Journal of Chromatography A, 1562 (2018) 27–35

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Multi-residue pesticide analysis in virgin olive oil by nanoflow liquid chromatography high resolution mass spectrometry☆,☆☆

David Moreno-González[a,*], Jaime Alcántara-Durán[a], Silvina M. Addona[b], Miriam Beneito-Cambra[a]

[a] Analytical Chemistry Research Group, Department of Physical and Analytical Chemistry, University of Jaén, 23071, Jaén, Spain
[b] Instituto de Ciencias Veterinarias del Litoral, Consejo Nacional de Investigaciones Científicas y Técnicas, Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 4 April 2018
Received in revised form 25 May 2018
Accepted 26 May 2018
Available online 28 May 2018

Keywords:
Nanoflow liquid chromatography
High resolution mass spectrometry
Pesticides
Olive oil
QuEChERS
Enhanced matrix removal-lipid

ABSTRACT

In this article, a nanoflow liquid chromatography system coupled to high resolution mass spectrometry (nanoflow LC/ESI Q-Orbitrap-MS) has been applied for the development of a multiresidue pesticide method for the determination of 162 multiclass pesticides in olive oil samples. Due to the relatively high lipid content of the raw QuEChERS acetonitrile extracts obtained from this type of fatty vegetable samples, a dispersive solid phase extraction (dSPE) sorbent proposed to retain both fatty acids and triglycerides, namely Enhanced Matrix Removal-Lipid (EMR-Lipid) has been implemented as additional cleanup step. The analytical performances of the proposed method were evaluated,achieving recoveries in the range 75–119% with relative standard deviations lower than 19% (n = 6). The dSPE sorbent allowed the removal of most coextracted interferences without a significant loss of analytes. Matrix effects were also evaluated, showing a negligible effect for most of the compounds tested, when a dilution factor of 50 was applied. Notably, despite the use of relatively high dilution factors (e.g. 1:50) to minimize matrix effects, the lowest concentration levels detected with this method – in the low $\mu g\,kg^{-1}$ range – are well below the corresponding maximum residue levels established by the current European legislation.

1. Introduction

Olive grove is amongst the most important agri-food sectors within the Mediterranean basin [1]. The implementation of new technologies has triggered remarkable improvements and benefits in production and quality, but also some issues derived from the control of pests and diseases [2,3]. Misusing of pesticides can affect the olive tree, posing also health risks to consumers through the exceeded presence of their residues. Consequently, the European Union (EU) have established maximum residue levels (MRLs) for pesticides in olives and to olive oil through the use of processing factors which are yet to be established (Annex VI of 396/2005) [4,5]. These MRLs are usually in the $\mu g\,kg^{-1}$ level. Consequently, the development of reliable analytical methods for the determination of pesticides in olive oils is therefore essential.

Sample treatment is undoubtedly the main challenge associated to the development of multiresidue methods for pesticides in olive oil. Due to the use of organic solvents such as acetonitrile, the obtained extracts typically contain a relatively large amount of coextracted fatty acids and tryglicerides together with low polar pigments [6]. Consequently, there is a need to include additional steps aiming at clearing the presence of fat in the final extract subjected to chromatography/mass spectrometry, and this, itself, constitutes a challenge, provided the wide array of pesticides and their different physicochemical properties that need to be addressed in current pesticide multiresidue methods. In order to isolate pesticides without the undesirable co-extraction of lipids, which may affect the detection system, several cleanup procedures have been developed in olive oil or other fatty samples including matrix solid-phase dispersion [7,8], gel permeation chromatography [9,10], liquid–liquid extraction [11–13], freezing/low-temperature cleanup procedures [14] or solid-phase extraction (SPE) [15,16]. Above all, the use of QuEChERS method (quick, easy, cheap, effective, rugged, and safe) has been extended as an straightforward sample preparation approach to determine multiresidue pesticides in fruits and vegetables as well as other complex matrices at trace levels [17–19]. QuEChERS method con-

☆ Selected paper from the 15th Instrumental Analysis Conference, 3–5 October 2017, Barcelona, Spain.
☆☆ Presented at XVII meeting of Spanish Society of Chromatography and related techniques, held in Barcelona, Spain, 3–5 October 2017 (SECyTA 2017).
* Corresponding author.
E-mail address: dmgonzal@ujaen.es (D. Moreno-González).

https://doi.org/10.1016/j.chroma.2018.05.053
0021-9673/

sists of a microscale extraction using generally acetonitrile (MeCN) and partitioning with magnesium sulfate alone or in combination with other salts, followed by a clean-up of the organic extract by dispersive SPE (dSPE), playing a crucial role in the case of pesticides analysis in olive oil [20,21]. Different dSPE sorbents including PSA, C18 and graphitized carbon black have been proposed [22,23]. Recent efforts have focused on the use of specific dSPE sorbents targeting the selective removal of lipids, such as the use of zirconium oxide-based sorbents (Z-Sep) [24,25]. However, despite its good capacity to remove lipid components of the sample, this sorbent presents high capacity to retain certain non-polar pesticides, obtaining poor recoveries [20]. Recently, a dSPE sorbent namely Enhanced Matrix Removal-Lipid sorbent (EMR-Lipid) have been proposed to remove major lipid classes from sample matrix without great effect on pesticide recoveries [26,27]. This sorbent has been applied for the determination of pesticides in this type of matrix in combination with gas chromatography (GC) or liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) [26–28]. These recently reported methods exhibited matrix effects ranging from negligible to strong (e.g. suppression of signals above 50%) though. Thus, it would be necessary to improve the performance for those pesticides affected by signal suppression due to these mentioned matrix effects. In this context, the use of nano flow LC-ESI–MS allows both a reduction of matrix effects [29,30] and the improvement on the ionization efficiency and method sensitivity [31]. This enhancement in sensitivity is based on nanospray is a more effective process than pneumatically assisted electrospray. Due to droplet sizes in nanospray are much smaller than conventional electrospray at higher flow rates, obtaining better ionization efficiency [32].This fact can be used to minimize matrix effects through the implementation of high dilution of the sample without compromising overall method sensitivity.

In this work, a nanoflow liquid chromatography system coupled to high resolution mass spectrometry (nanoflow LC/ESI Q-Orbitrap-MS) has been applied for the development of a multiresidue pesticide method for the determination of 162 multiclass pesticides in olive oil samples. The advantages of nano flow-LC have been combined with the use of a lipid-removal sorbent (EMR-lipid) as dSPE sorbent for QuEChERS sample treatment and a relatively high dilution factor of 1:50. The proposed method has been evaluated [33] in terms of recovery rates and matrix effects.

2. Experimental section

2.1. Chemicals and reagents

HPLC-grade methanol (MeOH) and HPLC-grade MeCN were obtained from Sigma-Aldrich (Madrid Spain). EMR-Lipid sorbent was obtained from Agilent Technologies (Santa Clara, CA, USA) whereas, sodium chloride, anhydrous magnesium sulfate and formic acid were supplied by Sigma-Aldrich. Acetic acid (HOAc) were bought from J.T. Baker (Center Valley, PA, USA).

The 162 multiclass pesticides typically used in olive grove, including insecticides, herbicides, fungicides and acaricides, used were obtained from Sigma-Aldrich or Dr. Ehrenstorfer (Augsburg, Germany). Each analytical quality standard (>99% purity) was purchased from individual stock solution (ca. 500 $\mu g\,mL^{-1}$) were prepared in MeCN or MeOH and kept at −20 °C. Then, a working solution containing the mixture of standards was prepared (5 $\mu g\,mL^{-1}$) in MeCN and also stored at −20 °C.

2.2. Sample treatment

Olive oil samples were purchased from the local market (Jaén, Spain). The samples were treated as described by López-Blanco et al. [34] with minor modifications. In brief, 3 g of olive oil, 7 mL H_2O Milli-Q water and 10 mL MeCN (1% HOAc) was weighed into 50 mL centrifuge tube and stirred by vortex for 30 s. Then, 4 g of $MgSO_4$ and 1 g of NaCl were added and shaken by hand for 1 min. The extract was centrifuged at 5000 rpm for 5 min, supernatant was preserved for the second clean-up step. First of all, 1 g of EMR sorbent, contained in other 50 mL centrifuge tube, was activated with 5 mL of H_2O Milli-Q water, shaking for 30 s, prior to use. Then, 5 mL of organic extract from sample partitioning were added, being the tube shaken by hand for 1 min and then centrifuged at 5000 rpm for 5 min. After then, 5 mL of the obtained supernatant were transferred with a Polish centrifuge tube, which contained 0.4 g of NaCl and 1.6 g of $MgSO_4$. Finally, it was shaken by hand for a minute and centrifuged for 5 min at 5000 rpm. The extract was filtrated through a 0.45 μm nylon syringe filter and diluted 1:15 with Milli-Q water, to obtain a final extract dilution 1:50, so that the final extract match the initial composition of the mobile phase of the chromatographic method.

2.3. Nanoflow liquid chromatography high resolution mass spectrometry

A Thermo Scientific EASY-nLC 1000 nano-LC system (Thermo Scientific, San Jose, USA) with an EASY-Spray PepMap® C18 column (75 μm x 150 mm, 3 μm particle size and 100 Å pore) with integrated emitter (Thermo Scientific, San Jose, USA) was used. Viper® zero-dead volume finger-tight fittings (Thermo Scientific, San Jose, USA) connections were used. The column was maintained at 25 °C, the injection volume was 1 μL and the flow rate was 200 nL min^{-1}. The following linear gradient was used: 0–5 min 2% B, 5–15 min 30% B, 15–25 min 100% B, 25–28 min 100% B, 28–33 min 2% B and 33–37 min 2% B. Mobile phases A and B were Milli-Q water and MeCN respectively, both of them with 0.1% (v/v) formic acid. The nano-LC system was connected to a Thermo Q-Exactive Orbitrap mass spectrometer equipped with an Easy-Spray nano-electrospray ion source was used. The positive polarity ESI parameters used were those optimized in previous studies [29,30]. For qualitative and quantitative analysis Xcalibur 3.0 and TraceFinder 3.3 (Thermo Scientific) were used. Retention time (RT) are included in Table 1.

3. Results and discussion

3.1. Method optimization: MS and nanoLC parameters

Taking into consideration previous works, ionization source temperature and spray voltage were kept at 250 °C and 2.2 KV, respectively [29,30]. Two product ions and a precursor ion – with a mass accuracy of less than 5 ppm- was established as criteria to obtain the unambiguous identification of the pesticides studied in the case of HRMS [33,35]. The complete MS/AIF combination per cycle consists of a complete MS scan event, where a full scan of the C-Trap is performed on a specific range of masses, and an AIF scan event where all ions were fragmented in high energy collision dissociation cells (HCD), and analyzed in orbitrap once trapped in C-trap [36]. According to previous studies [37], a resolution of 70000 was set to obtain, in terms of selectivity and total cycle acquisition, satisfactory results, obtaining a sweep rate of ca. 5 Hz in the FS experiment.

The AIF mode was carried out at 30 V, this value was enough to obtain a fragmentation pattern for most of studied compounds (**Table S1**). In all cases, the precursor ion was the protonated molecule $[M+H]^+$. Other studied parameters in positive mode were: S-lens RF level, 60; capillary temperature, 250° C; spray voltage, 2.2 KV. The mass spectrometer acquired two experiments with

Table 1
Analytical performance characteristics of the proposed method for each pesticide. MRL values for olive and olive oils for each pesticide are included.

Compound	Rt (min)	Precision		Recovery (n = 6)		LCL[a] (μg kg^{1})	S/N	MRL Olive[b] (μg Kg^{-1})	MRL Olive Oil[c] (μg Kg^{-1})	Matrix Effect[d]
		Intraday (%, n = 6)	Interday (%, n = 6)	(%)	%RSD					
3-hydroxycarbofuran	16.74	6	12	113	6.7	0.5	108	20	100	0
Acetamiprid	17.84	7	3	75	3.8	5	124	900	4500	−11
Alachlor	25.24	5	7	120	8.4	5	377	20	100	9
Aldicarb	26.08	6	5	111	7.5	0.5	69	20	100	−6
Aldicarb sulfone	3.36	18	9	75	8.9	0.05	268	20	100	7
Aldicarb sulfoxide	11.48	5	3	70	7.6	0.5	69	20	100	0
Ametoctradin	23.43	2	3	95	4.5	0.5	654	10	50	−7
Atrazine	22.35	4	8	71	4.7	0.5	511	500	2500	−9
Azinphos methyl	23.76	3	4	87	10.9	5	147	50	250	−2
Azoxystrobin	24.06	7	5	107	5.7	0.05	230	10	50	6
Benalaxyl	25.46	3	6	83	8.2	0.05	165	50	250	−5
Bifenazate	24.51	3	4	107	8.1	0.5	799	20	100	0
Bitertanol	12.80	6	7	97	8.1	5	52	20	100	−14
Boscalid	24.09	8	10	98	7.5	5	174	10	50	−9
Bromuconazole 1	23.91	6	6	100	14.5	50	341	50	250	−9
Bromuconazole 2	24.42	8	10	101	13.4	50	189	50	250	−9
Bupirimate	22.62	3	4	117	7.5	0.05	654	50	250	−8
Buprofezin	24.96	5	6	85	5.1	5	212	5000	25000	−9
Carbaryl	22.00	5	6	107	8.7	0.5	22	20	100	−7
Carbendazim	3.30	11	11	84	12.4	0.05	116	100	500	−7
Carbofuran	21.56	5	4	118	18.6	0.5	92	20	100	−2
Carfentazone ethyl	24.75	4	10	100	12.4	5	124	10	50	−5
Chlorantraniliprol	23.28	4	9	100	5.1	0.5	221	10	50	−7
Chlorfenvinphos	25.32	5	9	90	18.4	0.5	149	20	100	2
Chlorotoluron	22.07	4	7	115	6.6	0.5	219	20	100	8
Chlorpyrifosmethyl	25.89	6	11	117	6.1	5	147	50	250	7
Clofentezin	26.12	17	15	103	7.8	5	171	20	100	0
Clomazone	23.25	6	5	77	6.6	0.5	55	20	100	−7
Coumaphos	25.99	2	8	107	6.6	5	28	100	50	1
Cyazofamid	25.32	6	8	119	7.7	5	360	20	100	−17
Cymoxanil	24.07	4	8	108	14.5	0.5	264	50	250	−3
Cyproconazole	23.64	4	6	88	6.5	0.05	36	50	250	−10
Cyprodinil	22.84	10	13	86	6.3	5	220	20	100	0
Cyromazine	3.24	14	16	105	13.4	0.5	264	50	250	−10
Dazomet	22.09	7	12	105	5.2	0.5	37	20	100	9
DEET[e]	22.32	6	8	107	5.7	0.5	71	10	50	3
DemetonSmethylsulphon	4.11	4	13	111	10.1	0.05	233	10	50	−6
Diazinon	25.86	4	9	96	4.1	0.5	377	20	100	−6
Dichlorvos	20.64	3	9	119	17.7	0.05	117	10	50	−4
Dicrotophos	14.26	11	6	119	3.6	0.05	254	10	50	7
Diethofencarb	23.75	11	3	110	5.1	0.5	37	10	50	0
Difenoconazole	25.56	2	13	115	11.3	5	265	2000	10000	3
Diflubenzuron	21.68	7	12	105	18.4	0.5	185	50	250	−9
Dimethoate	17.33	7	4	82	12.2	0.5	117	2000	10000	−7
Dimethomorph E	23.17	5	5	97	8.1	0.5	267	20	100	−6
Dimethomorph Z	23.39	5	7	96	12.4	0.5	60	20	100	−16
Diniconazole	25.07	5	8	117	8.6	0.05	104	20	100	8
Diuron	22.54	19	8	75	6.1	0.05	796	20	100	−16
Epoxiconazole	24.2	6	8	114	6.2	0.5	264	50	250	−5
Ethion	26.82	7	20	102	18.6	5	147	10	50	1
Ethirimol	24.32	8	4	99	12.4	0.5	265	50	250	7
Ethoprophos	24.32	2	7	105	7.7	0.5	37	20	100	−11
Etofenprox	25.42	5	4	74	18.4	0.5	149	10	50	6
Fenamidone	24.08	4	3	89	5.2	0.5	366	10	50	6
Fenamiphos	23.98	9	6	89	9.1	0.05	377	50	250	−4
Fenamiphos sulfone	21.28	7	8	117	10.1	0.5	117	50	250	−11
Fenamiphos sulfoxide	21.67	8	12	116	11.4	0.5	162	50	250	7
Fenarimol	23.75	7	7	98	5.9	5	796	20	100	4
Fenazaquin	27.33	5	14	74	4.7	5	49	10	50	−8
Fenbuconazole	19.54	7	14	100	6.7	0.05	796	50	250	−7
Fenhexamid	31.02	5	7	102	6.3	0.05	308	20	100	−15
Fenoxycarb	25.5	5	10	107	12.2	0.05	25	3000	15000	8
Fenpropathrin	23.99	7	19	72	9.2	0.5	15	10	50	0
Fenpropimorph	19.19	5	12	79	8.3	5	267	50	250	−17
Fenthion	25.70	5	4	92	6.1	5	219	10	50	−1
Fenthion sulfone	22.97	9	4	99	7.2	0.5	215	10	50	7
Fenthion sulfoxide	22.97	4	6	95	6.3	5	89	10	50	1
Fipronil	25.29	2	4	98	8.3	0.5	99	10	25	−6
Flonicamid	30.54	5	4	101	7.5	5	221	60	300	−6
Fluazifop	21.99	4	4	100	7.5	0.5	610	10	50	−3
Flufenacet	24.9	7	8	117	7.3	5	268	50	250	−1
Flufenoxuron	27.05	4	20	105	3.7	50	366	50	250	−1
Fluopyram	24.28	6	6	112	8.9	0.5	13	10	50	−3
Fluquinconazole	32.01	2	23	85	7.6	0.05	52	50	250	5

Table 1 (*Continued*)

Compound	Rt (min)	Precision		Recovery (n = 6)		LCL[a] (μg kg^{1})	S/N	MRL Olive[b] (μg Kg^{-1})	MRL Olive Oil[c] (μg Kg^{-1})	Matrix Effect[d]
		Intraday (%, n = 6)	Interday (%, n = 6)	(%)	%RSD					
Flusilazol	24.53	5	14	85	9.2	0.5	104	10	50	7
Flutriafol	22.00	6	7	113	12.4	0.5	13	20	100	−7
Formetanate	10.32	5	11	78	5.6	0.5	308	10	50	−15
Fosthiazate	22.04	8	5	111	14.3	0.5	254	20	100	8
Haloxyfop	24.67	6	10	99	14.2	0.5	212	10	50	0
Hexaconazole	24.75	5	14	77	9.3	0.05	48	20	100	−1
Imazalil	18.06	8	4	86	5.9	0.5	407	50	250	−6
Imidacloprid	16.77	4	8	97	12.7	0.05	218	1000	5000	−6
Indoxacarb	26.16	3	7	100	6.1	5	230	20	100	−3
Iprodione	22.97	5	5	119	7.7	5	324	10	50	−17
Iprovalicarb	23.91	5	7	111	7.9	0.5	1102	20	100	2
Isocarbophos	23.34	7	4	90	13.3	0.5	876	10	50	−14
Isofenphos methyl	25.81	4	6	97	18.4	0.5	134	10	50	−14
Isoprocarb	22.58	3	4	96	7.2	0.5	13	10	50	7
Isoproturon	22.43	2	3	94	8.3	0.05	873	10	50	−9
Isoxaflutole	23.92	7	6	112	14.6	0.5	264	20	100	−10
Kresoxim methyl	25.36	5	6	111	7.6	0.5	57	200	1000	1
Linuron	23.89	3	6	96	4.4	5	101	50	250	9
Lufenuron	24.67	5	13	84	3.9	0.05	140	20	100	−11
Malaoxon	21.42	7	10	100	3	0.5	233	20	100	−4
Malathion	24.66	3	6	110	7.3	0.5	248	20	100	−20
Mandipropamid	24.17	5	5	99	5.2	0.5	159	10	50	0
Mepanipyrim	24.70	5	12	94	5.5	0.05	51	20	100	1
Metalaxyl	22.16	66	7	87	6.3	0.5	149	50	250	2
Metconazole	24.9	6	12	106	6.2	0.05	363	50	250	−9
Methamidophos	24.61	6	63	105	4.8	5	1721	20	100	−15
Methidathion	23.7	4	5	112	6.3	0.5	97	20	100	3
Methiocarb	23.61	3	6	92	8.5	0.5	159	200	1000	−2
Methiocarb sulfoxide	30.00	20	11	94	11.5	0.5	197	300	1500	−7
Methomyl	3.27	5	5	86	4.6	0.5	46	50	250	−12
Methoxyfenozide	24.58	19	6	91	4.1	0.5	508	10	50	0
Metobromuron	22.74	7	6	98	5.1	0.5	305	10	50	−11
Monocrotophos	4.13	3	20	83	8.9	0.5	22	20	100	−18
Myclobutanil	23.99	7	6	104	10.4	0.05	149	20	100	−8
Nitenpyram	3.48	3	9	99	7.6	5	264	10	50	−17
Norflurazon	22.75	5	6	105	12.8	0.5	230	10	50	−20
Omethoate	3.98	3	8	100	1.9	0.5	200	10	50	2
Oxadixyl	20.41	8	7	75	7.9	5	134	20	100	−7
Oxamyl	7.73	3	4	100	9.1	5	265	10	50	−14
Oxyfluorfen	24.65	9	10	95	5.8	5	187	1000	5000	1
Paclobutrazol	23.42	3	5	88	11.5	0.5	607	500	2500	−8
Paraoxon methyl	20.64	7	11	99	6.1	0.5	69	20	100	7
Penconazole	24.86	7	14	93	4.1	0.5	271	50	250	−13
Pencycuron	25.86	6	14	100	11.1	0.5	248	50	250	−4
Pendimethalin	22.98	3	4	75	6.1	0.5	69	50	250	−14
Penthiopyrad	25.51	7	4	100	7.7	5	268	10	50	7
Phenthoate	25.71	7	15	100	6.5	0.5	149	20	100	−17
Phosalone	26.12	3	14	87	3.6	5	305	20	100	−9
Phosmet	23.97	3	5	110	6.3	0.5	654	3000	15000	−9
Phoxim	26.08	12	6	103	6.9	0.5	511	20	100	−5
Pirimicarb	4.59	12	2	115	5.1	0.5	33	20	100	−6
Pirimicarbdesmethyl	13.36	7	9	100	8.3	0.05	230	20	100	−10
Pirimiphosmethyl	25.40	7	10	110	18.4	5	215	10	50	−19
Prochloraz	22.86	8	5	103	12	0.5	36	50	250	2
Procymidone	24.54	17	18	100	6.8	0.5	219	20	100	−3
Profenofos	26.5	19	13	105	9.7	5	145	20	100	7
Propamocarb	5.07	4	13	74	7.2	5	147	10	50	−1
Propaquizafop	26.41	6	11	96	10.3	5	873	50	250	−2
Propiconazole	24.82	2	11	120	4.7	5	1027	10	50	−20
Propoxur	21.38	10	9	98	3.9	0.5	55	50	250	−12
Propyzamide	24.39	5	7	84	8.8	0.5	51	10	50	−6
Pymetrozine	3.25	6	8	105	12.4	0.5	124	50	250	7
Pyridate	28.66	3	13	101	8.7	0.5	93	50	250	1
Pyrimethanil	21.12	5	5	99	5.6	5	525	20	100	−7
Pyriproxyfen	23.98	7	19	77	4.5	5	4046	50	250	−7
Quinmerac	20.42	5	14	80	5.6	0.5	230	100	500	7
Quinoclamine	20.72	9	12	96	17.3	5	147	20	100	8
Quinoxyfen	26.57	3	18	89	11.2	5	1031	20	100	4
Rotenone	24.96	7	12	114	6.5	0.5	39	20	100	−1
Simazine	20.83	5	9	119	7.2	0.5	1363	10	50	−6
Spirodiclofen	29.02	3	4	73	13.9	5	360	20	100	9
Spirotetramat	23.60	21	5	119	4.4	0.5	1027	4000	20000	7
Tebuconazole	24.48	2	7	119	12.3	0.5	573	50	250	8
Tebufenpyrad	26.30	6	17	87	4.1	5	324	50	250	−7
Terbuthylazine	23.83	4	13	115	5.3	0.5	42	50	250	−7

Table 1 (*Continued*)

Compound	Rt (min)	Precision		Recovery (n = 6)		LCL[a] (μg kg[1])	S/N	MRL Olive[b] (μg Kg^{-1})	MRL Olive Oil[c] (μg Kg^{-1})	Matrix Effect[d]
		Intraday (%, n = 6)	Interday (%, n = 6)	(%)	%RSD					
Terbuthylazinedesethyl	21.1	7	5	108	4.4	0.5	44	50	250	−9
TFNG	4.44	3	10	103	9.5	0.5	360	60	300	9
Thiabendazol	21.00	4	15	112	13.9	0.5	19	50	250	−19
Thiacloprid	19.86	4	6	93	8.4	0.5	174	4000	20000	9
Thiamethoxam	14.42	7	13	118	10.4	5	482	400	2000	−5
Thiobencarb	21.48	6	12	93	8.5	0.5	187	20	100	−2
Thiodicarb	26.13	8	5	117	4.9	0.5	197	10	50	6
Triadimenol 1	23.31	5	4	100	7.8	0.5	221	100	500	−13
Triadimenol 2	23.53	8	10	117	14.2	0.5	46	100	500	−13
Triazophos	24.87	7	8	118	7.1	5	305	10	50	−10
Trichlorfon	33.67	6	5	116	9.3	0.5	604	20	100	−5
Triticonazole	23.68	6	7	118	6.5	0.5	17	10	50	−2
Zoxamide	25.53	3	7	103	5.9	5	268	20	100	6

[f] 4-(Trifluoromethyl)nicotinoyl glycine, N-[[4-(Trifluoromethyl)-3-pyridinyl]carbonyl]glycine.

[a] Corresponds to the lowest concentration level tested giving S/N ratio equal or higher than 10.

[b] A MRL of 10 μg kg^{-1} has been also set as a default value for pesticide/commodity combinations not included in the regulation [4].

[c] In the case of olive oil the MRL is set by a correction factor of 5 with respect MRL established in olives for olive oil production.

[d] Matrix effects calculated as [(calibration curve slope in matrix/calibration curve slope in solvent)-1] × 100. Zero result means that signal or suppression or enhancement has not been observed.

[e] N,N-diethyl-meta-toluamide.

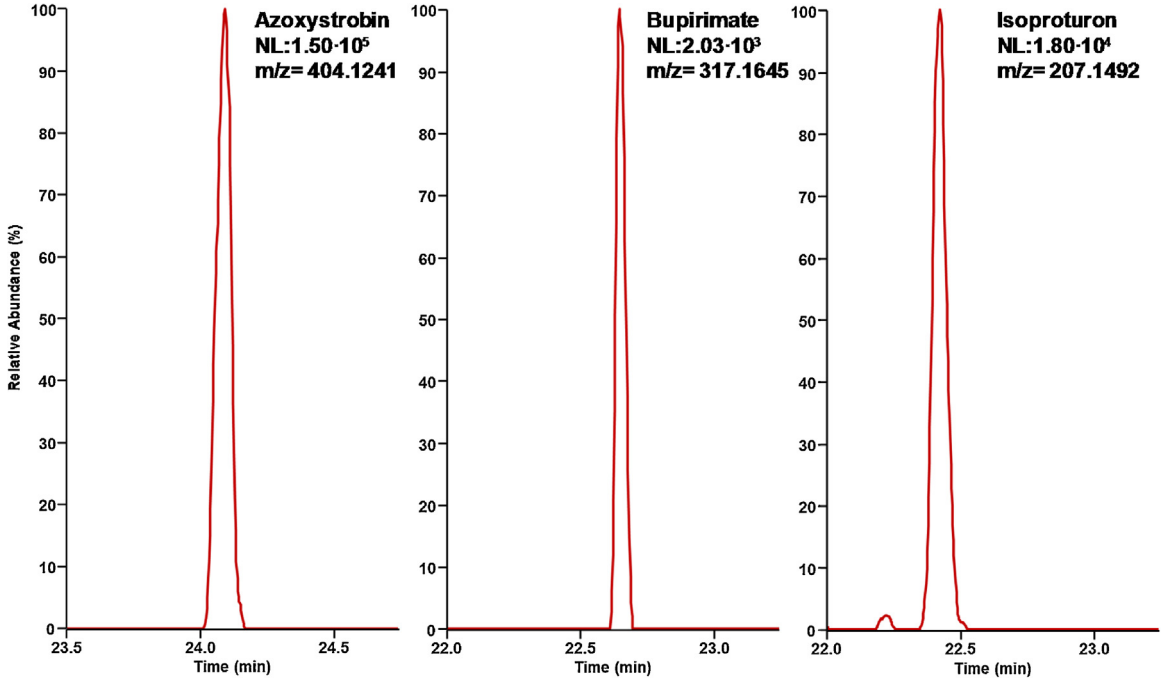

Fig. 1. Nanoflow LC–HRMS analysis of pesticide residues in olive oil. Extracted ion chromatograms of Azoxystrobin, Bupirimate and Isoproturon in olive oil spiked at 0.05 μg kg^{-1}.

a duty cycle of *ca.* 0.5 s/acquisition point. The first experiment was full-scan at a resolution of 70000 (at *m/z* 195), automatic gain control (AGC) target at $1 \cdot 10^6$; maximum injection time (IT), 200 ms and scan range, 100–750 *m/z*. Secondly was collision induced dissociation without precursor ion isolation or all-ion fragmentation (AIF) mode at a resolution of 17500, AGC target at $2 \cdot 10^5$, maximum IT, 50 ms and scan range: 100–750 *m/z*. Based on ± 5 ppm accurate-mass extraction windows, extracted ion chromatograms were reconstructed for identification and quantitation purposes, in addition to obtaining two product ions in AIF. According to the obtained results, it can be concluded that EU requirements in terms of diagnostic ions are widely fulfilled [32]. An an example, the theoretical *m/z* values for the identification of selected pesticides together with typical relative mass errors (in ppm) are included in **Table S1**.

The Rt data for the identification of selected pesticides are included in Table 1, whereas the distribution of the masses versus Rt is shown in Fig. S**1**. The mobile phases were Milli-Q water (A) and MeCN (B) both of them with 0.1% formic acid. It was observed that percentages of B at the beginning higher than 2% produced a poor retention of polar pesticides. Consequently, in order to improve the retention of these compounds, the initial gradient composition started with 2% of B. The precision of Rts was also evaluated, the RSD (%) values were calculated from 6 injections on different days (through 125 injections) in olive oil extracts spiked at 5 μg kg^{-1} for each pesticide. These values were lower than 0.2% (within ± 0.1 min), in compliance with the current quality control guidelines in all cases [33].

3.2. Analytical performance

In order to evaluate the performance of the proposed method, different analytical features including matrix effect, lowest concentration level (LCL), recoveries and precision were evaluated in olive oil samples. External (solvent) standard and Matrix-matched calibration curves were established, these were evaluated at five levels of concentration (0.05, 0.5, 5, 50 and 500 $\mu g\,kg^{-1}$). The analytical parameters of the proposed method are given in Table 1. The determination coefficients (R^2) were higher than 0.995 in all cases, showing that the linearity was adequate. To establish the LOQs of the proposed method, the S/N criterion was avoided. High-resolution data from Orbitrap provides higher S/N due to negligible chemical background in extracted ion chromatograms with an *m/z* window of ±5 ppm. So, the data from the lowest concentration level tested (Table 1) yielding S/N ratios distinctly higher than 10 were employed, thus, providing an insight into the actual sensitivity displayed by the method. As could be observed in Table 1, lowest concentration level tested were ranged from 0.05 to 50 $\mu g\,kg^{-1}$, being lower than their corresponding MRLs [4,5]. As an example, the extracted ion chromatograms of selected compounds at 0.05 $\mu g\,kg^{-1}$ are shown in Fig. 1.

The trueness of the proposed method was estimated by recovery studies in olive oil samples. Olive oil samples were spiked at 5 $\mu g\,kg^{-1}$ (n = 6). The absolute recoveries were calculated by comparing concentration of studied compounds in each sample spiked before the sample treatment procedure with concentration in extracts of each sample spiked after extraction and dilution step. As shown in Table 1, recovery rates ranged between 71% and 119% for all analytes with satisfactory precision for all analytes, being in

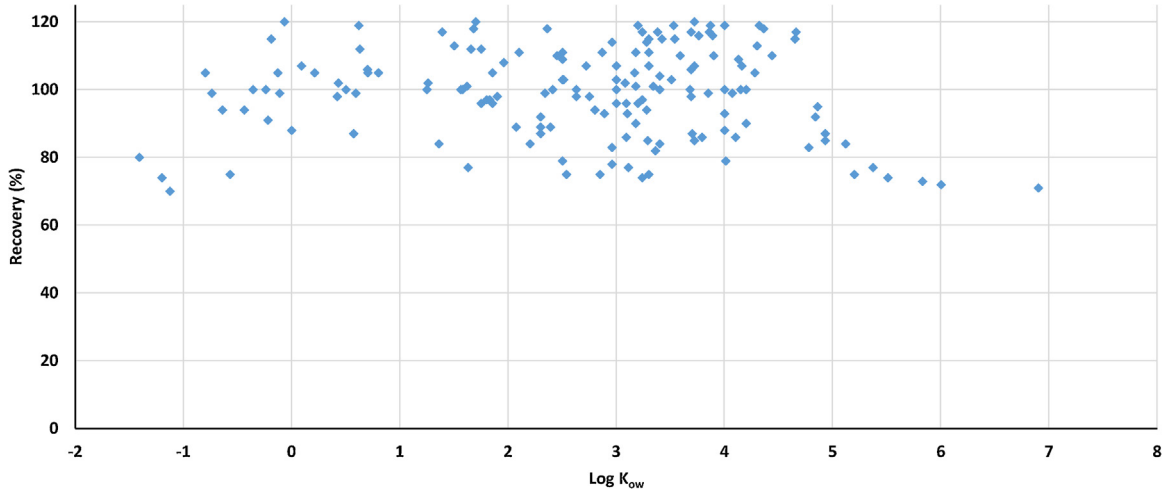

Fig. 2. 2D-Plot of the recovery rates/Log Kow in olive oil using EMR sorbent.

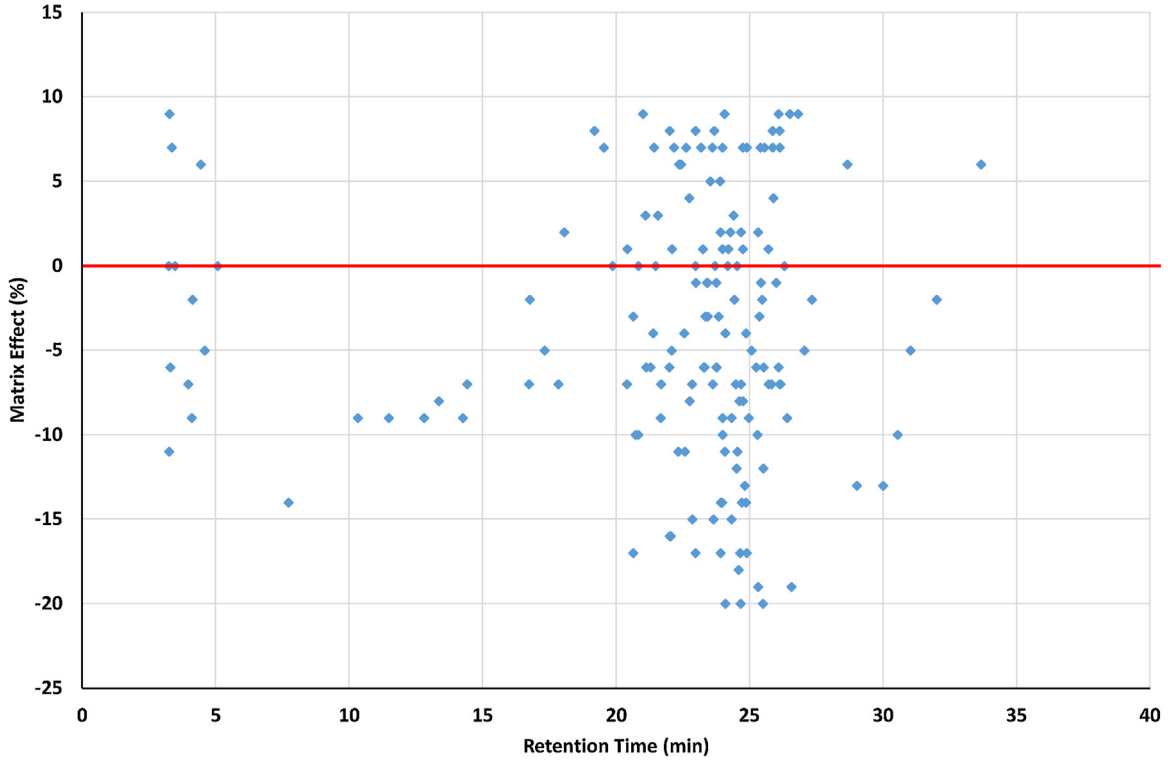

Fig. 3. 2D-Plot of the matrix effect of the pesticides/retention time in olive oil using EMR sorbent.

agreement with current guidelines [33]. To scrutinize the results obtained and unravel the behavior of the studied pesticides during extraction, a 2D plot representing the recovery rates versus the octanol/water partition coefficient (K_{OW}) of the analytes tested is shown in Fig. 2. In the case of propamocarb, aldicarb, aldicarb sulfoxide, quinmerac the low recoveries (around 70%) could be attributed to its polar nature ($\log K_{OW} < -1$). On the other hand, several authors have suggested that the low recoveries of highly lipophilic pesticides occurs because fat is insoluble in acetonitrile and forms an additional layer in the extraction step. Thus, a partition of lipophilic pesticides between acetonitrile and fat/oil layers is produced [38,39]. Then, there is a tendency to obtain low recoveries for pesticides with $\log K_{OW}$ equal to or greater than 5 such as etofenprox ($\log K_{OW}$ 6.9, 71%).

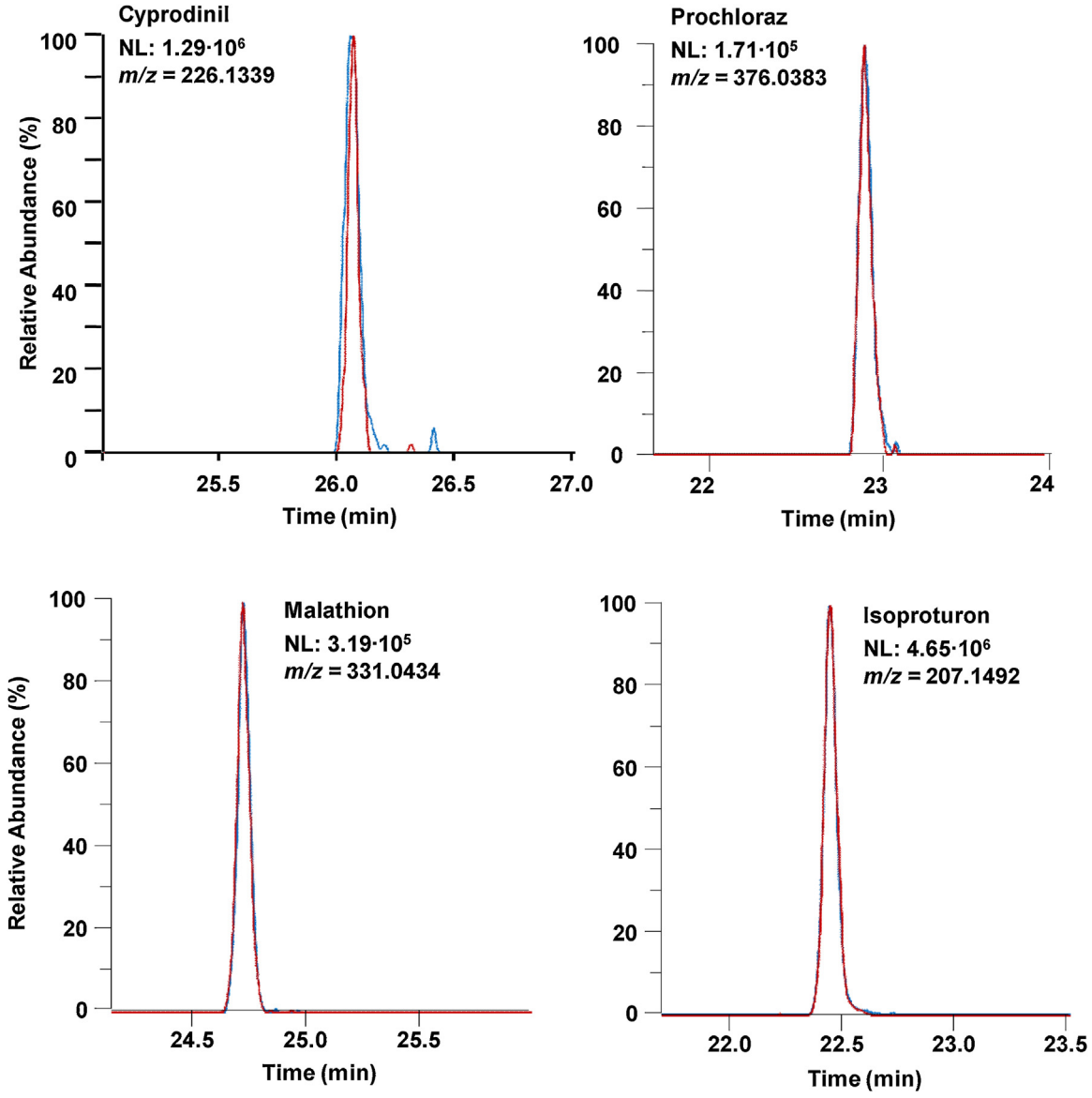

Fig. 4. Extracted ion chromatogram of Cyprodinil, Prochloraz, Malathion and Isoproturon corresponding to a standard in solvent (red) and spiked olive oil matrix (blue) at a concentration of 5 $\mu g\,kg^{-1}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Comparison of the proposed method with other reported methods for the determination of pesticides in olive oil samples using LC-ESI–MS and EMR sorbent.

Method	Precision	Matrix Effect	Recovery	Sensitivity ($\mu g\,kg^{-1}$)	Number of compounds	Reference
Nano flow LC-Q-Exactive Orbitrap	<20%	80% negligible 20% soft	75–119%	LCL: 0.05–50	162	Present Work
UHPLC-QqQ-MS/MS	<20%	51% negligible 30% soft 13% medium 5% strong	70–120%	LOQ: 0.1–91	67	[26]
UHPLC-QqQ-MS/MS	<20%	53% negligible 27% soft 12% medium 8% strong	70–120%	LOQ: 10–50	165	[28]

Matrix effect is a very relevant factor the determination of these compounds by LC-ESI–MS analysis. It describes the deviation between slope of matrix-matched calibration curves and the slope of external standard calibration curves, using the following equation [40]:

$$\text{Matrix effect}(\%) = \left(\left(\frac{\text{Calibration curve slope in matrix}}{\text{Calibration curve slope in solvent}} \right) - 1 \right) \times 100$$

A dilution factor of 1:15 was applied to the final extracts obtained from the sample treatment (a final dilution factor of 1:50 is obtained, considering the ratio 3 g/10 mL of QuEChERS). The matrix effects (Table 1**)** are illustrated as a 2D plot versus retention time in Fig. 3. Soft signal suppression ([−10%–20%]) was observed in the late eluting compounds (e.g. retention times > 21 min). This fact may be due to a higher concentration of co-extracted lipids. It should be noted that, 80% of compounds displayed a negligible matrix effect ([0%–±10%]), while soft/minor signal variation were obtained for the rest (between [±10%–±20%]), with signal suppression as predominant phenomenon. Fig. 4 shows the overlapped extracted ion chromatograms of selected compounds corresponding to a standard in solvent and spiked olive oil matrix at a concentration of 5 $\mu g\,kg^{-1}$. As could be observed, the signal was almost the same in both cases, showing that matrix effect is negligible. Thus, no isotopically labelled internal standard were employed in the present method, due its use is required when matrix effects is sizeable. Thus, isotopically labelled internal standard was not considered.

The precision of the method in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision) was evaluated for olive oil spiked at 5 $\mu g\,kg^{-1}$ with dilution factor of 50. Each sample was analyzed 6 times and injected into the nanoflow LC system. The precision expressed as%RSD of peak areas were satisfactory, obtaining%RSD lower than 20% in all cases (Table 1).

Finally, to highlight the value the analytical performance of the proposed method, a comparison with other reported methods for the determination of pesticides in olive oil samples using LC-ESI MS and EMR-lipid sorbent is shown in Table 2. The results in terms of sensitivity and recovery are similar to that reported by conventional LC-ESI–MS methods [26,28]. However, matrix effect values with the proposed method were significantly reduced by the use of nano flow LC-ESI–MS and high dilution factors, achieving a negligible effect for 80% of pesticides and soft for the rest. More specifically, matrix effect for several pesticides including aldicarb, fenthion, carbendazim, pymetrozine, carbaryl, fenarimol and cyromazine was reduced from medium ([±20%–±50%]) or strong ([±50%]) to negligible with the use of nano flow LC-ESI–MS. This fact is due to the advantages of nanospray in terms of tolerance to signal suppression as compared to conventional electrospray. Different studies have shown that the extent of matrix effects is related to the size of the droplet occurring in the Taylor cone. Thus, lower flow rates in the $nL\,min^{-1}$ scale generates lower droplet size, and these smaller droplets contain less number of molecules (interfering species) candidates to compete for ionization [41].

4. Concluding remarks

In this work, a multiresidue method using nanoflow LC MS has been proposed for the detection and quantification of 162 pesticides in olive oil sample. For accurate identification and quantification of the analytes, precise mass measurements combined with Rt information and AIF fragmentation were used. EMR-Lipid, in the clean-up step of a QuEChERS method, has been evaluated, applying a dilution factor of 50 to the sample, obtaining a negligible matrix effects for 80% of compound and soft for the other 20%. The sensitivity achieved with the proposed allow to achieve LCL lower than their MRLs established for olive oil.

Acknowledgements

The authors acknowledge funding from the Spanish Ministerio de Economía y Competitividad (MINECO) through Project Ref. CTQ-2015-71321-P, partially co-financed with FEDER funds. D.M.G. thanks the Spanish Ministerio de Economía y Competitividad (MINECO) for a Juan de la Cierva postdoctoral contract (Ref. FJCI-2014–19573). S.M.A thanks the Argentine National Council of Scientific and Technical Research (CONICET) for granting the scholarship pertaining to the Program of Partial Financing of Stays Abroad for Assistant Researchers. The authors acknowledge Servicios Centrales de Apoyo a la Investigación (SCAI-UJAEN) from University of Jaén and Dr. Juan Castro Mármol (SCAI-UJAEN) for technical support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.chroma.2018.05.053.

References

[1] Handbook of Olive Oil Analysis and Properties, Ramón Aparicio, John Harwood, New York (USA), second edition, ©Springer Science + Business Media, New York, 2013 (ISBN 978-1-4614-7776-1).
[2] Production techniques in olive growing, ©International Olive Council, Madrid (Spain), First edition: May 2007, ISBN: 978-84-931663-6-6.
[3] Ch. Lentza-Rizos, Insecticides authorized for use on olive tres and the relationship between their registration and residues in olive oil, Grasas y Aceites 47 (1996) 392–396.
[4] European. Commission, Regulation (EC) 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC, Off. J. Eur. Union L70 (2005) 1–16.
[5] European Commission Implementing Regulation (EU) 2015/595 of 15 April 2015 concerning a coordinated multiannual control programme of the Union for 2016, 2017 and 2018 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin, Off. J. Eur. Union L99 (2015) 7–20.
[6] B. Gilbert-López, J.F. García-Reyes, A. Molina-Díaz, Sample treatment and determination of pesticide residues in fatty vegetable matrices: a review, Talanta 79 (2009) 109–128.
[7] J. Zhan, J. Li, D. Liu, C. Liu, G. Yang, Z. Zhou, P. Wang, A simple method for the determination of organochlorine pollutants and the enantiomers in oil seeds based on matrix solid-phase dispersion, Food Chem. 194 (2016) 319–324.
[8] B. Gilbert-López, J.F. García-Reyes, A. Lozano, A.R. Fernández-Alba, Antonio Molina-Díaz, 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, J. Chromatogr. A 1217 (2010) 6022–6035.
[9] A.G. Sánchez, N.R. Martos, E. Ballesteros, Multiresidue analysis of pesticides in olive oil by gel permeation chromatography followed by gas chromatography-tandem mass-spectrometric determination, Anal. Chim. Acta 558 (2006) 53–61.
[10] J.L. Fernández-Moreno, F.J. Arrebola-Liébanas, A. Garrido-Frenich, J.L. Martínez-Vidal, Evaluation of different sample treatments for determining pesticide residues in fat vegetable matrices like avocado by low-pressure gas chromatography-tandem mass spectrometry, J. Chromatogr. A 1111 (2006) 97–105.
[11] M.A. Farajzadeh, B. Feriduni, M.R.A. Mogaddam, Determination of triazole pesticide residues in edible oils using air-assisted liquid–liquid microextraction followed by gas chromatography with flame ionization detection, J. Sep. Sci. 38 (2015) 1002–1009.
[12] A. Angioni, L. Porcu, F. Pirisi, LC/DAD/ESI/MS method for the determination of imidacloprid thiacloprid, and spinosad in olives and olive oil after field treatment, J. Agric. Food Chem. 59 (2011) 11359–11366.
[13] R. Nortes-Méndez, J. Robles-Molina, R. López-Blanco, A. Vass, A. Molina-Díaz, J.F. Garcia-Reyes, Determination of polar pesticides in olive oil and olives by hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry and high resolution mass spectrometry, Talanta 158 (2016) 222–228.
[14] L. Li, Y. Xu, C. Pan, Z. Zhou, S. Jiang, F. Liu, Simplified pesticide multiresidue analysis of soybean oil by low-temperature cleanup and dispersive solid-phase extraction coupled with gas chromatography/mass spectrometry, J. AOAC Int. 90 (2007) 1387–1393.
[15] F.A. Esteve-Turrillas, A. Pastor, M. de la Guardia, Determination of pyrethroid insecticide residues in vegetable oils by using combined solid-phases

extraction and tandem mass spectrometry detection, Anal. Chim. Acta 553 (2005) 50–57.
[16] S. López-Feria, S. Cárdenas, M. Valcárcel, One step carbon nanotubes-based solid-phase extraction for the gas chromatographic-mass spectrometric multiclass pesticide control in virgin olive oils, J. Chromatogr. A 1216 (2009) 7346–7350.
[17] B. Socas-Rodríguez, J. González-Sálamo, A.V. Herrera-Herrera, J. Hernández-Borges, M.A. Rodríguez-Delgado, Chapter Eleven – Recent advances and developments in the QuEChERS method, Comp. Anal. Chem. 76 (2017) 319–374.
[18] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce, J. AOAC Int. 86 (2003) 412–431.
[19] T. Rejczak, T. Tuzimski, A review of recent developments and trends in the QuEChERS sample preparation approach (Review), Open Chem. 13 (2015) 980–1010.
[20] E. Hakme, A. Lozano, C. Ferrer, F.J. Díaz-Galiano, A.R. Fernández-Alba, Analysis of pesticide residues in olive oil and other vegetable oils, TrAC Trends Anal. Chem. 100 (2018) 167–179.
[21] J.F. García-Reyes, C. Ferrer, M.J. Gómez-Ramos, A. Molina-Díaz, A.R. Fernández-Alba, Determination of pesticide residues in olive oil and olives, TrAC Trends Anal. Chem. 26 (2007) 239–251.
[22] C. Anagnostopoulos, G.E. Miliadis, Development and validation of an easy multiresidue method for the determination of multiclass pesticide residues using GC–MS/MS and LC–MS/MS in olive oil and olives, Talanta 112 (2013) 1–10.
[23] L. Polgár, B. Kmellár, J.F. García-Reyes, F. Fodor, Comprehensive evaluation of the clean-up step in QuEChERS procedure for the multi-residue determination of pesticides in different vegetable oils using LC–MS/MS, Anal. Methods 4 (2012) 1142–1148.
[24] T. Tuzimski, T. Rejczak, Application of HPLC-DAD after SPE/QuEChERS with ZrO2-based sorbent in d-SPE clean-up step for pesticide analysis in edible oils, Food Chem. 190 (2016) 71–79.
[25] D. Moreno-González, J.F. Huertas-Pérez, A.M. García-Campaña, L. Gámiz-Gracia, Determination of carbamates in edible vegetable oils by ultra-high performance liquid chromatography-tandem mass spectrometry using a new clean-up based on zirconia for QuEChERS methodology, Talanta 128 (2014) 299–304.
[26] R. López-Blanco, R. Nortes-Méndez, J. Robles-Molina, D. Moreno-González, B. Gilbert-López, J.F. García-Reyes, A. Molina-Díaz, Evaluation of different cleanup sorbents for multiresidue pesticide analysis in fatty vegetable matrices by liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1456 (2016) 89–104.
[27] P. Parrilla Vázquez, E. Hakme, S. Uclés, V. Cutillas, M. Martínez Galera, A.R. Mughari, A.R. Fernández-Alba, Large multiresidue analysis of pesticides in edible vegetable oils by using efficient solid-phase extraction sorbents based on quick, easy, cheap, effective, rugged and safe methodology followed by gas chromatography?tandem mass spectrometry, J. Chromatogr. A 1463 (2016) 20–31.
[28] J.V. Dias, V. Cutillas, A. Lozano, I.R. Pizzutti, A.R. Fernández-Alba, Determination of pesticides in edible oils by liquid chromatography-tandem mass spectrometry employing new generation materials for dispersive solid phase extraction clean-up, J. Chromatogr. A 1462 (2016) 8–18.
[29] D. Moreno-González, P. Pérez-Ortega, B. Gilbert-López, A. Molina-Díaz, J.F. García-Reyes, A.R. Fernández-Alba, Evaluation of nanoflow liquid chromatography high resolution mass spectrometry for pesticide residue analysis in food, J. Chromatogr. A 1512 (2017) 78–87.
[30] D. Moreno-González, J. Alcántara-Durán, B. Gilbert-López, J.F. García-Reyes, A. Molina-Díaz, Matrix-effect free quantitative LC-MS analysis in complex matrices using nanoflow LC with integrated emitter tip and high dilution factors, J. Chromatogr. A 1519 (2017) 110–120.
[31] Y. Shen, R. Zhao, S.J. Berger, G.A. Anderson, N. Rodriguez, R.D. Smith, High-Efficiency nanoscale liquid chromatography coupled on-line with mass spectrometry using nanoelectrospray ionization for proteomics, Anal. Chem. 74 (2002) 423–4249.
[32] R. Juraschek, T. Dülcks, M. Kara, Nanoelectrospray—more than just a minimized-flow electrospray ionization source, J. Am. Soc. Mass Spectrom. 10 (1999) 300–308.
[33] Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed. SANTE/11813/2017, European Commission, 2017.
[34] R. López-Blanco, D. Moreno-González, R. Nortes-Méndez, J.F. García-Reyes, A. Molina-Díaz, B. Gilbert-López, Experimental and theoretical determination of pesticide processing factors to model their behavior during virgin olive oil production, Food Chem. 239 (2018) 9–16.
[35] European Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Commission Decision 2002/657/EEC, Off. J. Eur. Commun. L221 (2002) 8–36.
[36] P. Kumar, A. Rúbies, F. Centrich, M. Granados, N. Cortés-Francisco, J. Caixach, R. Companyó, Targeted analysis with benchtop quadrupole-orbitrap hybrid mass spectrometer: application to determination of synthetic hormones in animal urine, Anal. Chim. Acta 780 (2013) 65–73.
[37] L. Rajski, M.M. Gómez-Ramos, A.R. Fernández_Alba, Large pesticide multiresidue screening method by liquid chromatography-Orbitrap mass spectrometry in full scan mode applied to fruit and vegetables, J. Chromatogr. A 1360 (2014) 119–127.
[38] S.C. Cunha, S.J. Lehotay, K. Mastovska, J.O. Fernandes, B.M.P.P. Oliveira, Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olives, J. Sep. Sci. 30 (2007) 620–632.
[39] S.J. Lehotay, K. Mastovska, S.J. Yun, Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes, J. AOAC Int. 88 (2005) 630–638.
[40] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS, Anal. Chem. 75 (2003) 3019–3030.
[41] A. Schmidt, M. Karas, T. Dülcks, Effect of different solution flow rates on analyte ion signals in nano-ESI MS, or: when does ESI turn into nano-ESI? J. Am. Soc. Mass Spectrom. 14 (2003) 492–500.