Journal of Chromatography A, 1217 (2010) 6022–6035

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Large-scale pesticide testing in olives by liquid chromatography–electrospray tandem mass spectrometry using two sample preparation methods based on matrix solid-phase dispersion and QuEChERS

Bienvenida Gilbert-López [a], Juan F. García-Reyes [a], Ana Lozano [b], Amadeo R. Fernández-Alba [b], Antonio Molina-Díaz [a,*]

[a] Analytical Chemistry Research Group, Department of Physical and Analytical Chemistry, University of Jaén, Campus Las Lagunillas, Edificio B-3, 23071 Jaén, Spain
[b] Pesticide Residue Research Group, Department of Hydrogeology and Analytical Chemistry, University of Almería, 04120 La Cañada de San Urbano, Almería, Spain

ARTICLE INFO

Article history:
Received 30 April 2010
Received in revised form 22 July 2010
Accepted 23 July 2010
Available online 1 August 2010

Keywords:
Pesticide testing
Food
Olives
Liquid chromatography
Tandem mass spectrometry
Non-segmented multiple reaction monitoring (MRM) mode
MSPD
QuEChERS

ABSTRACT

In this work we have evaluated the performance of two sample preparation methodologies for the large-scale multiresidue analysis of pesticides in olives using liquid chromatography–electrospray tandem mass spectrometry (LC–MS/MS). The tested sample treatment methodologies were: (1) liquid–liquid partitioning with acetonitrile followed by dispersive solid-phase extraction clean-up using GCB, PSA and C_{18} sorbents (QuEChERS method – modified for fatty vegetables) and (2) matrix solid-phase dispersion (MSPD) using aminopropyl as sorbent material and a final clean-up performed in the elution step using Florisil. An LC–MS/MS method covering 104 multiclass pesticides was developed to examine the performance of these two protocols. The separation of the compounds from the olive extracts was achieved using a short C_{18} column (50 mm × 4.6 mm i.d.) with 1.8 μm particle size. The identification and confirmation of the compounds was based on retention time matching along with the presence (and ratio) of two typical MRM transitions. Limits of detection obtained were lower than 10 μg kg^{-1} for 89% analytes using both sample treatment protocols. Recoveries studies performed on olives samples spiked at two concentration levels (10 and 100 μg kg^{-1}) yielded average recoveries in the range 70–120% for most analytes when QuEChERS procedure is employed. When MSPD was the choice for sample extraction, recoveries obtained were in the range 50–70% for most of target compounds. The proposed methods were successfully applied to the analysis of real olives samples, revealing the presence of some of the target species in the μg kg^{-1} range. Besides the evaluation of the sample preparation approaches, we also discuss the use of advanced software features associated to MRM method development that overcome several limitations and drawbacks associated to MS/MS methods (time segments boundaries, tedious method development/manual scheduling and acquisition limitations). This software feature recently offered by different vendors is based on an algorithm that associates retention time data for each individual MS/MS transition, so that the number of simultaneously traced transitions throughout the entire chromatographic run (dwell times and sensitivity) is maximized.

1. Introduction

In recent years, particular scientific interest has been focused on the health benefits associated to the Mediterranean diet. Virgin olive oil is the main source of fats in the Mediterranean diet, and has become an outstanding commodity due to its fatty acid composition, and the content of tocopherols and phenolic compounds, which have been reported to exert beneficial effects on cardiovascular diseases and cancer [1]. For this reason, olive oil consumption has increased worldwide in approx. 1.100 tons over the last 19 years [2].

European Union regulates the use of agrochemicals to control pests in olive groves [3]. Pesticide residues, which can persist up to the harvest stage, are transferred to the olives by different ways, causing the presence of trace amounts of pesticides in both olives and olive oil. Consequently monitoring the pesticide residue levels in this commodity is of great interest to ensure food safety. Both the Codex Alimentarius Committee on Pesticide residues and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) have established maximum pesticide residue limits in olives and olive oil [4]. In addition, in September 2008, a new European Union regulation was set harmonizing maximum residue levels (MRLs) of pesticides

* Corresponding author. Tel.: +34 953 212 147; fax: +34 953 212 940.
E-mail address: amolina@ujaen.es (A. Molina-Díaz).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.07.062

in or on food and feed of plant and animal origin [5], including olives for oil production. Besides the traditional European countries (i.e. Spain, Italy and Greece), in recent years other countries such as China, Turkey, Australia, United States, Peru, Chile and Argentina are emerging olive oil producers. The application of agrochemicals in olive plantations is regulated independently in each country, so that both pesticides and dosage used may be different in each country, even considering that the production is destined to exportation. In addition, there is a lack of worldwide harmonized MRLs. It makes necessary the development of large-scale multi-residue methods to cover a wide range of pesticides with different physicochemical properties (not only those regulated by European Union), in order to update the analysis methods according to the new worldwide olive oil producers scenario.

Pesticide multi-residue analysis in olives is a challenging task taking into account the inherent complexity of the matrix because of the high fat content. Methods applied to determine pesticide residues in fatty food can require many steps and analysis time. The procedure normally includes sample treatment (extraction and clean-up) and chromatographic determination. The sample treatment is a crucial step when working with complex fatty food matrixes, as olives and olive oil. The main problem lies in the extraction of pesticides without co-extracting lipids. Many approaches are being carried out in order to solve this challenge, as several reviews published recently demonstrate [6,7]. The proposed approaches usually rely on liquid–liquid partitioning with acetonitrile followed by solid-phase extraction (SPE)-based clean-up [8–10] (amongst them QuEChERS [8]), gel permeation chromatography (GPC) [11–14] or matrix solid-phase dispersion (MSPD) [15–18]. This sample treatment scheme is combined with hyphenated chromatographic–mass spectrometric techniques. The technique of choice for the determination and quantification of pesticide residues in fruits and vegetables (including those with high fat content) has been traditionally GC–MS [11–14,19]. Nowadays, liquid chromatography–mass spectrometry (LC–MS) is replacing gas chromatography/mass spectrometry (GC–MS) methodologies, since pesticides are often more polar and less volatile, and from all the LC–MS techniques, LC–tandem mass spectrometry (LC–MS/MS) using a triple quadrupole instrument operated in multiple reaction monitoring (MRM) mode is the adapted gold standard for target pesticide testing by official and routine laboratories [20,21].

LC–MS/MS instruments (either triple quadrupole or hybrid quadrupole linear ion trap (QTRAP)) operated in multiple reaction monitoring (MRM) mode provides the most sensitive and highly selective detection for target multi-residue analysis of pesticides in complex samples. The main drawback of this approach is that the overall analytical performance of the method is dependent upon the number of species targeted. Actually, when dealing with a large number of compounds to trace, in order to achieve adequate sensitivity, chromatographic peak shape and peak sampling (number of evenly spaced data points across the chromatographic peak), it is necessary to group the analytes into time segments according to their elution patterns. Therefore, the development of conventional LC–MS/MS methods require users to pre-define multiple time segments in order to reduce the number of concurrent MRM transitions and the overall cycle time for each MRM scan, so that there are more data points per peak. One challenge using time segments is that the change from one segment to the next must be done during a time when no peaks are eluting from the LC column, a very difficult task when a large number of analytes is monitored and many coelutions occur. Time segmentation involves the risk of losing the analytes that elute near or between time segments. Besides, the addition of new target compounds into a method may require complete redevelopment in order to adjust time segments. To solve these drawbacks, a new software feature for multiple reaction monitoring (MRM) acquisition mode for LC–MS/MS (for both triple quadrupole and hybrid quadrupole linear ion trap (QTRAP) mass analyzers) analysis has been introduced by several suppliers [22–24]. The main advantage of this advanced MRM methods relies on the fact that the restriction of defining time segments is circumvented.

In this work, we report the development of a large-scale multi-residue analysis of pesticides in olives using LC–MS/MS. Due to the complexity of the matrix, and the lack of large-scale multi-residue methods in olives, two sample treatment protocols have been compared: QuEChERS (fatty vegetable matrices modified protocol) [8] and MSPD [15]. To our knowledge, these two sample preparation methodologies were validated just for a short list of compounds in both olives and olive oil matrices [8,15]. In the present study, this approach is applied to a hundred of pesticides. The proposed methodologies were validated and compared paying special attention to features such as occurring matrix effects, recovery rates and precision at different concentration levels, sensitivity/limits of detection and ruggedness. Together with the examination of the sample treatment extraction methods, we have addressed the use of the recently introduced automated MRM software feature for non-segmented LC–MS/MS method development, including a detailed discussion of the advantages and disadvantages of this approach.

2. Experimental

2.1. Pesticide standards

Pesticide analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany), certified quality, and from Riedel de Haën (Seelze, Germany), Pestanal® quality. Individual pesticide stock solution (*ca.* 500 $\mu g\,mL^{-1}$) were prepared in methanol and stored at −20 °C. Then, a working solution containing the mixture of standards was prepared (10 $\mu g\,mL^{-1}$) in methanol and also freezed.

A total number of 104 pesticides were selected for this study. Some of them (27) are included in Annex I of the European Union Directive 91/414/EEC concerning the placing of plant protection products on the market [3]. The rest of pesticides targeted and metabolites have been selected for their important health implications and their allowance in countries outside European Union, or for their appearance in fruits and vegetables [25]. Some of the studied pesticides are representative of a family of compounds with similar physicochemical properties.

European Regulation (EC) 396/2005 established (annexes II and III) MRLs for the appearance of some of the studied pesticides in olives for oil production. The default MRL was set in 10 $\mu g\,kg^{-1}$ for those residues that do not appear in annexes II and III of its Regulation. Codex Alimentarius has also set MRLs up to 100 $\mu g\,kg^{-1}$ for the presence of nine pesticides in olives samples [4].

2.2. Reagents

HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Formic acid (puriss) and anhydrous magnesium sulphate (reagent grade) were obtained from Fluka (Buchs, Switzerland). Primary–secondary amine (Supelclean™ PSA SPE Bulk packing, 50 μm) and graphitized carbon black – GCB – (Supelclean™ ENVI-Carb™ SPE Bulk packing, 120–400 mesh) were purchased from Supelco (Bellefonte, PA, USA). 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. Ethyl acetate and petroleum ether were from Riedel de Haën (Seelze, Germany), Pestanal® quality. Sodium acetate (reagent grade) was from Sigma–Aldrich (Madrid, Spain), and sodium chloride (reagent grade) was from J.T. Baker (Phillips-

burg, NJ, USA). Florisil cartridges (2 g, 50 μm, 12 mL) and C_{18} sorbent (50 μm) were from Análisis Vínicos (Tomelloso, Ciudad Real, Spain). Aminopropyl sorbent (Bondesil-NH_2 Bond Elut, particle size 40 μm) was obtained from Varian Inc. (Palo Alto, CA, USA).

2.3. Sample treatment

2.3.1. Pretreatment (mill)

Approximately 500 g of olives (including the kernel) were first crushed by means of a mill manufactured by Talleres Lopera (Priego de Córdoba, Córdoba, Spain) and designed specially for crushing up olives (*molino triturador-reductor* (*M-R*), 45 cm (length) × 51.5 cm (height) × 35 mm (width), 40 kg (weight)). The mill consisted in a hopper that led the olives to a worm gear connected to a rotor (1.1 kW). This rotor rips the olives and olive kernel, then obligating them to pass through a sieve of small orifices (5.0 mm i.d.). As a result, a homogenized paste is obtained and collected in a plastic food-container. The olive paste finally is frozen until its treatment for the analysis.

2.3.2. Procedure I: QuEChERS (quick, easy, cheap, effective, rugged and safe) procedure for fatty vegetable matrixes [8]

The employed method comprised the following steps: a representative 10 g portion of previously homogenized sample (crushed olives) was weighed in a 50 mL PTFE centrifuge tube. Then 10 mL of acetonitrile were added together with 4 g of anhydrous magnesium sulphate and 1 g of sodium chloride, and immediately the tube was vigorously shaken for 1 min to prevent coagulation of $MgSO_4$. The extract was then centrifuged (3700 rpm, 1377 g (rcf)) for 1 min. 5 mL of the supernatant (acetonitrile phase) were pipetted and transferred to a 15 mL graduated centrifuge tube containing 250 mg of PSA, 250 mg of C_{18} sorbent, and 250 mg of GCB, together with 750 mg of $MgSO_4$. Then, it was manually shaken for 30 s. After that, the extract was centrifuged again (3700 rpm, 1377 g (rcf)) for 1 min. 1 mL of this extract were evaporated to near dryness, and taken up with 500 μL with MeOH and 500 μL mQ H_2O. Prior to LC/MS analysis the extract was filtered through a 0.45 μm PTFE filter (Millex FG, Millipore, Milford, MA, USA) and transferred into a vial. In order to obtain cleaner samples, the extracts were diluted 1:2 with mQ water before they were injected in the HPLC–MS instrument. Thus, the injected extracts finally contained 75% of water.

2.3.3. Procedure II: matrix solid-phase dispersion (MSPD)

1 g of crushed olives was placed in a glass mortar and gently blended and homogenized together with 2 g of aminopropyl sorbent (Bondesil-NH_2) until a dry and homogeneous powder was obtained. This mixture was then transferred to a commercially available 12 mL SPE cartridge containing 2 g of florisil, connected to a vacuum system. The elution step was carried out with 2 × 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 (to facilitate the filtration step and avoid the formation of murky extracts), reaching a final volume of 1 mL. The extract finally contains the equivalent of 1 g of sample per mL and was filtered through a 0.45 μm PTFE filter (Millex FG, Millipore, Milford, MA, USA) and transferred into a vial, prior to LC/MS analysis. In order to obtain cleaner samples, the extracts were diluted 1:2 with mQ water before they were injected in the HPLC–MS instrument. Thus, the injected extracts finally contained 75% of water.

2.3.4. Spiking procedure

For recovery studies, the samples were spiked with the studied pesticides before the corresponding extraction procedure. A representative 100 g portion of homogenized crushed olives sample was weighted and fortified homogeneously with appropriate volume of working standard solution to reach 10 μg kg^{-1} of the studied pesticides in the spiked sample. The mixture was then gently blended for 1 h, to better ensure the homogeneity of the spiked sample. Then the sample was incubated at room temperature for 6 h, to make sure the solvent evaporated. Next, six extractions of 10-g portions from the spiked sample were prepared following the procedure described in Section 2.3.2. Besides, six extractions of 1-g portions from the spiked sample were prepared following the procedure described in Section 2.3.3. The same procedure was followed in order to perform the recovery studies at 100 μg kg^{-1} concentration level. Taking into account the dilution step at the final stage of sample treatment – using both protocols, QuEChERS and MSPD-, the extracts injected in the LC–MS/MS instrument contained, respectively, 5 and 50 μg L^{-1} of the studied pesticides.

2.3.5. Standard addition calibration curves

Matrix-matched standards of the studied pesticides (in olive matrix) were prepared using both sample treatment methods, by adding known amount of working pesticides solution to the olives extracts in order to attain the desired concentration range. Blank extracts of olives were also measured to ensure they did not contain the studied compounds.

2.4. Liquid chromatography/triple quadrupole mass spectrometry

2.4.1. Chromatography

The separation of the species from the extracts was carried out using an HPLC system consisting of vacuum degasser, autosampler and a binary pump (Agilent Series 1200, Agilent Technologies, Santa Clara, CA, USA). This was equipped with a reversed phase rapid resolution C_{18} analytical column of 50 mm × 4.6 mm i.d. and 1.8 μm particle size (RR Zorbax Eclipse XDB-C_{18}). 10 μL of extract were injected in each study. Mobile phases A and B were water with 0.1% formic acid and acetonitrile respectively. The chromatographic method held the initial mobile phase composition (10% B) constant for 1 min, followed by a linear gradient to 100% B at 11 min. Then, 100% B was passing during 4 min. The flow-rate used was 0.6 mL min^{-1}.

2.4.2. Electrospray triple quadrupole mass spectrometry

The HPLC system was connected to a triple quadrupole mass spectrometer Agilent 6410 Triple-Quad LC/MS (Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray interface operating in positive ion mode, using the following operation parameters: capillary voltage: 5000 V; nebulizer gas: 50 psig; gas flow: 12 L min^{-1}; gas temperature: 325 °C. Nitrogen served as the nebulised and collision gas. Specific MRM transitions, fragmentor voltage and collision energy were optimized for each compound analyzed (Table 1). Agilent MassHunter Data Acquisition software was used for method development and data acquisition. Agilent MassHunter Qualitative Analysis and Quantitative QQQ Analysis software were used for data processing, including the MassHunter Optimizer and the Dynamic Multiple Reaction Monitoring Mode (DMRM) software features. Automatic optimization of both fragmentor voltage and collision energy (CE) is accomplished (a ramp with different experimental values) – with or without using an HPLC column – after determining the *m*/*z* values for precursor and product ions.

Table 1
MRM parameters of the LC–MS/MS method developed for 104 pesticides, including quantifier (Q) (bolded) and qualifier (q) transitions, including optimized values for fragmentor and collision energy voltages.

Pesticide	Elemental composition parent ion	*m/z* parent	*m/z* fragments (Q/q)	fragmentor (V)	CE (V)	RT (min)
Methamidophos	C2H9NO2PS	142.1	125.0	90	10	1.28
			94.1			
Acephate	C4H11N1O3PS1	184.1	**143.0**	90	5	1.49
			125.0		15	
Thiocyclam	C5H12NS3	182.0	**137.0**	90	15	1.59
			73.0		20	
Omethoate	C5H13NO4PS	214.1	**183.0**	90	5	1.77
			125.0		20	
Carbendazim	C9H10N3O2	192.0	**160.0**	150	15	2.66
			132.0		20	
Butoxycarboxim	C7H15N2O4S	223.0	166.0	90	5	3.33
			106.0			
Aldicarb sulfone	C7H15N2O4S	223.0	148.0	120	5	3.73
			86.0		10	
Oxamyl	C7H13N3O3SNa	237.0	**72.0**	60	10	3.75
			90.0		5	
Methomyl	C5H10N2O2NaS	185.0	**128.0**	90	5	4.20
			99.0		10	
Monocrotophos	C7H15NO5P	224.0	**127.0**	60	10	4.25
			98.0		15	
Thiamethoxam	C8H11ClN5O3S	292.0	**211.0**	90	10	5.00
			181.0		20	
Pirimicarb	C11H19N4O2	239.2	182.1	150	15	5.28
			72.2		20	
Metamitron	C10H11N4O	203.0	**175.0**	120	15	5.45
			104.0		20	
Fenuron	C9H13N2O	165.0	120.0	90	15	5.60
			72.0		20	
Chloridazon	C10H9ClN3O	222.0	**104.0**	120	20	5.70
			92.0			
Imidacloprid	C9H11ClN5O2	256.0	209.0	90	15	5.77
			175.0			
Dimethoate	C5H13NO3PS2	230.0	**199.0**	90	5	5.94
			171.0		10	
Acetamiprid	C10H12ClN4	223.0	**126.0**	120	20	6.05
			56.0		15	
Thiacloprid	C10H10ClN4S	253.0	**126.0**	120	20	6.68
			186.0		10	
Aldicarb	C7H15N2O2S	213.0	116.0	120	10	6.83
			89.0		15	
Bromacil	C9H14BrN2O2	261.0	**205.0**	90	10	6.99
			188.0		20	
Imazalil	C14H15Cl2N2O	297.0	255.0	150	15	6.99
			159.0		20	
Monuron	C9H12ClN2O	199.0	126.0	120	20	7.11
			72.0		15	
Oxadixyl	C14H19N2O4	279.0	**219.0**	90	5	7.11
			133.0		20	
Simazine	C7H13N5Cl	202.0	**132.0**	120	20	7.15
			124.0			
Desethyl terbuthylazine	C7H13ClN5	202.0	**146.0**	120	15	7.30
			110.0		20	
Dichlorvos	C4H8Cl2O4P	221.1	144.9	150	10	7.30
			109.0		15	
Lenacil	C13H19N2O2	235.0	**153.0**	90	10	7.37
			136.0		20	
Carbofuran	C12H16NO3	222.0	165.0	90	10	7.75
			123.0		20	
XMC	C10H14NO2	180.1	**123.0**	60	5	7.91
			95.1		20	
Chlorotoluron	C10H14N2OCl	213.0	**72.0**	120	20	7.96
			140.0			
Fluometuron	C10H12F3N2O	233.0	**72.0**	120	20	7.96
			160.0			
Carbaryl	C12H12NO2	202.0	**145.0**	140	10	8.02
			127.0		20	
Pyrimethanil	C12H14N3	200.0	**107.0**	120	20	8.03
			183.0			
Atrazine	C8H15ClN5	216.0	**174.0**	120	15	8.11
			146.0		20	
Isoproturon	C12H19N2O	207.0	**72.0**	120	20	8.14
			165.0		10	
Deet	C12H18NO	192.1	**119.0**	120	15	8.17
			91.1		20	

Metalaxyl	C15H22NO4	280.0	**220.0**	120	10	8.17
			160.0		20	
Diuron	C9H11Cl2N2O	233.0	160.0	120	20	8.21
			72.0			
Ethiofencarb	C11H16NO2S	226.0	164.0	60	5	8.21
			107.0		15	
Difenoxuron	C16H19N2O3	287.0	123.0	90	20	8.23
			72.0			
Monolinuron	C9H12ClN2O2	215.0	148.0	120	15	8.26
			126.0			
Isoprocarb	C11H16NO2	194.1	**95.1**	90	15	8.47
			152.0		5	
Metobromuron	C9H12BrN2O2	260.0	**149.0**	120	10	8.51
			171.0		20	
Flazasulfuron	C13H13F3N5O5S	408.0	**182.0**	120	20	8.63
			301.0		10	
Dimethomorph	C21H23ClNO4	388.0	**301.0**	150	20	8.65, 8.81
			165.0			
Triadimenol	C14H19ClN3O2	296.2	227.0	60	5	8.79
			70.2		10	
Ethiprole	C13H10Cl2F3N4OS	396.9	**351.0**	120	15	8.90
			255.1		20	
Propazine	C9H17ClN5	230.0	188.0	120	20	8.90
			146.0			
Cyproconazole	C15H19ClN3O	292.0	125.0	120	20	9.04
			70.0			
Prochloraz	C15H17Cl3N3O2	376.0	**308.0**	90	10	9.12
			266.0		15	
Terbuthylazine	C9H17ClN5	230.0	**174.0**	120	15	9.19
			146.0		20	
Fenobucarb	C12H18NO2	208.1	**95.0**	90	10	9.20
			152.1		5	
Methidathion	C6H11N2O4NaPS3	303.0	145.0	60	5	9.25
			85.0		15	
Diethofencarb	C14H22NO4	268.2	**226.2**	90	5	9.29
			180.2		15	
Bupirimate	C13H25N4O3S	317.0	**166.0**	150	20	9.30
			108.0			
Fenamiphos	C13H23NO3PS	304.0	**217.0**	120	20	9.30
			234.0		15	
Fenarimol	C17H13Cl2N2O	331.1	268.2	150	20	9.30
			259.1			
Linuron	C9H11Cl2N2O2	249.0	**160.0**	90	20	9.30
			182.0		15	
Bromuconazole	C13H13BrCl2N3O	378.0	**159.0**	120	20	9.32, 9.68
			70.0			
Myclobutanil	C15H18ClN4	289.2	125.1	150	20	9.35
			70.2		15	
Promecarb	C12H18NO2	208.0	151.0	60	5	9.35
			109.0		15	
Azoxystrobin	C22H18N3O5	404.0	**372.0**	120	10	9.40
			344.0		20	
Dimethylvinphos	C10H11Cl3O4P	330.9	127.1	90	10	9.42
			205.0		20	
Chlorbromuron	C9H11BrClN2O2	293.0	204.0	120	20	9.43
			182.0		15	
Triadimefon	C14H17ClN3O2	294.2	225.0	150	10	9.54
			197.1			
Fenhexamid	C14H18Cl2NO2	302.0	**97.0**	90	25	9.57
			55.0		30	
Pyridaphenthion	C14H18N2O4PS	341.1	205.1	120	20	9.58
			189.2			
Tebuconazole	C16H23ClN3O	308.0	**70.0**	90	20	9.64
			125.0			
Methoxyfenozide	C22H29N2O3	369.3	**149.2**	90	15	9.79
			133.1		20	
Diflubenzuron	C14H10ClF2N2O2	311.0	**158.0**	120	10	9.80
			141.0		20	
Penconazole	C13H16Cl2N3	284.0	159.0	90	20	9.93
			70.0		15	
Iprodione	C13H14Cl2N3O3	330.0	**245.0**	90	15	9.95
			101.0		20	
Chromafenozide	C24H31N2O3	395.2	**175.1**	90	10	9.97
			339.2		5	
Malathion	C10H20O6PS2	331.0	127.0	90	10	10.01
			99.0		20	

Table 1 (*Continued*)

Pesticide	Elemental composition parent ion	*m/z* parent	*m/z* fragments (*Q*/*q*)	fragmentor (V)	CE (V)	RT (min)
Triazophos	C12H17N3O3PS	314.1	286.2	150	10	10.03
			162.2		20	
Fenoxycarb	C17H20NO4	302.2	116.2	90	5	10.10
			88.2		20	
Metolachlor	C15H23ClNO2	284.0	**252.0**	120	10	10.10
			176.0		20	
Alachlor	C14H21ClNO2	270.0	238.0	90	15	10.20
			162.0			
Triflumizole	C15H16ClF3N3O	346.0	**278.0**	90	5	10.20
			73.0		10	
Azinphos-ethyl	C12H17N3O3PS2	368.1	160.2	150	10	10.30
			132.2		15	
Neburon	C12H17Cl2N2O	275.0	**88.0**	120	15	10.30
			114.0		10	
Tebufenozide	C22H29N2O2	353.2	296.9	150	5	10.30
			133.1		15	
Edifenphos	C14H16O2PS2	311.0	283.1	120	10	10.35
			173.0		5	
Chlorfenvinphos	C12H15Cl3O4P	359.0	**155.1**	120	10	10.40
			126.9		15	
Aclonifen	C12H10ClN2O3	265.1	**248.1**	120	15	10.50
			218.1		20	
Difenoconazole	C19H18Cl2N3O3	406.0	337.0	120	15	10.50
			251.0		20	
Kresoxim-methyl	C18H20NO4	336.2	**246.2**	150	15	10.50
			229.2		20	
Triflumuron	C15H11ClF3N2O3	359.0	**156.0**	120	15	10.50
			139.0		20	
Benalaxyl	C20H24NO3	326.0	**294.0**	120	5	10.60
			208.0		15	
Quinalphos	C12H16N2O3PS	299.1	163.2	150	20	10.60
			147.2			
Anilofos	C13H20ClNO3PS2	368.0	**199.0**	120	10	10.80
			171.0		20	
Isofenphos methyl	C14H23NO4PS	231.0	199.0	90	15	10.93
			121.0			
Diazinon	C12H22N2O3PS	305.0	**169.0**	120	15	10.99
			153.0		20	
Indoxacarb	C22H18ClF3N3O7	528.1	**249.1**	150	15	11.20
			150.2			
Pirimiphos-methyl	C11H21N3O3PS	306.2	**164.2**	150	20	11.20
			108.2			
Fluacrypyrim	C20H22F3N2O5	427.1	**145.1**	90	20	11.30
			205.1		5	
Triclocarban	C13H10Cl3N2O	315.0	162.0	120	20	11.30
			128.0		15	
Trifloxystrobin	C20H20F3N2O4	409.2	206.2	120	10	11.30
			186.2		20	
Buprofezin	C16H24N3OS	306.0	**201.0**	120	10	11.40
			116.0		15	
Flufenoxuron	C21H12ClF6N2O3	489.0	306.0	120	15	11.90
			158.0			
Ethion	C9H23O4P2S4	385.1	**199.0**	90	5	12.20
			171.0		10	
Hexythiazox	C17H22ClN2O2S	353.1	228.2	120	10	12.30
			168.2		20	
Fenazaquin	C20H23N2O	307.3	**161.3**	150	15	12.50
			147.2			

3. Results and discussion

3.1. Liquid chromatography–tandem mass spectrometry method development

3.1.1. Separation and identification of the targeted pesticides by LC–electrospray-MS/MS

The separation of the targeted species was achieved in 16 min, obtaining satisfactory resolution with average peak widths of 10 s, which compares well against the typical analytical columns (i.e. 150 mm × 4.6 mm i.d., 5 μm particle size) usually 20–40 s average of peak width at baseline. Thus, the average base-line peak width is reduced 2-fold, which involves an increase in analyte S/N ratio at low concentrations, thus improving the limits of detection of the method. This improvement is based on the use of a short column (i.e. 5 cm) with small particle size (1.8 μm). In addition, the use of organic solvent (acetonitrile) is minimized *ca*. 60% with regards to classic analytical size columns, being therefore a more environmentally friendly LC method.

Standard conditions for small molecule analysis were set for electrospray source main parameters (nebulizer pressure, drying gas flow, gas temperature and capillary voltage) to provide the best possible sensitivity in positive ionization mode, since the effect of all these parameters in the commonly studied ranges did not affect significantly the signal of the analytes. In contrast, analyte-dependent MS parameters (such as MRM transitions, fragmentor voltage and collision energy) were carefully studied and optimized for each target compound individually. In most cases, the

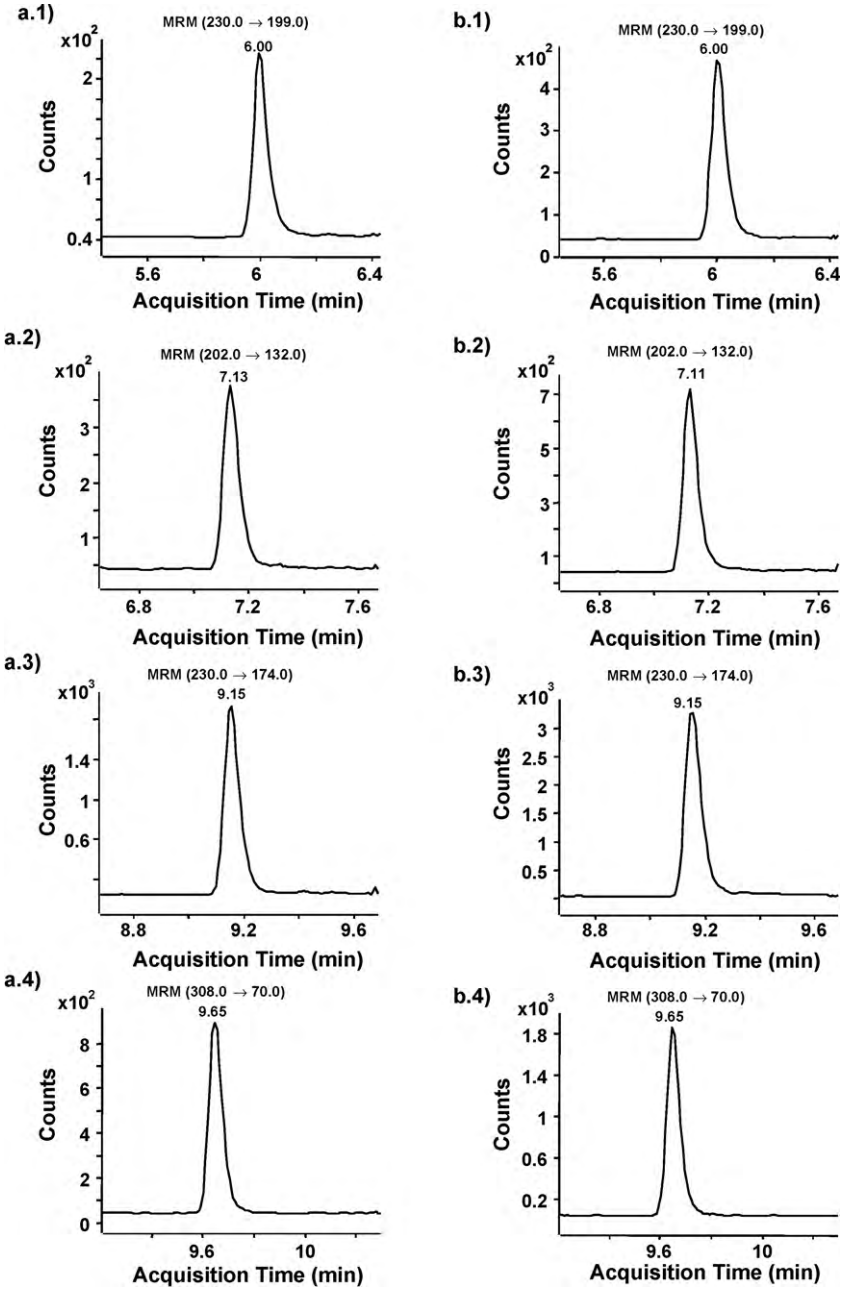

Fig. 1. MRM chromatogram corresponding to the selected quantitation transition (in DMRM mode) of (1) dimethoate, (2) simazine, (3) terbuthylazine and (4) tebuconazole from (a) a QuEChERS matrix-matched standard at 10 μg kg^{-1} concentration level, and (b) a MSPD matrix-matched standard at 10 μg kg^{-1} concentration level.

protonated molecules ($[M+H]^+$) were selected as precursor ions, except when the relative intensity of a sodium adduct ($[M+Na]^+$) was higher than that of the protonated molecule in the selected ESI+ conditions (methidathion, methomyl and oxamyl). In addition, two MRM transitions involving the formation of a product ion with higher m/z value and with a higher relative intensity were chosen, as far as possible. The optimization was carried out by the injection of 1 μL of the individual pesticide standard solution (1–3 ng μL^{-1} in acetonitrile) directly into the mass spectrometer into a constant flow of acetonitrile/water (50:50) of 0.2 mL min^{-1}. Fragmentor voltage was studied in the range 60–150 V (60, 90, 120 and 150 V) while collision energy was investigated in the range 5–20 eV (5, 10, 15 and 20 eV). In order to establish the best possible conditions, different combinations of fragmentor and collision energy voltages were assayed automatically using MassHunter Optimizer software (Agilent Technologies). Optimized parameters are listed in Table 1.

The identification of pesticide residues in olive extracts was carried out using the retention time matching and two specific MRM transitions, being the most intense transition used as a quantifier (Q) and the other one used as qualifier (q) peak for the confirmatory analysis. The ratio between these transitions (Q/q) is also used for confirmatory purposes, considering 20% of variability (tolerance). This criterion is in compliance with the DG SANCO European Quality Control guidelines [30], based on ion-ratio statistics for the transitions monitored. In the case of iprodione, kresoxim-methyl and methomyl, the calculation of the Q/q ratios was not possible because the intensity of the qualifier transition was very low. The rest of studied compounds presented variability of Q/q ratio lower than 20% in the linear concentration range. As an example, chromatographic peaks obtained for dimethoate, simazine, terbuthylazine and tebuconazole in matrix-matched standards at low concentration level (10 μg kg^{-1}) are shown in Fig. 1.

Table 2
Evaluation of the performance of the sample treatment procedures: recovery studies and matrix effects. Matrix effects are expressed as the ratio between the calibration curve slopes of matrix-matched standards and solvent-based standards. The first line in the recovery study corresponds to 10 μg kg^{-1} concentration level, and the second line corresponds to a 100 μg kg^{-1} spiked level.

Pesticide	t_R (min)	Matrix effect QuEChERS[a] (Δ%)	Matrix effects MSPD[a] (Δ%)	Recovery QuEChERS[b] %Rec (RSD%)	Recovery MSPD[b] %Rec (RSD%)
Methamidophos	1.28	0.76 (−24%)	1.03 (+3%)	89.24 (6.86)	<LOQ
				92.38 (5.57)	92.04 (10.76)
Acephate	1.49	1.00 (0%)	3.24 (+224%)	92.96 (6.94)	LOD
				87.69 (9.46)	–
Thiocyclam	1.59	1.09 (+9%)	0.54 (−46%)	70.80 (16.22)	75.18 (11.25)
				77.15 (4.45)	61.25 (12.60)
Omethoate	1.77	0.87 (−13%)	1.61 (+61%)	89.52 (5.97)	<LOQ
				96.30 (14.64)	60.44 (18.58)
Carbendazim	2.66	0.64 (−36%)	0.82 (+18%)	54.94 (4.61)	53.46 (8.88)
				52.57 (7.70)	41.19 (19.52)
Butoxycarboxim	3.33	0.83 (−17%)	1.05 (+5%)	116.25 (9.00)	71.23 (8.56)
				121.27 (5.67)	62.26 (9.83)
Thiabendazole	3.68	0.72 (−28%)	1.10 (+10%)	<LOD	<LOD
				44.09 (19.00)	40.81 (8.40)
Aldicarb sulfone	3.90	0.96 (−4%)	1.30 (+30%)	122.26 (8.43)	74.04 (7.23)
				119.33 (4.51)	62.86 (9.09)
Oxamyl	3.80	1.18 (+18%)	1.62 (+62%)	117.87 (7.05)	69.43 (9.38)
				111.54 (5.20)	62.24 (8.57)
Methomyl	4.20	0.30 (−70%)	0.39 (−61%)	LOD	69.52 (4.68)
				99.92 (5.88)	72.21 (4.17)
Monocrotophos	4.30	0.86 (−14%)	1.13 (+13%)	<LOD	<LOD
				108.40 (4.19)	58.81 (9.49)
Thiamethoxam	5.00	0.44 (−56%)	0.55 (−45%)	86.22 (7.01)	61.27 (7.29)
				92.68 (3.30)	51.59 (2.43)
Pirimicarb	5.28	0.72 (−28%)	0.80 (−20%)	92.61 (7.41)	79.88 (11.30)
				92.65 (4.03)	65.07 (8.17)
Metamitron	5.45	0.53 (−47%)	0.75 (−25%)	87.67 (15.6)	55.13 (8.69)
				71.75 (8.10)	35.02 (6.58)
Fenuron	5.60	0.42 (−58%)	0.79 (−21%)	70.13 (8.73)	61.48 (8.39)
				79.41 (9.26)	55.22 (5.00)
Chloridazon	5.70	0.34 (−66%)	0.50 (−50%)	82.96 (8.01)	57.75 (10.24)
				75.58 (6.68)	48.62 (2.75)
Imidacloprid	5.77	0.33 (−67%)	0.52 (−48%)	<LOQ	51.61 (9.77)
				85.08 (4.44)	43.67 (2.86)
Dimethoate	5.94	0.45 (−55%)	0.60 (−40%)	91.96 (9.55)	65.69 (11.67)
				91.75 (4.01)	55.73 (3.50)
Acetamiprid	6.05	0.42 (−58%)	0.74 (−26%)	87.75 (5.08)	63.27 (10.93)
				85.14 (3.99)	51.70 (1.66)
Thiacloprid	6.68	0.84 (−16%)	0.54 (−46%)	<LOQ	59.01 (12.30)
				75.06 (1.75)	45.83 (2.62)
Aldicarb	6.83	0.3 (−70%)	0.40 (−60%)	<LOD	<LOD
				60.82 (9.31)	48.95 (7.46)
Bromacil	6.99	0.41 (−59%)	0.71 (−29%)	97.72 (4.08)	66.99 (9.86)
				92.55 (6.75)	53.29 (7.96)
Imazalil	6.99	0.66 (−34%)	0.83 (−17%)	55.61 (11.66)	36.56 (18.04)
				54.33 (7.19)	24.41 (7.92)
Monuron	7.11	0.54 (−46%)	0.72 (−28%)	89.99 (9.66)	68.42 (11.94)
				93.58 (5.69)	58.70 (5.65)
Oxadixyl	7.11	0.68 (−32%)	0.90 (−10%)	108.94 (6.71)	72.68 (12.20)
				111.64 (5.63)	61.31 (7.09)
Simazine	7.15	0.50 (−50%)	0.95 (−5%)	98.81 (5.13)	69.02 (10.69)
				98.43 (4.86)	53.60 (4.46)
Desethyl terbuthylazine	7.30	0.58 (−42%)	0.70 (−30%)	105.76 (8.72)	72.48 (10.95)
				94.74 (3.79)	52.47 (6.02)
Dichlorvos	7.30	1.01 (+1%)	2.87 (+187%)	87.60 (3.81)	–
				94.93 (4.63)	–
Lenacil	7.37	0.53 (−47%)	0.87 (−13%)	93.10 (7.64)	68.79 (10.81)
				94.32 (5.51)	53.98 (6.96)
Carbofuran	7.75	0.86 (−14%)	1.02 (+2%)	118.12 (8.33)	77.86 (10.96)
				115.91 (5.75)	65.73 (8.52)
XMC	7.91	1.79 (+79%)	2.11 (+111%)	106.20 (7.12)	72.88 (8.21)
				110.94 (4.62)	70.29 (5.52)
Chlorotoluron	7.96	0.44 (−56%)	0.95 (−5%)	98.52 (3.97)	64.27 (8.93)
				99.88 (4.37)	56.59 (7.24)
Fluometuron	7.96	0.54 (−46%)	0.74 (−26%)	106.66 (6.87)	68.75 (10.06)
				110.96 (3.34)	59.87 (8.42)
Carbaryl	8.02	1.80 (+80%)	4.79 (+379%)	95.08 (8.82)	68.86 (11.72)
				107.76 (4.65)	61.65 (5.93)
Pyrimethanil	8.03	0.55 (−45%)	0.85 (−15%)	<LOD	<LOQ
				49.25 (4.43)	53.20 (9.44)
Atrazine	8.11	0.61 (−39%)	0.84 (−16%)	96.74 (11.78)	77.43 (9.20)
				105.56 (4.75)	55.65 (7.69)

Table 2 (*Continued*)

Pesticide	t_R (min)	Matrix effect QuEChERS[a] (Δ%)	Matrix effects MSPD[a] (Δ%)	Recovery QuEChERS[b] %Rec (RSD%)	Recovery MSPD[b] %Rec (RSD%)
Isoproturon	8.14	0.57 (−43%)	0.94 (−6%)	111.10 (5.79)	67.64 (13.14)
				113.43 (2.90)	56.90 (6.86)
Deet	8.12	0.68 (−32%)	0.72 (−28%)	104.07 (4.18)	89.78 (7.83)
				106.47 (6.32)	66.99 (4.93)
Metalaxyl	8.17	0.63 (−37%)	0.82 (−18%)	109.14 (9.97)	74.57 (9.73)
				127.66 (3.58)	59.74 (8.17)
Diuron	8.21	0.25 (−75%)	0.33 (−67%)	<LOD	73.13 (7.54)
				105.22 (3.85)	58.21 (6.83)
Ethiofencarb	8.21	0.87 (−13%)	1.12 (+12%)	88.32 (5.74)	52.77 (8.26)
				88.04 (4.87)	45.02 (6.64)
Difenoxuron	8.23	0.50 (−50%)	0.65 (+33%)	108.67 (9.97)	66.55 (10.45)
				114.58 (3.96)	56.20 (3.51)
Monolinuron	8.26	0.49 (−51%)	0.47 (−53%)	107.89 (3.52)	75.19 (6.25)
				116.83 (6.77)	56.58 (4.80)
Isoprocarb	8.47	0.49 (−51%)	0.71 (−29%)	101.10 (7.71)	87.37 (11.66)
				107.93 (6.61)	70.66 (10.77)
Metobromuron	8.51	0.65 (−35%)	1.25 (+25%)	<LOD	LOD
				102.05 (13.20)	56.97 (6.36)
Flazasulfuron	8.63	1.52(+52%)	2.38 (+138%)	98.00 (11.24)	28.50 (8.29)
				77.99 (17.84)	21.39 (12.41)
Dimethomorph	8.65, 8.81	0.77 (−23%)	1.04 (+4%)	109.34 (9.04)	77.36 (12.27)
				108.62 (3.25)	56.95 (8.58)
Triadimenol	8.79	0.65 (−35%)	0.78 (−22%)	LOD	82.40 (9.39)
				104.20 (4.00)	63.26 (7.52)
Ethiprole	8.90	0.53 (−47%)	0.75 (−25%)	104.80 (6.29)	84.47 (12.65)
				108.34 (2.70)	64.71 (7.06)
Propazine	8.90	0.57 (−43%)	0.85 (−15%)	95.35 (13.17)	72.89 (11.25)
				85.99 (3.96)	53.43 (9.10)
Cyproconazole	9.04	0.50 (−50%)	0.86 (−14%)	101.61 (4.45)	69.75 (9.46)
				102.10 (4.74)	54.11 (9.67)
Prochloraz	9.12	0.62 (−38%)	0.94 (−6%)	91.54 (9.65)	70.54 (14.64)
				71.07 (4.62)	54.04 (5.94)
Terbuthylazine	9.19	0.46 (−54%)	0.93 (−7%)	92.58 (2.10)	73.25 (14.39)
				87.00 (3.43)	54.05 (7.62)
Fenobucarb	9.20	0.56 (−44%)	0.71 (−29%)	100.42 (7.57)	81.40 (7.40)
				110.80 (4.81)	67.58 (6.42)
Methidathion	9.25	1.17 (+17%)	1.47 (+47%)	115.22 (7.62)	69.21 (7.18)
				121.59 (7.55)	61.79 (6.05)
Diethofencarb	9.29	0.52 (−48%)	0.85 (−15%)	102.98 (8.16)	89.83 (14.59)
				111.56 (3.32)	69.43 (6.42)
Bupirimate	9.30	0.74 (−26%)	1.02 (+2%)	101.82 (3.26)	73.58 (11.37)
				89.82 (5.78)	53.94 (7.74)
Fenamiphos	9.30	0.60 (−40%)	0.83 (−17%)	118.11 (5.29)	71.92 (9.19)
				108.54 (2.23)	59.73 (8.09)
Fenarimol	9.30	0.51 (−49%)	0.53 (−47%)	80.71 (12.61)	78.68 (10.66)
				84.04 (5.41)	60.02 (7.29)
Linuron	9.30	0.49 (−51%)	0.71 (−29%)	121.08 (13.89)	69.54 (12.49)
				107.37 (4.69)	63.08 (8.60)
Bromuconazole	9.32, 9.68	0.67 (−33%)	0.82 (−18%)	109.43 (20.07)	72.75 (8.86)
				100.35 (2.56)	57.40 (9.85)
Myclobutanil	9.35	0.70 (−30%)	0.76 (−24%)	103.67 (9.53)	85.47 (10.32)
				106.58 (4.79)	61.05 (6.27)
Promecarb	9.35	0.87 (−13%)	1.30 (+30%)	121.14 (8.83)	74.10 (8.89)
				115.36 (3.64)	61.72 (8.79)
Azoxystrobin	9.40	0.80 (−20%)	0.39 (−61%)	125.81 (6.84)	76.08 (9.99)
				128.89 (2.04)	62.55 (5.75)
Dimethylvinphos	9.42	1.04 (+4%)	1.08 (+8%)	93.60 (9.47)	77.77 (6.90)
				104.95 (1.53)	62.62 (9.49)
Chlorbromuron	9.43	0.76 (−24%)	1.15 (+15%)	116.26 (8.87)	68.27 (10.05)
				104.24 (5.86)	63.61 (7.67)
Triadimefon	9.54	0.82 (−18%)	0.80 (−20%)	112.30 (11.20)	86.01 (8.61)
				106.41 (4.65)	66.01 (10.21)
Fenhexamid	9.57	0.68 (−32%)	0.91 (−9%)	104.31 (7.65)	–
				95.46 (2.44)	–
Pyridaphenthion	9.58	0.92 (−8%)	1.00 (0%)	98.26 (9.05)	84.34 (9.07)
				105.38 (2.49)	66.01 (10.21)
Tebuconazole	9.64	0.68 (−32%)	0.85 (−15%)	97.68 (2.45)	75.48 (9.78)
				93.90 (1.99)	54.60 (8.21)
Methoxyfenozide	9.79	0.83 (−17%)	0.89 (−11%)	115.26 (9.32)	84.53 (12.30)
				110.57 (1.75)	63.73 (7.92)
Diflubenzuron	9.80	0.64 (−36%)	0.79 (−21%)	77.06 (18.86)	67.23 (14.71)
				71.00 (5.63)	47.45 (3.10)
Penconazole	9.93	0.59 (−41%)	0.89 (−11%)	98.79 (8.10)	69.88 (12.06)
				95.59 (3.57)	51.19 (8.91)

Table 2 (*Continued*)

Pesticide	t_R (min)	Matrix effect QuEChERS[a] (Δ%)	Matrix effects MSPD[a] (Δ%)	Recovery QuEChERS[b] %Rec (RSD%)	Recovery MSPD[b] %Rec (RSD%)
Iprodione	9.95	1.20 (+20%)	1.07 (+7%)	123.92 (20.72)	–
				150.24 (8.85)	–
Chromafenozide	9.97	0.86 (−14%)	0.93 (−7%)	107.12 (12.03)	81.36 (11.26)
				103.53 (1.47)	62.06 (6.89)
Malathion	10.01	1.19 (+19%)	1.60 (+60%)	44.40 (6.11)	28.12 (13.08)
				61.87 (4.96)	26.27 (12.24)
Triazophos	10.03	0.92(−8%)	0.79 (−21%)	103.42 (3.14)	82.64 (9.69)
				100.05 (3.22)	67.61 (7.52)
Fenoxycarb	10.10	0.73 (−27%)	1.33 (+33%)	92.31 (2.55)	73.46 (5.83)
				85.82 (4.93)	59.15 (7.52)
Metolachlor	10.10	0.72 (−28%)	0.94 (−6%)	107.66 (5.78)	71.56 (10.63)
				105.27 (3.34)	57.90 (6.43)
Alachlor	10.20	0.74 (−26%)	1.07 (+7%)	<LOD	<LOQ
				106.20 (5.66)	54.64 (9.74)
Triflumizole	10.20	3.02 (+202%)	4.20 (+320%)	66.43 (5.58)	67.65 (9.30)
				57.03 (6.59)	47.08 (7.61)
Azinphos-ethyl	10.30	0.17 (−83%)	0.31 (−69%)	<LOQ	<LOQ
				56.07 (2.47)	47.61 (7.76)
Neburon	10.30	0.71 (−29%)	0.95 (−5%)	77.30 (9.92)	66.98 (14.54)
				84.22 (2.54)	53.69 (11.57)
Tebufenozide	10.30	0.56 (−44%)	0.60 (−40%)	106.02 (13.87)	88.22 (10.75)
				109.09 (4.64)	70.13 (7.63)
Edifenphos	10.35	2.42 (142%)	2.62 (+162%)	93.25 (4.66)	58.80 (5.90)
				89.66 (4.42)	53.27 (9.19)
Chlorfenvinphos	10.40	0.58 (−42%)	0.76 (−24%)	105.01 (6.36)	78.36 (14.54)
				99.13 (3.20)	60.55 (8.60)
Aclonifen	10.50	0.63 (−37%)	0.85 (−15%)	<LOQ	72.38 (4.57)
				60.35 (5.29)	61.75 (15.19)
Difenoconazole	10.50	0.74 (−26%)	0.87 (−13%)	88.49 (3.19)	72.02 (10.19)
				72.25 (7.31)	51.52 (6.51)
Kresoxim-methyl	10.50	1.04 (4%)	1.61 (+61%)	<LOD	LOD
				<LOQ	61.11 (18.76)
Triflumuron	10.50	0.52 (−48%)	0.84 (−16%)	79.57 (8.52)	64.12 (14.65)
				66.71 (6.36)	47.50 (7.21)
Benalaxyl	10.60	0.73 (−27%)	0.93 (−7%)	109.68 (4.22)	71.27 (13.64)
				98.01 (2.58)	60.11 (10.14)
Quinalphos	10.60	0.87 (−13%)	0.82 (−8%)	81.35 (10.27)	77.18 (10.07)
				80.98 (2.51)	70.95 (7.86)
Anilofos	10.80	0.48 (−52%)	0.62 (−38%)	97.53 (6.71)	80.09 (9.64)
				82.47 (5.78)	63.22 (8.65)
Isofenphos methyl	10.93	0.59 (−41%)	0.84 (−16%)	98.89 (8.91)	72.18 (11.76)
				107.74 (3.37)	59.31 (8.54)
Diazinon	10.99	0.81 (−19%)	1.19 (+19%)	105.40 (3.98)	75.29 (11.42)
				97.34 (1.93)	57.67 (8.28)
Indoxacarb	11.20	1.21 (+21%)	1.21 (+21%)	94.68 (9.24)	68.81 (8.54)
				80.18 (10.43)	57.75 (11.49)
Pirimiphos-methyl	11.20	0.83 (−17%)	0.96 (−4%)	91.14 (6.00)	74.34 (12.42)
				68.10 (3.78)	58.58 (7.92)
Fluacrypyrim	11.30	0.51 (−49%)	0.54 (−46%)	96.31 (3.73)	81.98 (11.32)
				82.75 (2.91)	65.55 (7.58)
Trifloxystrobin	11.30	0.94 (−6%)	0.84 (−16%)	84.61 (6.43)	79.28 (9.47)
				69.63 (5.26)	64.46 (6.44)
Buprofezin	11.40	0.61 (−39%)	0.81 (−19%)	63.97 (3.32)	59.25 (11.89)
				50.92 (9.49)	43.05 (9.75)
Flufenoxuron	11.90	0.70 (−30%)	0.76 (−24%)	<LOD	LOD
				–	41.66 (10.28)
Ethion	12.20	0.75 (−25%)	0.82 (−18%)	56.21 (7.28)	72.15 (14.26)
				40.11 (19.97)	57.68 (5.64)
Hexythiazox	12.30	0.89 (−11%)	0.82 (−18%)	41.48 (11.69)	65.05 (15.40)
				31.46 (17.02)	42.07 (6.95)
Fenazaquin	12.50	0.61 (−39%)	0.81 (−19%)	–	75.39 (9.49)
				–	52.63 (15.07)

[a] Matrix effects were estimated by calculating the value of matrix-matched calibration slope/solvent calibration slope ratios.
[b] Recovery studies were carried out by analyzing six replicates of spiked samples by the described method.

3.1.2. Use of MRM method development software feature based on dynamic time segment acquisition windows

When using the MRM software feature, there is no need to set time window segments for selected group of transitions, bearing in mind also the total number of target species and the dwell time/duty cycle. Actually, "virtual" time segments are automatically constructed by the software during the analysis (like a time window in continuous motion throughout the course of the run). The "dynamic MRM (DMRM)" software used in this study automatically constructs DMRM timetables based in analyte retention times with a detection window (Delta RT) to prevent analyte losses due to peak shifting, and a constant scan cycle time (to provide enough number of data points across all detected peaks) [22–24]. In our study, Delta RT value was set at 1.0 min, in order to consider peak shifting. Scan cycle time (per transition) is not a user-defined parameter; it is calculated by the software feature. Dynamic MRM software groups ion transitions into small tables (timetables), on the basis of analyte retention time, detection windows (Delta RT)

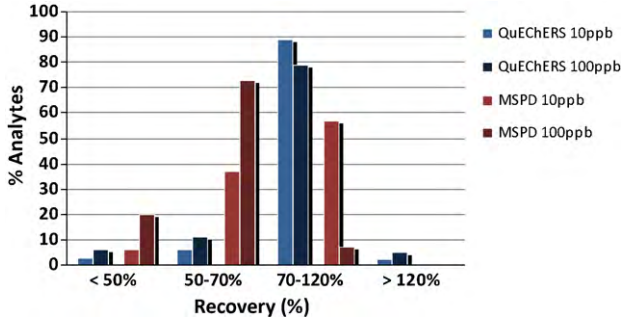

Fig. 2. Distribution of mean recoveries percentages of the studied pesticides in spiked olives analyzed using both tested sample treatment procedures at two concentration levels (10 μg kg^{-1} and 100 μg kg^{-1}).

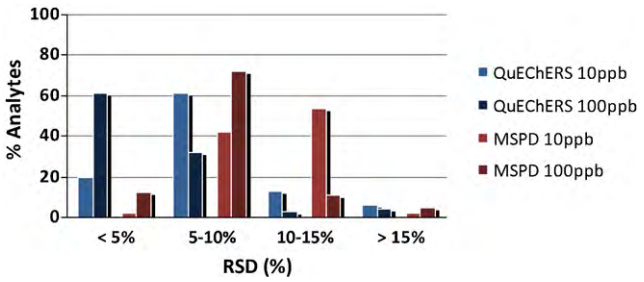

Fig. 3. Precision study. Mean relative standard deviation (RSD) percentages of the 104 studied pesticides in spiked olives analyzed using both tested sample treatment procedures at two concentration levels (10 μg kg^{-1} and 100 μg kg^{-1}).

and cycle time. These tables are similar to conventional MRM time segments but contain fewer ion transitions. The software allows up to 4000+ ion transitions (200 max/table) in a dynamic MRM method. Within each DMRM timetable, the cycle time remains constant, while dwell times vary for each timetable to ensure that all analytes are quantitatively sampled (with a minimum number of data points across chromatographic peak (i.e. >10)).

Drawbacks of conventional MRM analysis related to including new compounds in an existing MRM method, or the risk of analyte losses due to retention time shifting (especially peaks eluting near or between time segment boundaries) are eliminated when using this automated MRM approach. For instance, in a LC–MS/MS multiresidue method for the determination of 46 pesticides in wines [26], 10.9% of target species eluted near time segment boundaries, which may prompt to peak loss. The percentage of analytes near time segment boundaries increases with the number of target species, increasing therefore the probability of analyte loses (i.e. 26.9% of analytes in a 160 pesticide multi-residue in fruits and vegetables [27]). The common practice to minimize analyte losses near time segment boundaries in conventional MRM approach is to duplicate transitions in consecutive time segments, in some cases partially overlapped [28]. However, duplicate transitions are not the solution for peak shifting in many cases, thus involving time-wasting by the instrument while scanning MRM transitions that led to a non-valid peak for integration purposes, or a false missing peak. From our experience of using this software tool, we realized it may have a main drawback. A small change on the method (the deletion or addition of analytes, etc) may change the entire analytical features of the method, particularly the calibration curve slopes, thus requiring a new calibration when a change on the acquisition data parameters is provided. This would happen only in the window time segment affected by the change in a conventional MRM approach. The rest of features of this approach are advantaging, since it makes easier method development and optimization.

3.2. Evaluation of the sample treatment procedures

To evaluate the efficiency of the proposed extraction procedures, recovery studies were carried out at two different concentration levels: 10 and 100 μg kg^{-1}, using both sample treatment methodologies. The results are detailed in Table 2. In general, better recoveries were observed when QuEChERS protocol is used for the extraction of the studied pesticides. As can be seen in Fig. 2, in which the recovery rate data from both sample treatment procedures and fortification levels are included, better recoveries were obtained in the case of QuEChERS. Considering the average value from both concentration levels, 86% of the analytes are recovered in the range 70–120% when using QuEChERS procedure, while using MSPD-based procedure the percentage of analytes recovered in the same range is 20%. Most of studied pesticides (65%) were recovered in the range 50–70% when MSPD is the choice for sample treatment. Note in both cases that the recovery rates were higher for the higher concentration level experiment (100 μg kg^{-1}) with both methods.

On the other hand, both flufenoxuron and fenazaquin were not recovered by QuEChERS protocol, probably because they were retained in GCB solid phase in the clean-up step, due to their planar structures. Employing MSPD methodology, recoveries for both compounds are around 60%. In contrast, acephate, dichlorvos, fenhexamid and iprodione were not recovered with MSPD extraction while they were quantitatively recovered using QuEChERS procedure. In view on these results, QuEChERS methodology seems to be more appropriate as extraction procedure for large-scale multiresidue analysis in olives.

Besides the recovery study, the precision of the methodologies (sample preparation + LC–MS/MS) analysis was also studied. Relative standard deviation ($n = 6$) from both sample treatment methodologies at both fortification levels are shown in Table 2 and also represented in Fig. 3. It can be observed that better precision results were obtained with the QuEChERS method (with 85–90% of the compounds with RSD (%) of 10% or lower). In contrast, with MSPD, at the lower concentration level (10 μg kg^{-1}), the RSD values were significantly higher than with QuEChERS, with values ranging between 5% and 15%. Note also that the precision study percentages were very significantly lower at the higher fortification level tested with both methods.

Finally, with regards to the cleanliness of the extracts and matrix effects, there were not significant differences between the extracts obtained with both methods. With the diluted extract (0.5 g matrix per mL of extract), there were not problems during long sequences and neither the source condition nor the signal stability/sensitivity were affected over the course of long batches of olive samples.

3.3. Analytical performance

The linearity of the method was evaluated with matrix-matched standards using both sample treatment methodologies, at nine concentration levels ranging 2–2000 μg kg^{-1}. The calibration curves showed correlation coefficients higher than 0.995 for 79.8% of target compounds using QuEChERS extraction procedure, and for 81.7% of analysed compounds using the MSPD one. Linear dynamic ranges (LDRs) are shown in Table 3. In the case of dimethomorph and bromuconazole, the standards contained two isomers. Therefore, for these compounds, the calibration curves were obtained plotting the sum of both areas (corresponding to each isomer quantitation transition) versus the concentration of the standards.

Limits of detection (LODs) were estimated from the injection of matrix-matched standard solutions at 0.5 μg kg^{-1} concentration level. LODs and LOQs were assigned taking into account signal-to-noise (S/N) ratio criterion (S/N = 3 and 10 for LOD and LOQ respectively) in the qualifier MRM transition. The results obtained for each pesticide are included in Table 3.

Table 3
Analytical performance of the proposed methods using LC–MS/MS: linearity, detection (LODs) and quantitation limits (LOQs), expressed in $\mu g\,kg^{-1}$.

Pesticide	QuEChERS			MSPD		
	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	LDR ($\mu g\,kg^{-1}$)	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	LDR ($\mu g\,kg^{-1}$)
Methamidophos	3.00	9.90	3–500	6.00	19.80	6–250
Acephate	2.00	6.60	2–500	10.00	33.00	10–250
Thiocyclam	2.00	6.60	2–2000	2.00	6.60	2–500
Omethoate	0.40	1.32	0.4–2000	6.00	19.80	6–500
Carbendazim	4.00	13.20	4–2000	0.50	1.65	0.5–2000
Butoxycarboxim	4.00	13.20	4–500	2.00	6.60	2–500
Thiabendazole	20.00	66.00	20–500	20.00	66.00	20–2000
Aldicarb sulfone	2.00	6.60	2–500	1.00	3.30	1–500
Oxamyl	1.00	3.30	1–2000	1.00	3.30	1–2000
Methomyl	10.00	33.00	10–500	2.00	6.60	2–500
Monocrotophos	30.00	99.00	30–500	20.00	66.00	20–500
Thiamethoxam	1.40	4.62	1.4–500	0.50	1.65	0.5–2000
Pirimicarb	0.40	1.32	0.4–2000	0.16	0.53	0.16–2000
Metamitron	3.00	9.90	3–2000	2.00	6.60	2–2000
Fenuron	2.40	7.92	2.4–2000	1.00	3.30	1–500
Chloridazon	1.00	3.30	1–500	0.50	1.65	0.5–500
Imidacloprid	4.00	13.20	4–2000	1.00	3.30	1–2000
Dimethoate	1.40	4.62	1.4–2000	0.60	1.98	0.6–2000
Acetamiprid	0.50	1.65	0.5–500	0.40	1.32	0.4–250
Thiacloprid	3.60	11.88	3.6–2000	1.00	3.30	1–2000
Aldicarb	20.00	66.00	20–500	14.00	46.20	14–500
Bromacil	3.00	9.90	3–2000	1.40	4.62	1.4–500
Imazalil	2.00	6.60	2–2000	1.00	3.30	1–2000
Monuron	1.60	5.28	1.6–500	0.50	1.65	0.5–500
Oxadixyl	1.20	3.96	1.2–500	0.50	1.65	0.5–500
Simazine	0.50	1.65	0.5–2000	0.20	0.66	0.2–500
Desethyl terbuthylazine	0.20	0.66	0.2–2000	0.10	0.33	0.1–2000
Dichlorvos	5.00	16.50	5–2000	1.00	3.30	1–100
Lenacil	1.00	3.30	1–2000	0.50	1.65	0.5–500
Carbofuran	0.20	0.66	0.2–500	0.20	0.66	0.2–1000
XMC	4.00	13.20	4–500	4.00	13.20	4–250
Chlorotoluron	1.00	3.30	1–500	1.00	3.30	1–100
Fluometuron	1.00	3.30	1–500	0.20	0.66	0.2–500
Carbaryl	2.00	6.60	2–500	2.00	6.60	2–50
Pyrimethanil	14.00	46.20	14–2000	6.00	19.80	6–1000
Atrazine	0.50	1.65	0.5–500	0.50	1.65	0.5–500
Isoproturon	2.00	6.60	2–500	0.40	1.32	0.4–250
Deet	0.20	0.66	0.2–500	0.20	0.66	0.2–500
Metalaxyl	0.20	0.66	0.2–500	2.00	6.60	2–500
Diuron	14.00	46.20	14–500	2.00	6.60	2–500
Ethiofencarb	4.00	13.20	4–500	0.50	1.65	0.5–500
Difenoxuron	0.20	0.66	0.2–500	0.20	0.66	0.2–500
Monolinuron	0.50	1.65	0.5–500	0.50	1.65	0.5–2000
Isoprocarb	2.00	6.60	2–2000	0.50	1.65	0.5–500
Metobromuron	20.00	66.00	20–2000	10.00	33.00	10–250
Flazasulfuron	1.00	3.30	1–2000	0.50	1.65	0.5–1000
Dimethomorph	0.50	1.65	0.5–500	0.50	1.65	0.5–500
Triadimenol	10.00	33.00	10–2000	0.50	1.65	0.5–1000
Ethiprole	1.00	3.30	1–2000	0.50	1.65	0.5–500
Propazine	0.50	1.65	0.5–2000	0.20	0.66	0.2–1000
Cyproconazole	0.50	1.65	0.5–2000	0.20	0.66	0.2–500
Prochloraz	2.00	6.60	2–2000	1.40	4.62	1.4–1000
Terbuthylazine	0.20	0.66	0.2–2000	0.06	0.20	0.06–500
Fenobucarb	6.00	19.80	6–500	1.00	3.30	1–250
Methidathion	1.00	3.30	1–500	0.50	1.65	0.5–500
Diethofencarb	0.20	0.66	0.2–500	0.20	0.66	0.2–100
Bupirimate	0.50	1.65	0.5–500	0.20	0.66	0.2–500
Fenamiphos	0.20	0.66	0.2–500	0.20	0.66	0.2–500
Fenarimol	2.00	6.60	2–2000	1.00	3.30	1–500
Linuron	1.00	3.30	1–500	1.00	3.30	1–250
Bromuconazole	2.00	6.60	2–2000	0.40	1.32	0.4–2000
Myclobutanil	1.00	3.30	1–2000	0.20	0.66	0.2–2000
Promecarb	0.60	1.98	0.6–500	0.20	0.66	0.2–250
Azoxystrobin	0.20	0.66	0.2–500	0.20	0.66	0.2–500
Dimethylvinphos	2.00	6.60	2–500	0.50	1.65	0.5–500
Chlorbromuron	2.00	6.60	2–500	2.00	6.60	2–250
Triadimefon	2.00	6.60	2–2000	2.00	6.60	2–2000
Fenhexamid	1.60	5.28	1.6–500	0.60	1.98	0.6–500
Pyridaphenthion	0.20	0.66	0.2–500	0.20	0.66	0.2–500
Tebuconazole	0.50	1.65	0.5–500	0.50	1.65	0.5–1000
Methoxyfenozide	0.20	0.66	0.2–500	0.20	0.66	0.2–1000
Diflubenzuron	1.00	3.30	1–2000	2.00	6.60	2–1000

Table 3 (*Continued*)

Pesticide	QuEChERS			MSPD		
	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	LDR ($\mu g\,kg^{-1}$)	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	LDR ($\mu g\,kg^{-1}$)
Penconazole	0.30	0.99	0.3–2000	0.20	0.66	0.2–1000
Iprodione	2.00	6.60	2–1000	2.00	6.60	2–1000
Chromafenozide	2.00	6.60	2–500	6.00	19.80	6–500
Malathion	1.60	5.28	1.6–500	0.20	0.66	0.2–500
Triazophos	2.00	6.60	2–500	1.00	3.30	1–2000
Fenoxycarb	2.00	6.60	2–500	6.00	19.80	6–1000
Metolachlor	0.20	0.66	0.2–500	0.06	0.20	0.06–500
Alachlor	20.00	66.00	20–500	6.00	19.80	6–250
Triflumizole	1.00	3.30	1–2000	4.00	13.20	4–1000
Azinphos-ethyl	6.00	19.80	6–500	6.00	19.80	6–100
Neburon	1.40	4.62	1.4–500	0.50	1.65	0.5–500
Tebufenozide	1.00	3.30	1–500	2.00	6.60	2–500
Edifenphos	0.50	1.65	0.5–500	0.20	0.66	0.2–500
Chlorfenvinphos	0.50	1.65	0.5–500	0.50	1.65	0.5–500
Aclonifen	6.00	19.80	6–2000	2.00	6.60	2–500
Difenoconazole	1.00	3.30	1–2000	0.20	0.66	0.2–2000
Kresoxim-methyl	50.00	165.00	50–500	10.00	33.00	10–250
Triflumuron	1.00	3.30	1–500	0.60	1.98	0.6–250
Benalaxyl	0.50	1.65	0.5–500	0.20	0.66	0.2–500
Quinalphos	0.40	1.32	0.4–2000	0.20	0.66	0.2–2000
Anilofos	0.20	0.66	0.2–2000	0.20	0.66	0.2–500
Isofenphos methyl	1.00	3.30	1–2000	0.50	1.65	0.5–1000
Diazinon	0.06	0.20	0.06–500	0.06	0.20	0.06–500
Indoxacarb	1.00	3.30	1–2000	0.40	1.32	0.4–2000
Pirimiphos-methyl	0.06	0.20	0.06–2000	0.06	0.20	0.06–1000
Fluacrypyrim	0.10	0.33	0.1–500	0.06	0.20	0.06–500
Trifloxystrobin	0.20	0.66	0.2–250	0.20	0.66	0.2–1000
Buprofezin	0.06	0.20	0.06–500	0.06	0.20	0.06–500
Flufenoxuron	20.00	66.00	20–2000	10.00	33.00	10–2000
Ethion	1.00	3.30	1–1000	0.40	1.32	0.4–1000
Hexythiazox	1.00	3.30	1–1000	1.00	3.30	1–1000
Fenazaquin	0.10	0.33	0.1–500	0.20	0.66	0.2–250

Limits of detection obtained were below 10 $\mu g\,kg^{-1}$ for 89% of selected compounds, being as low as 0.1 $\mu g\,kg^{-1}$ or lower for pirimiphos-mehyl, diazinon or buprofezin, using both methods. In addition, most used – and detected – pesticides in olive harvesting have limits of detection below 1 $\mu g\,kg^{-1}$ (simazine, terbuthylazine and its metabolite). These results demonstrate enough sensitivity for both evaluated methods to be applied to the quantitative analysis of trace pesticide residues in olives. Note that recent improvements in ionization and ion transmission steps have resulted in newer state-of-the-art instruments that may provide better sensitivity that the one used in this study by a factor of 10.

Matrix effects in LC–MS with electrospray ionization source are very important for the determination of pesticides in complex food matrixes. The response of the analytes can be reduced or enhanced, compared to solvent-based standards. This is due to coeluting species presented in the matrix can interfere in the ionization of the target compounds. To evaluate these possible effects, the slopes obtained in the calibration with matrix-matched standards were compared with those obtained with solvent-based standards, calculating matrix/solvent slope ratios for each pesticide. As it can be seen in Table 2, the signal is affected for the matrix in most cases (slope ratio $\neq$ 1), using both sample treatment procedures.

Most of studied pesticides displayed signal suppression with both extraction methods. Nevertheless differences are observed in the intensity of matrix effects with each sample treatment protocol. Fig. 4 shows the matrix effects represented in percentage. As it is shown in the figure, when matrix solid-phase dispersion is employed, 51% of analytes presented soft matrix effect (equal or less than 20%, which corresponds with slope ratios between 0.80 and 1.20 in Table 2). Besides, 24% of analytes presented matrix effect as strong as 50% or higher, that means, the response of these analytes is enhanced or suppressed to a half or more, compared to the signal obtained in solvent-based standards. Otherwise, when QuEChERS methodology is used to extract the olives, matrix effects

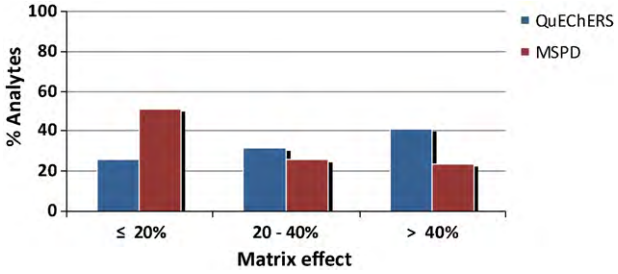

Fig. 4. Distribution of matrix effects – expressed in terms of absolute percentage of signal enhancement or suppression – displayed by the selected 105 pesticides, obtained with extracts using the two studied sample treatment procedures.

values are reversed: soft matrix effect was observed for 26% of pesticides while 41% of pesticides showed strong matrix effect (equal or up to 50%). These results indicate the need of matrix-matched standards as the European guide DG SANCO recommends [29]. Finally, the proposed method was applied to two olives samples collected in different regions of the province of Jaén, in the south-eastern of Spain. Positive results on simazine, terbuthylazine and its metabolite (desethyl terbuthylazine) were usually found using both sample treatments as it has been previously described in olive oil in the literature [30].

4. Conclusions

Taking into account the lack of large-scale multi-residue methods in olives and the inherent complexity of the matrix, two sample treatment protocols widely used for the extraction of pesticides in fruits and vegetables (and recently validated for few compounds in olives and olive oil matrixes) have been compared: QuEChERS and MSPD. In view of the performance of

the studied methods in terms of recovery yields and matrix effects (signal suppression), QuEChERS method was found to be more appropriate than MSPD as extraction procedure for large-scale multi-residue analysis in olives. The proposed methods were successfully applied to the analysis of real olives samples, showing the potential applicability of the proposed methodology and revealing the presence of some of the target species in the $\mu g\,kg^{-1}$ range. Since most of the studied pesticides are representative for a family of compounds with similar physico-chemical properties, the methodology presented here could be suitable for the extraction of a large number of pesticides (i.e. 250–300).

In this work, we also addressed the evaluation of a new MRM method development software feature recently introduced by various vendors [22–24], for the fast method development of large-scale LC–MS methodologies using multiple reaction monitoring (MRM) mode. It is based on the use of so-called "dynamic" time segment windows. This methodology provides several practical advantages during method development with regards to the classic approach based on the elaboration of different time segment windows with duplicated transitions for the frontier compounds. The use of dynamic MRM approach supposes a great improvement in the development of LC–MS/MS quantitative methods. Method creation and optimization is simplified and less time-consuming because the requirement of pre-define fixed time segments containing groups of MRM transitions is avoided. Drawbacks of monitoring coeluting species or including new compounds in an existing MRM method are also eliminated when using DMRM. Moreover, the risk of analyte losses due to retention time shifting is avoided. In the present study, this approach has been successfully applied to the development of a large-scale multi-residue method for the analysis of over one hundred multiclass pesticides in a complex food matrix such as olives.

Acknowledgments

The authors acknowledge funding support from Regional Government of Andalusia (Spain) "Junta de Andalucía" (Research Groups FQM-323, AGR-159, Project FQM-01463), the Spanish "Ministerio de Educación y Ciencia" (Project BQU-2006-15066) and the Spanish "Ministerio de Ciencia e Innovación" (project CTQ2009-10897). B.G.-L. also acknowledges a scholarship from Junta de Andalucía (FQM-01463).

References

[1] U. Wahrburg, M. Kratz, P. Cullen, Eur. J. Lipid Sci. Technol. 104 (2002) 698.
[2] Extracted from data provided by IOC webpage about world statistics on olive oil consumption: http://www.internationaloliveoil.org/web/aa-ingles/corp/AreasActivitie/economics/AreasActivitie.html.
[3] Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC) and subsequents updates.
[4] Codex Alimentarius Committee on Pesticide Residues, Joint FAO/WHO Meeting on Pesticide Residues, http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp. Last update, 30 March 2009.
[5] Regulation (EC) No. 396/2005 of the European Parliament and of the council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EC, and subsequent updates.
[6] B. Gilbert-López, J.F. García Reyes, A. Molina-Diaz, Talanta 79 (2009) 109.
[7] J.F. García-Reyes, C. Ferrer, M.J. Gomez-Ramos, A. Molina-Díaz, A.R. Fernández-Alba, Trends Anal. Chem. (TrAC) 26 (2007) 239.
[8] S.C. Cunha, S.J. Lehotay, K. Mastovska, J.O. Fernandes, M.B.P.P. Oliveira, J. Sep. Sci. 30 (2007) 620.
[9] M.D. Hernando, C. Ferrer, M. Ulaszewska, J.F. García-Reyes, A. Molina-Díaz, A.R. Fernández-Alba, Anal. Bioanal. Chem. 389 (2007) 1815.
[10] A. Garrido-Frenich, J.L. Martínez-Vidal, E. Pastor-Montoro, R. Romero-González, Anal. Bioanal. Chem. 390 (2008) 947.
[11] A. Garrido-Frenich, J.L. Fenández-Moreno, J.L. Martínez-Vidal, F.J. Arrebola Liébanas, J. Agric. Food Chem. 55 (2007) 8346.
[12] M. Guardia-Rubio, M.R. Marchal-López, M.J. Ayora-Cañada, A. Ruiz-Medina, J. Chromatogr. A 1145 (2007) 195.
[13] A. García Sánchez, N. Ramos Martos, E. Ballesteros, Anal. Chim. Acta 558 (2006) 53.
[14] S. Barrek, O. Paisse, M.-F. Grenier-Loustalot, Anal. Bioanal. Chem. 376 (2003) 355.
[15] C. Ferrer, M.J. Gómez, J.F. García-Reyes, I. Ferrer, E.M. Thurman, A.R. Fernández-Alba, J. Chromatogr. A. 1069 (2005) 183.
[16] S.A. Barker, J. Biochem. Biophys. Methods 70 (2007) 151.
[17] S.A. Barker, J. Chromatogr. A 880 (2000) 63.
[18] S.A. Barker, J. Chromatogr. A 885 (2000) 115.
[19] M.A. Aramendía, V. Borau, F. Lafont, A. Marinas, J.M. Moreno, F.J. Urbano, Food Chem. 105 (2007) 855.
[20] Y. Picó, C. Blasco, G. Font, Mass Spectrom. Rev. 23 (2004) 45.
[21] L. Alder, K. Greulich, G. Kempe, V. Bärbel, Mass Spectrom. Rev. 25 (2006) 838.
[22] Agilent Technologies Technical Note 5990-3595, 2009.
[23] C. Hunter, B. Simons, Applied Biosystems Sciex Technical Note 114TN 52-02, 2008.
[24] P.D. Rainville, J. Mather, R.S. Plumb, Waters Application;1; Note. 720002569EN, 2008, pp. 2008.
[25] www.pesticide-online.com database. Last accessed, November 2009.
[26] A. Economou, H. Botitsi, S. Antoniou, D. Tsipi, J. Chromatogr. A 1216 (2009) 5856.
[27] B. Kmellár, P. Fodor, C. Ferrer, M.A. Martínez-Uroz, A. Valverde, A.R. Fernandez-Alba, J. Chromatogr. A 1215 (2008) 37.
[28] M. Hiemstra, A. de Kok, J. Chromatogr. A 1154 (2007) 3.
[29] Guidance document on method validation and quality control procedures for pesticide residues analysis in food and feed. Document No. SANCO/10684/2009, 1 January 2010.
[30] J.F. García-Reyes, C. Ferrer, E.M. Thurman, A.R. Fernandez-Alba, I. Ferrer, J. Agric. Food Chem. 54 (2006) 6493.