 °C (keeping 180 °C for 8 min), 4 °C/min to 220 °C ((keeping 220 °C for 3 min) and 6 °C/min to 300 °C (keeping 300 °C for 5 min). The transfer line temperature was set at 280 °C.

Typical MS operating conditions were optimised by the autotuning software. EI mass spectra were obtained at 70 eV of electron energy, and monitored from *m/z* 50 to 400. The ion source and quadrupole analyser temperatures were fixed at 230 and 106 °C, respectively. Analyses in the NCI mode used methane as reagent gas. The autotuning software performed the reagent gas flow adjustment and the lens and electronic tuning. The quadrupole temperature was fixed at 106 °C and the ion-source temperature at 150 °C.

2.3. Liquid chromatography Ion-trap mass spectrometry

The method was developed using an HPLC system (consisting of vacuum degasser, autosampler and a binary pump) (Agilent Series 1100, Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed phase C_8 analytical column of 150 mm × 4.6 mm and 5 μm particle size (Zorbax Eclipse XDB-C8). Column temperature was maintained at 25 °C. The injected sample volume was 50 μl. Mobile phases A and B were water with 0.1% formic acid and acetonitrile respectively. A gradient elution was made using binary gradient of LC as follows: isocratic conditions for 5 min at 10% of solvent B, then linear gradient from 10 to 100% of solvent B, from 5 to 30 min. The flow-rate used was kept at 0.6 ml/min. A 12-min post-run time was used after each analysis.

This HPLC system was connected to an ion-trap mass spectrometer Agilent MSD Trap (Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray interface operating in positive ion mode. Ions were detected in ion charged control (ICC) (target: 50,000 ions) with an accumulation time of 200 ms, using the following operation parameters: capillary exit voltage (fragmentor): 50 V; capillary voltage: 4000 V; nebulizer pressure: 40 psig; drying gas: 9 $l\, min^{-1}$; gas temperature: 300 °C.

2.4. Sample treatment

2.4.1. Spiking procedure

A representative 50 g portion of olives (including the seed of the crop) previously homogenised was weighted and transferred to a glass mortar, where it was fortified homogenously with 5 ml of the working standard solution. The mixture was then gently blended in the mortar for 1 h, to asses the homogeneity of the sample. The sample was then allowed to stand at room temperature for one hour, before it was kept at −18 °C, until analysis.

2.4.2. Matrix solid-phase dispersion (MSPD) based extraction procedure

A methodology based on MSPD was used for the extraction of the selected herbicides from both the olive oil and the olives samples. The olive oil method added a preliminary liquid–liquid extraction before the MSPD step. In Fig. 1, the main steps of the procedure are schematically depicted.

2.4.2.1. Olives. A representative 1g portion of sample previously homogenised was weighted and transferred to the mortar, where it was gently blended and homogenized together with 2 g of aminopropyl (Bondesil-NH_2, 40 μm particle size, Varian Inc., Middleburg, The Netherlands) until obtaining a fine powder. A glass mortar was used in order to avoid analyte losses, as it had been already reported, with the use of materials such as porcelain [11]. This mixture was then transferred to a commercially available minicolumn containing 2 g of florisil (12 ml Bond-Elut-Varian minicolumn, Varian Inc.). This minicolumn was connected to a vacuum system for solid phase extraction adjusting the flow to 3 ml/min. The elution step was carried out with 2 ml × 5 ml of acetonitrile. The first aliquot of the eluting solvent was used to backwash both the mortar and the pestle. The final extract was evaporated until near dryness, being then dissolved in 1:1 acetonitrile:water

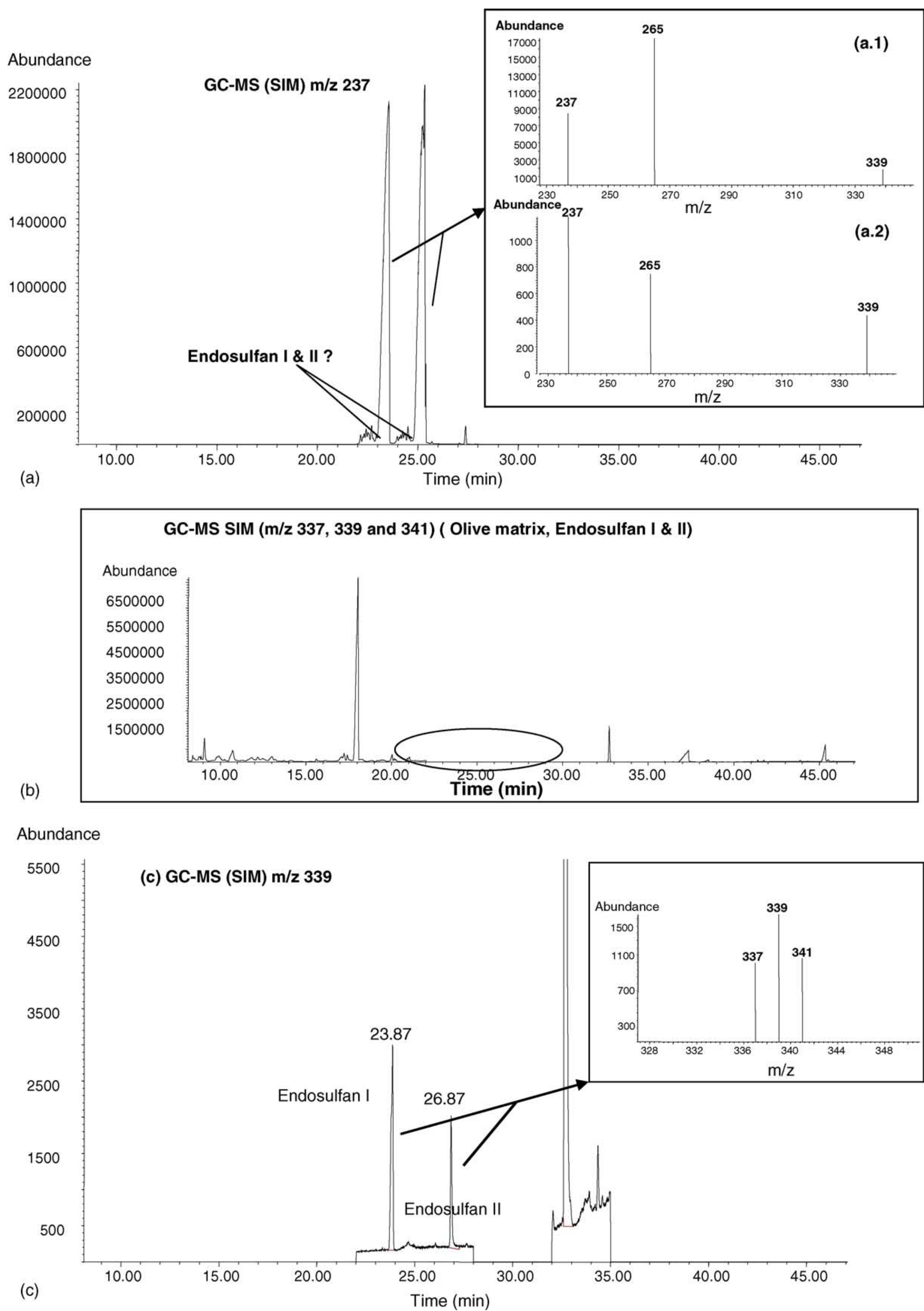

Fig. 2. Figure SIM mode in endosulfan in olives samples: use of different ions for the identification of endosulfan I and II in olives and olive oil.

for LC–MS analyses, and in acetonitrile for GC–MS. Prior to mass spectrometric analysis, the obtained extract was filtered through a 0.45 μm PTFE filter (Millex FG, Millipore, Milford, MA, USA).

2.4.2.2. Olive oil. An aliquot of approximately 5 g (ca. 5.5 ml) of olive oil sample was weighted in a 50 ml beaker. 15 ml of petroleum ether saturated with acetonitrile (see experimental section) were added and the mixture was then transferred to a 100 ml separation funnel, in which a two-step liquid–liquid extraction was undertaken. The first one with 25 ml of acetonitrile saturated with petroleum ether. The funnel was shaken vigorously for 3 min, being then the remaining acetonitrile phase separated from the petroleum ether one. After that, another 10 ml of acetonitrile saturated with petroleum ether were added to the petroleum ether extract, and the mixture was shaken for 3 min again, being both acetonitrile phases collected together. With a 10 ml pipette, a 7 ml aliquot of the acetonitrile extract was taken and transferred to a 10 ml glass test tube. The extract was then carefully evaporated up to a final volume of about 2 ml. This remaining extract was transferred to a glass mortar to be subject to the same treatment described above.

3. Results and discussion

3.1. Identification and quantitation of pesticides in olives and olive oil

3.1.1. Gas chromatography–mass spectrometry

The selected pesticides analysed by gas chromatography were: simazine, terbuthylazine, methyl-parathion, methyl-pirimiphos, endosulfan I, endosulfan II, endosulfan sulphate, cypermethrin and deltamethrin. The GC–MS analyses were performed in SIM mode. Three ions were used for identification and quantitation purposes. The selected ions chosen for identification and quantitation along with their relative abundances and the typical retention times are summarized in Table 1. As general criterion, the mass spectrometry conditions were carefully selected to provide a compromise solution between sensitivity, selectivity and structural information for quantitation purposes, being the most abundant peak chosen for quantitation purposes. However, due to the complexity of the olives matrix, the most abundant ion could not be used in all cases, because of the effect of interferences from the matrix. It happened in the case of both endosulfan I and II, which were determined in olives and olive oil, with different ions. In Fig. 2(a), the GC–MS SIM (*m*/*z* 237) chromatogram used for the quantitation of both endosulfan I and II is represented. The peak width confirms the presence of interfering species from the matrix. In addition, the relative intensity of the selected ions of the mass spectrum of this peak (Fig. 2(a.1) does not compare well with the relative intensities obtained with standards (Fig. 2(a.2)), which evidences the presence of masking species from the matrix. This makes not reliable the use of the most abundant ion of endosulfan for quantitation purposes in olives. Therefore, a more selective (and less sensitive) ion should be used. As can be seen in Fig. 2(b), where the GC–MS SIM (*m*/*z* 337, 339 and 341) chromatogram of the olive matrix is shown, this matrix has no interfering species at the retention times of endosulfan I and II, thus making possible the use of these ions for their proper identification and quantitation in olives (see Fig. 2(c)). For this reason, in olives, the quantitation ion used is *m*/*z* 339, which is not the most abundant, but rather more selective than the most abundant *m*/*z* 237, used in olive oil. For the quantitation of the rest of the pesticides, as can be noticed in Table 1, the most abundant ion (except for deltamethrin) was selected.

3.1.2. Liquid chromatography Ion-trap mass spectrometry

The selected pesticides analysed by LC–MS were: dimethoate, simazine, atrazine, diuron and terbuthylazine. All of them were analysed in positive ionization mode.

Table 1
Identification and quantitation by GC–MS

Pesticide	Mw[a]	Time (min)	SIM ions (RA[b], %)			
			Identification			Quantitaton
Simazine	201	13.0	186 (69)	201 (100)	203 (33)	201
Terbuthylazine	229	13.8	214 (100)	216 (33)	229 (24)	214
Parathion-methyl	263	17.3	109 (93)	125 (100)	263 (70)	125
Pirimiphos-methyl	305	19.0	276 (90)	290 (100)	305 (69)	290
Endosulfan I	404	23.7	237 (100)	265 (58)	339 (26)	237
			337 (63)	339 (100)	341 (65)	339[c]
Endosulfan II	404	26.8	237 (100)	265 (58)	339 (26)	237
			337 (63)	339 (100)	341 (65)	339[c]
Endosulfan sulfate	420	29.6	272 (100)	274 (84)	387 (65)	272
Cypermethrin	416	38.9	163 (83)	165 (54)	181 (100)	181
Deltamethrin	505	41.7	181 (100)	253 (81)	255 (36)	253

[a] Mw: molecular weight.
[b] RA: relative abundance.
[c] Selected quantitation ions in olives samples (for details see text).

Preliminary studies with matrix matched standards, accomplished in single MS mode evidenced the presence of numerous isobaric interferences in both olive oil and olives matrices. For this reason, in order to increase selectivity, the LC–MS method to determine the selected pesticides in olives and olive oil was optimised in MS–MS mode, isolating the precursor ion (molecular ion) using a narrow isolation mass window of *m*/*z* 2 and an optimised fragmentation in order to enhance both sensitivity, selectivity and signal-to-noise ratio, lowering thus, the detection limits when compared with those obtained in MS mode. For fragmentation purposes, the study of the amplitude voltage was undertaken in the range from 0.5 to 1.2 V. The triazines atrazine and terbuthylazine showed a similar fragmentation pattern yielding respectively *m*/*z* 216 -> 174 and *m*/*z* 230->174, using an amplitude voltage of 0.8 V. The optimized transition for diuron was *m*/*z* 233->72, using 0.8 V. However, simazine gave a poor fragmentation, yielding unstable and low abundant fragments corresponding to *m*/*z* 124 and 132, which could not be used for quantitative purposes. In fact, no studies have been reported regarding the determination of simazine by LC–ESI–MS–MS using an ion trap instrument. It has been accomplished only with an APCI source [14,15]. Therefore, in this case, for quantitation purposes, we isolated only the molecular ion of simazine (*m*/*z* 202), without fragmenting it.

In the case of dimethoate, the quantitation was carried out using multistage mass spectrometry. We noticed that MS–MS mode (transition *m*/*z* 230->199) was not selective enough for the analyses of dimethoate in olive samples, due to the complexity of the olive sample. The peak obtained for dimethoate was overlapped with species from the matrix. In Fig. 3(a), the total ion chromatogram of an olive sample (spiked with

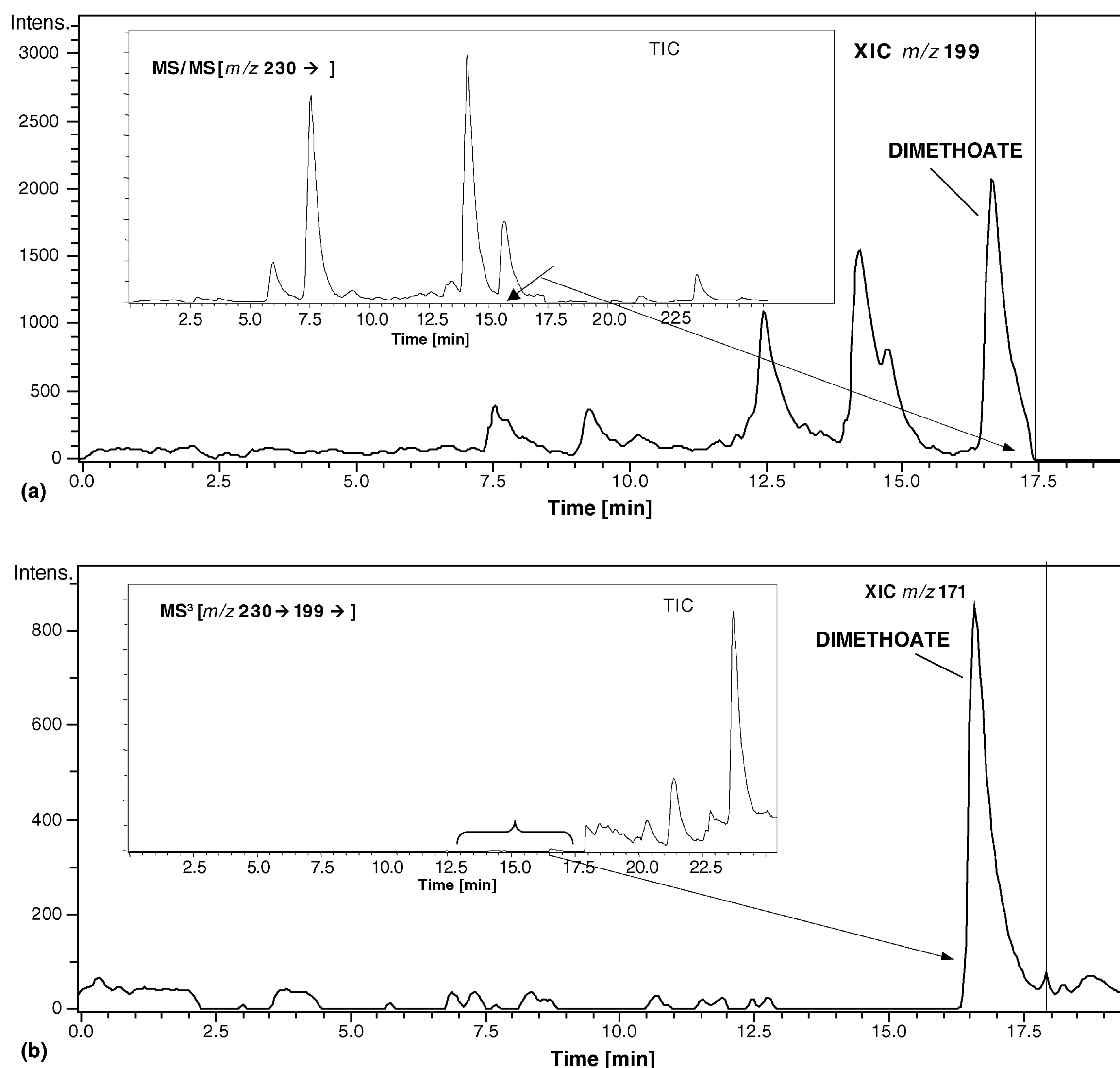

Fig. 3. Analysis of dimethoate by liquid chromatography multistage mass spectrometry: (a) extracted ion chromatogram of *m*/*z* 199 (dimethoate MS–MS fragment) in an olives sample spiked with 10 μg kg^{-1} of dimethoate, performed in MS–MS mode (*m*/*z* 230); (inset) corresponding total ion chromatogram (TIC); (b) extracted ion chromatogram of *m*/*z* 171 (dimethoate MS^3 fragment) of the extract, performed in MS^3 mode (*m*/*z* 230 → 199); (inset) TIC. (For details, see text).

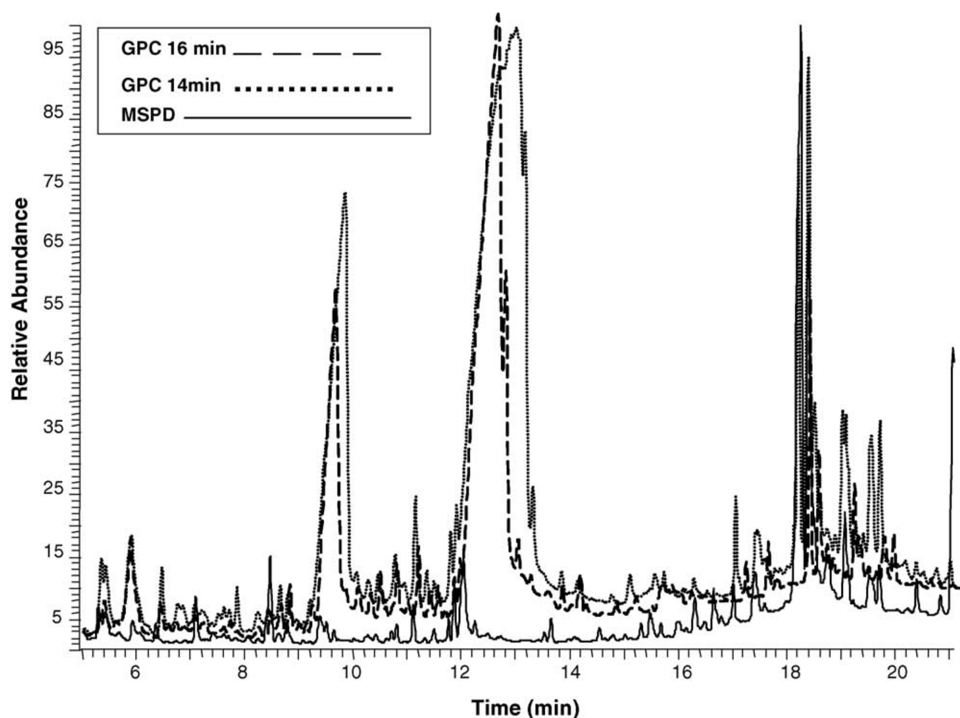

Fig. 4. Comparison of GC–MS full-scan olive oil matrix chromatograms obtained using gel permeation chromatography (GPC) and matrix solid-phase dispersion (MSPD) extractions.

0.025 mg kg^{-1} of dimethoate) obtained in MS–MS mode (isolating the molecular ion of dimethoate (*m/z* 230)) is shown together with the extracted ion chromatogram of the characteristic fragment ion of dimethoate (*m/z* 199)((a) Inset). The peak corresponding to dimethoate (t_R 16.7 min) was partially overlapped with interfering species from the matrix. In fact, the peak area of dimethoate was about five-fold higher than that obtained in pure solvent, when the transition *m/z* 230->199 was used. However, as can be seen in Fig. 3(b), the use of multi-stage mass spectrometry (*m/z* 230->199->171) provided enhanced selectivity, being then the matrix interferences easily circumvented. In fact, the use of two consecutive transitions yielded a total ion chromatogram without interfering peaks (see TIC from Fig. 4). For this reason, the MS^3 transition *m/z* 230->199->171 was selected, using amplitude voltages of 0.5 and 0.75 V respectively. Using this selective transition, the matrix interferences were completely removed. Moreover, the signal-to-noise ratio was increased, providing enhanced detection limits. The detection limit obtained for dimethoate in olives with multistage mass spectrometry was 4 μg kg^{-1}. Using single MS mode (using the ion trap in full-scan mode), the detection limit was 70 μg kg^{-1}.

3.2. Evaluation of matrix solid-phase dispersion based extraction procedure

3.2.1. Preliminary studies

The MSPD procedure is schematically depicted in Fig. 1. Due to the complexity of the sample with a high fat content, a clean-up step prior to analysis was mandatory, especially in olive samples. The developed extraction method involved a clean-up stage, due to the use of a co-column packed with florisil, in the elution step. Commercially available 12-ml florisil columns packed with 2 g of florisil were used to perform the elution step along with the clean-up. Three different materials were evaluated for the clean up: florisil, silica and alumina. Alumina was discarded, because of the low effectiveness of this clean-up, obtaining dirty extracts, which were not suitable for GC–MS analyses. Silica and florisil, provided clean extracts, so a study to evaluate the feasibility

Table 2
Comparative study of silica and florisil for the MSPD clean-up step in spiked olives (concentration level: 500 μg kg^{-1})

Pesticide	Silica		Florisil	
	Recovery (%)	RSD (%)[a]	Recovery (%)	RSD (%)[a]
Terbuthylazine	88.2	2	106.6	6
Endosulfan I	71.6	7	95	5
Endosulfan II	89.2	7	98.4	7
Endosulfan Sulfate	102.8	3	113	4

[a] $n = 3$.

Table 3
Study of the preliminary liquid–liquid extraction of olive oil method

Pesticide	Single liquid–liquid		Two-step liquid–liquid	
	Recovery (%)	RSD (%)[a]	Recovery (%)	RSD (%)[a]
Terbuthylazine	60.1	7.6	123.8	6.8
Simazine	50.1	10	108.8	4.5
Dimethoate	58.1	8.1	101.5	7.4
Endosulfan I	49.2	3.5	81.7	6.3
Endosulfan II	49.3	8.6	98.4	7.0
Endosulfan Sulfate	47.5	6.8	123	4.0

Single liquid–liquid extraction was accomplished with 25 ml (5 min); two-step liquid–liquid extraction was accomplished with 25 + 10 ml (4 + 3 min).
[a] $n = 5$.

Table 4
Recovery and RSD studies of the pesticides analysed by LC–MS in olives and olive oil

Pesticide	Amount added			
	$10\ \mu g\ kg^{-1}$		$100\ \mu g\ kg^{-1}$	
	Recovery (%)	RSD (%)[a]	Recovery (%)	RSD (%)[a]
Olives				
Dimethoate	95	8	88	7
Simazine	96	6	99	6
Atrazine	81	9	111	8
Diuron	88	7	86	6
Terbuthylazine	86	10	108	7
Olive oil				
Dimethoate	83	9	91	9
Simazine	88	7	102	6
Atrazine	103	7	104	8
Diuron	84	8	93	10
Terbuthylazine	96	5	103	6

[a] $n = 5$.

of the clean up step with each material were then accomplished, by performing recovery studies with some of the targeted pesticides, spiked in both olives and olive oil samples at a concentration level of $500\ \mu g\ kg^{-1}$. As can be seen in Table 2, both materials gave similar recoveries, except for endosulfan I, in which the values obtained with florisil were better than those obtained with silica. For this reason, florisil was chosen for further studies.

Another part of the extraction method which had a strong effect on the performance of the extraction, was the preliminary liquid–liquid extraction carried out in olive oil. We studied the use of a single liquid–liquid extraction using a volume of 25 ml, with an extraction time of 5 min, or a two-stage liquid–liquid extraction, using two aliquots of solvent (25 ml + 10 ml) with an extraction time of 4 min + 3 min. This study was carried out to evaluate the effect of the extraction step, using the rest of optimised method conditions. The results obtained from the recovery studies performed spiking olive oil with a concentration level of $500\ \mu g\ kg^{-1}$ are listed in Table 3. As it can be seen, the mean recoveries obtained using the single extraction method were lower than those obtained with the two-stage method. For this reason, two-step

Table 5
Recovery and RSD studies of the pesticides analysed by GC–MS in olive oil

Pesticide	Amount added			
	$80\ \mu g\ kg^{-1}$		$200\ \mu g\ kg^{-1}$	
	Recovery (%)	RSD (%)[a]	Recovery (%)	RSD (%)[a]
Simazine	101.5	3.3	129.7	4.0
Terbuthylazine	103.1	3.8	122.6	3.7
Methyl-parathion	93.4	4.9	109	14.6
Methyl-pirimiphos	84.0	3.9	118.4	4.6
Endosulfan I	73.2	5.8	91.4	3.1
Endosulfan II	95.4	5.7	119.5	4.8
Endosulfan sulfate	82.4	10.0	112.7	23
Cypermethrin	102.8	8.0	125.9	11.5
Deltamethrin	100.1	6.0	129.3	13.9

[a] $n = 5$.

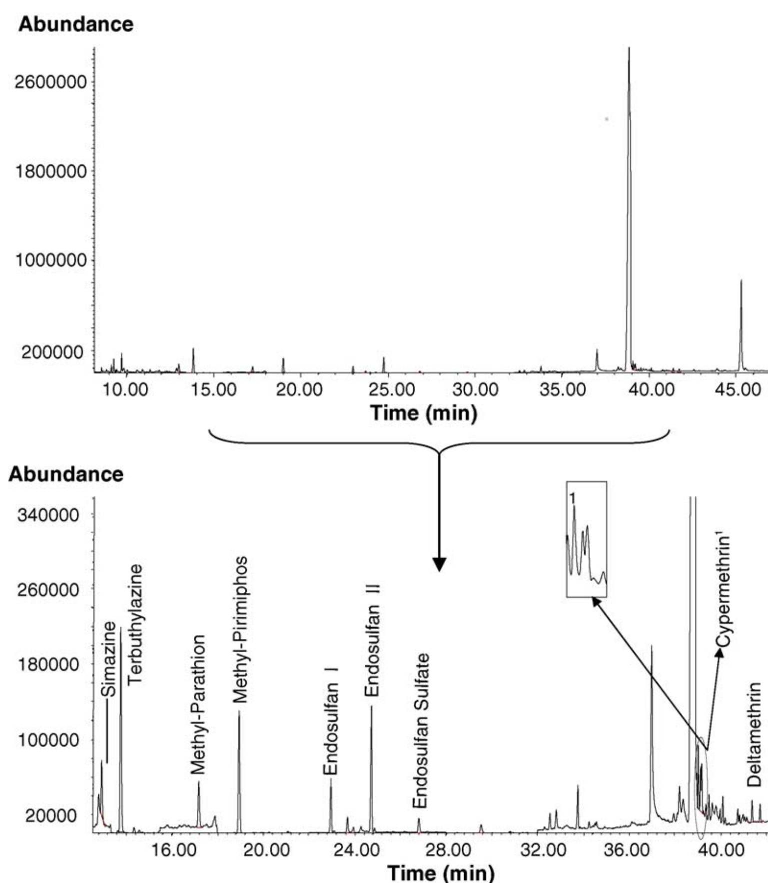

Fig. 5. Chromatograms corresponding to the GC–MS (SIM) analysis of an olive oil sample spiked with 100 μg kg^{-1} of the selected pesticides.

liquid–liquid extraction was adopted for the final extraction method.

3.2.2. *Performance of the extraction procedure*

The most widely used extraction procedure for olive oil is gel permeation chromatography. However, the main pitfalls associated with this methodology are both the use of large amount of organic solvents and the lack of flexibility to change from one method to another. Moreover, the separation of the pesticide fraction (which has low molecular weights) from the whole fatty matrix (constituted mainly by triglycerides) is very difficult to attain using GPC, because those two

Table 6
Analytical parameters

	Concentration range (mg kg$^-$1)	Linearity (regression coefficient)		Limits of detection (μg kg^{-1})		RSD (%)[a]	
		Olive oil	Olives	Olive oil	Olives	Olive oil	Olives
LC–MS–MS							
Dimethoate	0.005–0.5	0.988	0.999	3	4	5.8	4.8
Simazine	0.005–0.5	0.990	0.993	1	1	7.6	6.1
Atrazine	0.005–0.5	0.990	0.992	0.5	0.8	4.6	5.4
Diuron	0.005–0.5	0.994	0.995	2	2	4.8	3.7
Terbuthylazine	0.005–0.5	0.991	0.996	0.2	0.4	5.5	4.9
GC–MS							
Simazine	0.025–0.5	0.995	0.997	10	15	3.3	5.1
Terbuthylazine	0.025–0.5	0.997	0.996	3	8	3.8	3.6
Methyl-parathion	0.025–0.5	0.999	0.994	50	60	4.9	4.0
Methyl-pirimiphos	0.025–0.5	0.999	0.998	15	25	3.9	4.7
Endosulfan I	0.025–0.5	0.999	0.999	35	30	5.8	5.4
Endosulfan II	0.025–0.5	0.998	0.999	30	30	5.7	4.6
Endosulfan sulfate	0.025–0.5	0.994	0.999	30	40	9.9	6.1
Cypermethrin	0.025–0.5	0.996	0.997	50	70	8.2	5.4
Deltamethrin	0.025–0.5	0.997	0.999	60	80	6.0	3.5

[a] $n = 5$.

fractions are partially overlapped. Normally, as a compromise between cleanness of the extract (minimising the amount of fat in the pesticide fraction) and appropriate pesticide recoveries has to be chosen. This usually involves the lost of some of the pesticides (i.e. acrinathrin) [16], yielding, thus, lower mean recovery percentages. These drawbacks can be partly circumvented with the use of the proposed matrix solid-phase dispersion extraction method, which involves minor reagent consumption and waste generation and provides more flexibility to work. In addition, the resultant extracts are cleaner than those usually obtained by GPC, as can be seen in Fig. 4, where the full-scan GC–MS olive oil matrix chromatogram obtained using GPC is compared with that obtained using the method proposed in this work. The chromatogram of the MSPD method is much cleaner than those obtained with GPC using two different times to collect the pesticides fraction. This illustrates the capabilities of the proposed MSPD method to provide clean extracts of these complex matrices with a high fat content. To evaluate the effectiveness of the extraction method, different recovery studies were accomplished by spiking both olive oil and olives matrices at different concentration levels of the targeted analytes, being then analysed with the developed GC–MS and LC–MS methods. As can be seen in Tables 4 and 5, recoveries between 80 and 120% were obtained for the pesticides assayed in both olive and olive oil with RSD values below 10% ($n = 5$) in most cases. These results evidence the feasibility of the studied extraction method.

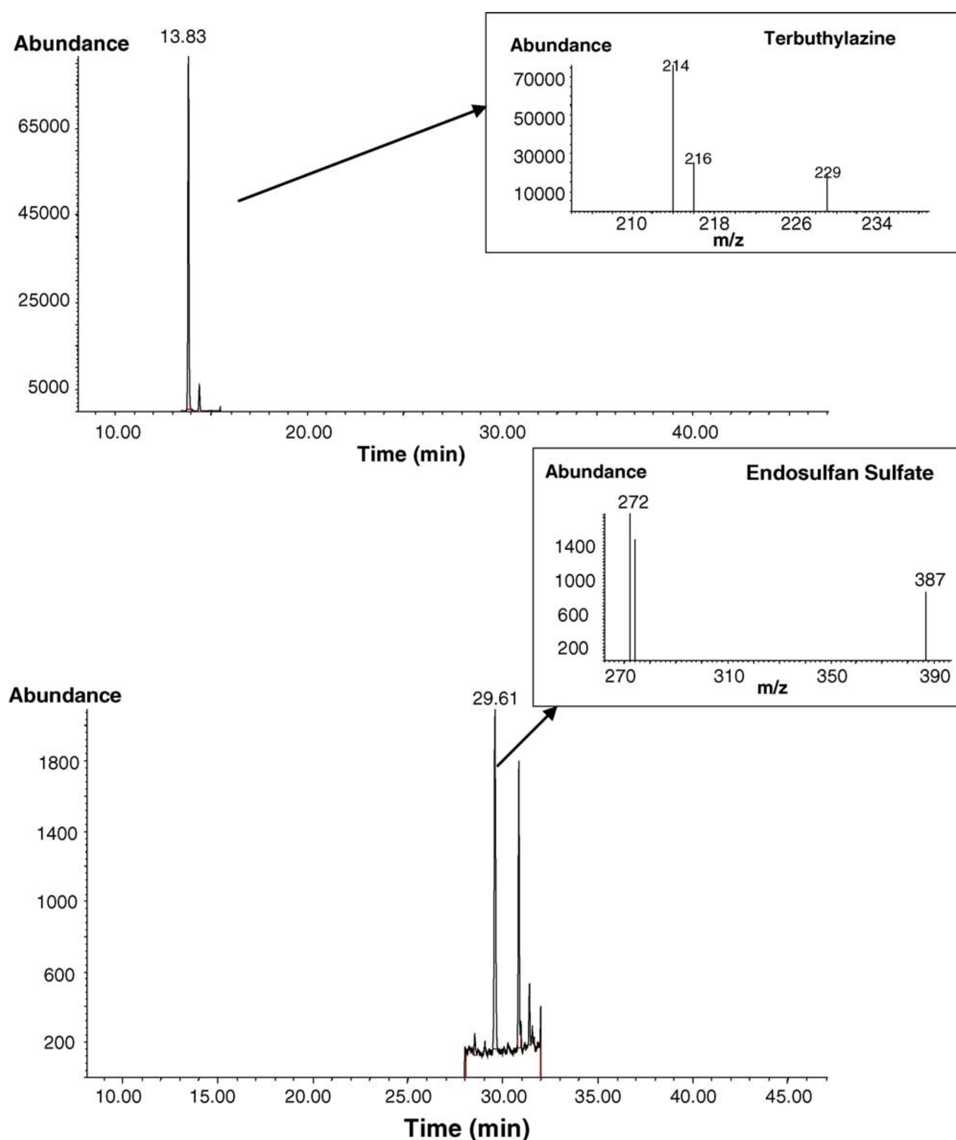

Fig. 6. GC–MS (SIM) Chromatograms, with their respective mass spectra (inset) corresponding to the analysis of an olive oil sample, where terbuthylazine and endosulfan sulfate have been detected at concentrations of 80 and 38 $\mu g\,kg^{-1}$, respectively.

3.3. Analytical features

The analytical performance of the proposed method was studied in order to evaluate its usefulness for quantitative analyses in the studied matrices. A preliminary study to evaluate the signal suppression was undertaken by comparison of calibration curve slopes obtained with solvent-based standards and with matrix matched standards. In LC–MS, both the olives and the olive oil samples suffer from matrix induced signal suppression, with a decrease in the signal up to 20% in olive oil and 35% in olives samples, depending on each individual pesticide. In GC–MS, the matrix produced either suppression or enhancement with fluctuations of up to about ± 15% depending also on both the matrix and each individual species. For this reason, the calibration was carried out using matrix matched standards. Linearity was evaluated by analyzing these matrix matched standards solutions, prepared at different concentration levels in the range 0.005–0.5 mg kg^{-1}. The quantitation was carried out using the extracting the chromatogram of the characteristic fragment ions analysed by LC–MS–MS and the selected ions used for quantitation purposes in GC–MS (see Table 1). The results obtained are summarized in Table 6. As can be observed, the linearity of the analytical response within the studied range was suitable, with correlation coefficients better than 0.99 in most cases. In addition, run-to-run RSD values obtained in both olives and olive oil were below 5 % in most cases. As an example, a typical chromatogram obtained by GC–MS in SIM mode of a 0.1 mg kg^{-1} matrix-matched standard from an olive oil sample is shown in Fig. 5.

The limits of detection (LOD) were estimated from the injection of matrix-matched standard solutions with concentration levels giving a signal-to-noise ratio of about 3. The results obtained by LC–MS and GC–MS analyses in both matrices are also included in Table 6. The limits of detection obtained are remarkable since, in most cases, they are far below the maximum residue level regulations established for these pesticides. In this sense, LC–MS analyses benefits of the use of mass spectrometry in MS–MS mode, which results in enhanced signal-to-noise ratio, providing, thus lower detection limits.

3.4. Analysis of pesticide residues in olives and olive oil samples

To evaluate the effectiveness of the proposed method, it was applied to the analysis of several samples of olive oil and olives. As an example, the GC–MS (SIM) chromatogram of a "positive" olive oil sample which contained both terbuthylazine and endosulfan sulphate is included in Fig. 6. The results obtained by LC–MS agreed with those obtained for GC–MS in the case of the pesticides analysed in both methods (simazine and terbuthylazine).

The proposed method was also applied to the analysis of a spiked olive sample from an internal inter-laboratory comparison test organized by TestQual® (www.testqual.com) for pesticide residue analysis performed by different laboratories in which the proposed method is being implemented for routine purposes. The results obtained are summarized in Table 7. These preliminary results confirm the feasibility of the proposed sample treatment strategy, which can be easily implemented in routine laboratories.

Table 7
Results obtained from an internal inter-comparison exercise carried out using the proposed method in a spiked olive sample

Pesticide	Amount found (mg kg^{-1})	Mean value (mg kg^{-1})[a]	RSD (%)[a]
Dimethoate	0.85	0.82	21
Endosulfan I	0.35	0.41	9
Endosulfan II	0.26	0.28	12
Deltamethrin	0.16	0.15	16

[a] Average value (3 laboratories).

4. Conclusions

In this study, we have applied, for the first time, matrix solid-phase dispersion as a sample treatment strategy to extract pesticide residues in matrices with a high fat content such as olive oil and olives. The proposed MSPD methodology was combined with mass spectrometric identification and quantitation by both GC–MS and LC–MS–MS, providing thus remarkable analytical features which allow the proposed methodology to be applied for the monitoring of pesticide residues in such commodities at low μg kg^{-1}. Moreover, MSPD offers various attractive advantaging features compared with gel-permeation chromatography; it provides cleaner extracts with remarkable mean recoveries (typically 85–110%), circumventing the main problems related with GPC methods with the collection of fractions (with the loss of part of some target species). In addition, it also involves both minor solvent consumption and waste generation.

The usefulness of the proposed approach has been assessed by various routine laboratories with remarkably good results. The application of this sample treatment strategy could be extended to other kind of samples with a relatively high fat content (i.e. avocado). More studies are being accomplished on our laboratory to explore the capabilities of MSPD in pesticide residue analysis.

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