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Analytical Methods

Application of HPLC–DAD after SPE/QuEChERS with ZrO_2-based sorbent in d-SPE clean-up step for pesticide analysis in edible oils

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ABSTRACT

In this study, the solid-phase extraction/quick, easy, cheap, effective, rugged and safe (SPE/QuEChERS) technique was adapted to develop a simple sample treatment for multi-residue pesticide analysis of edible oils. The proposed method is based on liquid–liquid partitioning with acetonitrile followed by dispersive solid phase extraction using zirconia-coated silica particles for extract purification. To evaluate the described method, 21 pesticides belonging to different chemical classes were analysed using high performance liquid chromatography with diode-array detection (HPLC–DAD). For validation purposes, recovery studies were performed at 75 ng g^{-1}, 125 ng g^{-1}, 250 ng g^{-1}, 500 ng g^{-1} and 1000 ng g^{-1} levels. Recoveries were over the range of 50–130% for most of the analytes, with relative standard deviations less than 15% being observed. HPLC–DAD provided suitable linearity, precision and accuracy. The validated method was successfully applied to the analysis of edible oil samples selected from the market.

1. Introduction

One of the trends in pesticide residue analysis is the development of rapid, highly sensitive, and highly accurate methods for reliable identification and quantification of analytes in complicated matrices (e.g., food commodities) at trace levels. To ensure the safety of food and to regulate international trade, legislation, such as the European Union (EU) directives, describe the maximum residue levels (MRLs) for pesticides permitted in products of plant or animal origin intended for human or animal consumption (Regulation (EC) No. 396/2005). Many sample preparation techniques are used in pesticide analysis, and the selection of the appropriate method depends on the complexity of the sample, the nature of the matrix, the properties of the analytes, and the available analytical techniques (Majors, 2013).

QuEChERS (quick, easy, cheap, effective, rugged, and safe) is a sample preparation approach used for the extraction of multi-class, multiresidue analytes, especially pesticides in fruit and vegetables and many other samples with complicated matrices (Chamkasen, Ollis, Harmon, Lee, & Mercer, 2013; Eitzer, Hammack, & Filigenzi, 2014; Koesukwiwat, Lehotay, Maštovská, Dorweiler, & Leepipatpiboon, 2010). The method has achieved worldwide acceptance due to its simplicity and high throughput, enabling laboratories to process significantly more samples in a given time compared with earlier methods (Anastassiades et al., 2002). Two-step extraction techniques are based on salting out (extraction) followed by dispersive solid phase extraction (d-SPE) (clean-up). Although NaCl was used for salting out in the original QuEChERS method (Anastassiades, Lehotay, Stainbaher, & Schenk, 2003), refinements were achieved when it was discovered that recovery of some base-sensitive compounds, such as fungicides (chlorothalonil and captan), was poor. This discovery led to the development of two buffered methods (AOAC QuEChERS 2007.01 and EN 15662) for the initial extraction step.

Recent efforts have focused on QuEChERS modifications to improve the scope of analytes and matrices covered (Frenich, Fernández, Moreno, Vidal, & Gutiérrez, 2012; Sapoznhnikova & Lehotay, 2013; Zheng et al., 2013). The modified QuEChERS procedure was compared with aqueous acetonitrile extraction and pure acetonitrile extraction by Lacina et al. (2012). Versions with various buffers have been compared by Lehotay, Maštovská, and Lightfield (2005). Both of the groups report that different versions performed reasonably well for most pesticides; the most pronounced differences were noted for two pH-dependent pesticides. It is well known (Geis-Asteggiante, Lehotay, & Heinzen, 2012; Koesukwiwat, Lehotay, & Leepipatpiboon, 2011; Koesukwiwat, Lehotay, Miao, & Leepipatpiboon, 2010; Lehotay et al., 2010) that when QuEChERS is used for sample preparation, some analytes,

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such as captan, folpet, chlorothalonil, and captafol, remain problematic due to degradation during sample preparation and gas chromatography/mass spectrometry (GC/MS) analysis.

There are two factors affecting the results of QuEChERS in terms of recovery, as follows: (i) the solvent chosen for extraction and (ii) the type, quantity and purity of salts and sorbents for d-SPE. These factors are mutually related to each other and have often been studied together by various authors. For the first factor, Savant et al. compared various solvents and estimated their effect on the recovery values of pesticides (Savant et al., 2010). In the QuEChERS experiments, these authors substituted MeCN with EtOAc and applied primary-secondary amine (PSA) and graphitized carbon black (GCB) for d-SPE, which increased recoveries to 70% or higher for some difficult analytes, such as captan, folpet and captafol (Savant et al., 2010). Garrido Frenich et al. optimized QuEChERS with acidified acetonitrile for multiresidue pesticide analysis of tuber and root commodities by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS) (Frenich et al., 2012).

For d-SPE, the sorbent is chosen to retain the matrix and undesired components and to allow the analytes of interest to remain in the liquid phase. Application of specific d-SPE kits depends on the nature of the fruit and/or vegetable sample and the type of matrix, such as high water content (e.g., tomato), high acidic content (e.g., citrus), high sugar content (i.e., raisins) and high fat content (olives or avocado) (Majors, 2013). The most complex matrices are those with high fat content because it is challenging to extract pesticides without the co-extraction of lipids, which are difficult to remove from the extract and may affect the detection system (García-Reyes, Ferrer, Gomez-Ramos, Molina-Díaz, & Fernández-Alba, 2007; Gilbert-López, García-Reyes, Fernández-Alba, & Molina-Díaz, 2010; Gilbert-López, García-Reyes, & Molina-Díaz, 2009; Lehotay, Maštovská, & Jong, 2005).

In d-SPE, a portion of the raw extract is purified using common sorbents, such as octadecyl (C18), PSA, and GCB. New d-SPE sorbents have been developed to enhance sample clean-up for complex matrices by removing interference and overcoming problems associated with traditional QuEChERS dispersive phases (e.g., an affinity for planar analytes to GCB or reduced recovery rates of non-polar analytes after C18 application). These sorbents have been reported in the literature along with more commonly used sorbents.

Zheng et al. described QuEChERS with magnetic GCB and PSA as an adsorbent for pesticide residue analysis in vegetables (Zheng et al., 2013). Another innovation, described by Cerqueira et al., was the use of chitin as an alternative sorbent for clean-up in the d-SPE step for simultaneous extraction of different organic contaminants in sludge samples by liquid chromatography coupled with electrospray ionization mass spectrometry (LC–ESI-MS/MS) (Cerqueira, Caldas, & Primel, 2014). The modified QuEChERS sample preparation with amine-modified graphene as a reversed-dispersive solid phase extraction material and application methodology to determine the pesticide residues in four oil crops by LC–MS/MS was described by Guan et al. (2013). Two sorbents containing ZrO_2 (Z-Sep and Z-Sep+) were tested as a d-SPE clean-up in combination with the QuEChERS and ethyl acetate multiresidue method in the pesticide residues extraction in avocado (Guan et al., 2013). Sapoznhnikova and Lehotay described a multiresidue method for the analysis of different analytes in fish by fast low-pressure gas chromatography triple quadrupole tandem mass spectrometry (LP-GC/MS-MS) after a QuEChERS sample preparation with extraction with acetonitrile and d-SPE clean-up with a zirconium-based sorbent (Sapoznhnikova & Lehotay, 2013).

Currently, HPLC or GC coupled with MS and/or tandem MS (MS/MS) appear to be the primary techniques for pesticide residue analysis in food commodities with relatively high fat content, e.g., in olive oils and olives (Gilbert-López et al., 2009). Anagnostopoulos and Miliadis described procedures, based on acetonitrile extraction according to the European Union guidelines (Document (EU) N° SANCO/12571/2013), suitable for the monitoring of multiclass pesticides in olives and olive oil by GC–MS/MS and LC–MS/MS (Anagnostopoulos & Miliadis, 2013).

Application of high performance liquid chromatography with diode-array detection (HPLC–DAD) can be useful for the correct identification of pesticides in complicated mixtures and the separation of analytes from the components of the matrix with high content of lipids, e.g., in sunflower seed samples (Tuzimski & Rejczak, 2014). The use of DAD may be useful in the analysis of samples with complicated matrices by obtaining UV spectra and evaluating the purity of the peaks on the chromatograms (Tuzimski, 2009; Tuzimski & Rejczak, 2014; Tuzimski & Sobczyński, 2009). The LC–DAD is a less expensive technique than LC–MS/MS, but the latter offers the advantage of identifying the compounds of interest with a high level of confidence (not always the case in DAD with interfering substances and/or compared with published reference spectra). The LC–DAD is a sufficiently sensitive technique for samples that were purified and concentrated before chromatographic analysis. The success of this technique is possible through the application of new types of sorbents during the purification (d-SPE) step of samples in the QuEChERS method.

The objective of the present work was to develop and validate the HPLC–DAD method following the QuEChERS/SPE procedure for the identification and quantitative analysis of 21 pesticides belonging to different classes in high oil matrices. The goal of this study was also to evaluate the so-called Z-Sep sorbent as the clean-up material in the d-SPE step of the QuEChERS extraction procedure for pesticide analysis in grapeseed oil and extra virgin olive oil samples.

2. Experimental

2.1. Pesticide standards

Standards for the pesticides under investigation (Table 1), such as fenuron, methabenzthiazuron, isoproturon, terbutryn, procymidone, fenitrothion, neburon, chlorfenvinphos, lufenuron, flufenoxuron, trifluralin and α-cypermethrin, were obtained from Dr. Ehrenstorfer-Schäfers (Augsburg, Germany); those of monuron, fluometuron, dimethomorph, linuron and clofentezine were obtained from Sigma–Aldrich (Supelco, Bellefonte, PA, USA); those of propazine, propachlor, terbuthylazine and bromopropylate were obtained from the Institute of Organic Industry (IPO, Warsaw, Poland). The standard purity indicated by the manufacturers in all of the cases was $\geqslant$97%.

All of the standards were dissolved in methanol, except for propachlor, clofentezine, lufenuron, and flufenoxuron, which were dissolved in acetone.

2.2. Solvents and mobile-phase solution

Acetonitrile (MeCN), methanol (MeOH), and acetone (Ac) were pro chromatography grade and were obtained from E. Merck (Darmstadt, Germany).

2.3. Reagents for dispersive-SPE (d-SPE) and SPE

Anhydrous magnesium sulphate ($MgSO_4$) and sodium chloride were obtained from POCH (Gliwice, Poland).

Tubes for d-SPE (Supel™ QuE – QuEChERS; Z-Sep 500 mg, No. 55403-U) were obtained from Sigma–Aldrich (Supelco, Bellefonte, PA, USA).

Table 1
Method validation parameters for the quantification of pesticides by the proposed HPLC–DAD method.

Pesticide	Dissociation constant (pKa) at 25 °C	Octanol–water partition coefficient at pH 7, 20 °C (log *P*)	t_r (min)	λ (nm)	LOD (μg/mL)	LOQ (μg/mL)	Range (μg/mL)	*r*
Fenuron	–	0.98	3.190–3.376	202	0.03	0.11	0.1–4.8	0.9999
				240	0.05	0.15	0.1–4.8	0.9999
Monuron	–	1.79	6.161–6.269	202	0.03	0.09	0.1–4.8	1
				212	0.04	0.13	0.3–4.8	1
				240	0.03	0.10	0.15–4.8	1
Methabenzthiazuron	–	2.64	7.932–8.071	202	0.05	0.16	0.15–3.6	0.9998
				222	0.04	0.13	0.1–3.6	0.9999
				240	0.06	0.18	0.3–3.6	0.9999
Fluometuron	Not applicable (no dissociation)	2.28	9.107–9.222	202	0.09	0.28	0.15–4.8	0.9996
				212	0.10	0.32	0.3–4.8	0.9996
				240	0.10	0.32	0.3–4.8	0.9996
Isoproturon	Not applicable (no dissociation)	2.5	9.643–9.788	202	0.04	0.11	0.1–3.6	0.9999
				222	0.04	0.14	0.6–3.6	1
				240	0.04	0.12	0.15–3.6	0.9999
Propachlor	–	1.6	11.501–11.656	202	0.03	0.09	0.15–4.8	1
				212	0.03	0.10	0.3–4.8	1
Dimethomorph	−1.3 (very strong acid)	2.68	12.278–12.390[a]	202	0.05	0.14	0.3–4.8	0.9999
			12.928–13.019	212	0.06	0.19	0.6–4.8	0.9999
Propazine	1.7 (very weak base)	3.95	12.329–12.785	212	0.02	0.05	0.15–5	1
				222	0.02	0.06	0.075–5	1
				240	0.01	0.04	0.3–5	1
Terbuthylazine	1.9	3.4	13.748–13.920	212	0.06	0.18	0.1–3.6	0.9997
				222	0.03	0.10	0.1–3.6	0.9999
				240	0.05	0.16	0.3–3.6	0.9999
Linuron	Not applicable (no dissociation)	3.0	14.819–14.951	222	0.05	0.16	0.3–4.8	1
				240	0.05	0.16	0.3–4.8	0.9999
Terbutryn	4.3 (weak base)	3.66	16.911–17.001	212	0.02	0.06	0.15–4.8	1
				222	0.02	0.06	0.1–4.8	1
				240	0.02	0.07	0.15–4.8	1
Procymidone	Not applicable (no dissociation)	3.3	19.094–19.243	202	0.05	0.15	0.1–3.6	0.9997
				212	0.05	0.16	0.1–3.6	0.9997
				222	0.05	0.16	0.3–3.6	0.9998
Fenitrothion	Not applicable (no dissociation)	3.32	19.609–19.735	202	0.10	0.32	0.3–4.8	0.9998
				212	0.10	0.32	0.3–4.8	0.9994
Neburon	–	3.8	19.921–20.065	202	0.07	0.22	0.3–6	0.9998
				212	0.06	0.17	0.1–6	0.9998
				240	0.08	0.25	0.3–6	0.9998
Chlorfenvinphos	–	3.8	20.417–20.496	202	0.03	0.11	0.15–3.6	0.9999
				212	0.03	0.10	0.2–3.6	0.9999
				240	0.05	0.16	0.6–3.6	1
Clofentezine	Unstable – cannot be determined	3.1	24.075–24.217	202	0.26	0.79	0.5–9	0.9998
				212	0.26	0.78	0.5–9	0.9998
				222	0.26	0.77	0.5–9	0.9998
Lufenuron	10.2 (very weak acid)	5.12	28.530–28.724	202	0.06	0.19	0.15–2.4	0.9996
				222	0.09	0.28	0.15–3.6	0.9994
				240	0.06	0.18	0.3–3.6	0.9998
Bromopropylate	–	5.4	29.838–29.912	202	0.05	0.16	0.3–4.8	0.9999
				212	0.06	0.18	0.3–4.8	0.9999
				222	0.07	0.22	0.3–4.8	0.9998
Flufenoxuron	10.1 (very weak acid)	5.11	30.327–30.529	202	0.06	0.17	0.1–4.8	0.9998
				212	0.07	0.20	0.15–4.8	0.9998
				222	0.09	0.27	0.3–4.8	0.9997
Trifluralin	Not applicable (no dissociation)	5.27	30.998–31.121	202	0.05	0.15	0.3–4.8	0.9999
				212	0.06	0.17	0.3–4.8	0.9999
				222	0.05	0.16	0.3–4.8	0.9999
α-Cypermethrin	5.0 (weak acid)	5.5	34.432–34.464[a]	202	0.06	0.20	0.1–3.6	0.9997
			34.540–34.596	212	0.05	0.17	0.05–3.6	0.9997
				222	0.08	0.25	0.15–3.6	0.9997

[a] Compound with two peaks.

Octadecyl SPE cartridges (C18 2000 mg/6 mL, no. 7020–08) were obtained from Bakerbond (J.T. Baker, Deventer, The Netherlands).

2.4. Samples

Samples of olive oil (Carbonell – Extra Virgin Olive Oil, Spain; Costad'Oro – Extra Virgin Olive Oil Integrale, Italy) and grapeseed oil (Olitalia – dal Vinacciolo, Italy) were purchased locally.

2.5. Spiked samples

Extraction efficiency was examined by spiking blank samples. Olive oil samples were spiked with the pesticides under investigation at concentrations ranging from 75 to 1000 ng g^{-1}. Three 6 g portions of an olive oil sample were weighed and fortified with the appropriate volume of the working standard solutions mixture and were incubated at room temperature for 12 h to ensure that the solvent was completely evaporated. Recovery studies were performed based on three replicates from the spiking procedure.

2.6. Edible oil samples selected from a market

An expanded procedure was employed for pesticide residue analysis in oil samples purchased from the local market. Pesticide residues were determined in twenty-one 6 g-portions of virgin olive oil and grapeseed oil using the proposed method. Five replicates were performed for each oil sample.

2.7. Sample treatment

The proposed method was composed of the three following steps: salting out, SPE and clean-up step using Z-Sep sorbent.

In the first step, 14 mL re-distilled water and 20 mL MeCN were added into 50-mL polypropylene (PP) centrifugation tubes containing 6 g of a spiked or natural (not spiked) oil portion. The tubes were closed and vigorously shaken manually for approximately 1 min. Next, 2 g NaCl and 8 g anhydrous $MgSO_4$ were added and shaken immediately to prevent coagulation of $MgSO_4$. Then, the tubes were centrifuged (Centrifuge MPW-223e, Warsaw, Poland) for 5 min (6000 rpm, 3480 rcf). The acetonitrile layer of each tube was then obtained with a pipette, combined (from three or twenty-one tubes for spiked or natural samples, respectively) and evaporated to dryness under a fume hood.

In the second step, the evaporated extracts were reconstituted in 5 mL MeCN for the three spiked oil portions or in 35 mL MeCN for the twenty-one natural oil samples. Twice-distilled (re-distilled) water was added to the reconstituted extracts to obtain a 5% solution in water (e.g., 95 mL water was added to 5 mL extract). SPE was performed using Bakerbond (J.T. Baker, Deventer, The Netherlands) cartridges containing octadecyl sorbent (C18 2000 mg/6 mL, no. 7020–08) and a Baker SPE-12G SPE chamber (J.T. Baker, Philipsburg, USA). Before use, each cartridge was conditioned with 3×2 mL MeCN and 3×2 mL twice-distilled water. After loading the cartridges with the oil sample extract (100 mL per cartridge, flow rate 10 mL/min, and pressure 85 mmHg), the cartridges were washed with 3×2 mL of a 2.5% solution of MeCN – water (v/v), dried for one minute (left under vacuum pressure in the SPE chamber) and then eluted with 5 mL MeCN. Extracts eluted after SPE were evaporated to dryness under a fume hood (for natural samples, extracts eluted from seven cartridges were combined and then evaporated).

The clean-up step was performed using d-SPE tubes (Supel™ QuE – QuEChERS; Z-Sep 500 mg, no. 55403-U). Extracts were reconstituted in 1.2 mL or 8.4 mL MeCN and transferred into a 12-mL PP tube containing 0.5 g or 3.5 g Z-Sep sorbent for the spiked or natural samples, respectively. The tubes were centrifuged (4000 rpm, 2320 rcf) for 8 min. The supernatants were collected and directly analysed by RP-HPLC–DAD (step A). The supernatant of the natural samples remaining after injection was evaporated to concentrate in a smaller volume of MeCN (step B) or to perform an extra clean-up step with another 0.5 g Z-Sep (step C).

2.8. RP-HPLC procedure

For the identification of pesticides in oil samples, after the sample preparation, the extracts were injected onto a C18 column and analysed by HPLC–DAD. The extracts were analysed at 22 °C using an Agilent Technologies 1200 series chromatograph equipped with a quaternary gradient pump with a degasser set at a flow rate of 1 mL/min and with a DAD. Extracts were injected onto the column using a Rheodyne 20 μL injector. The HPLC apparatus was equipped with a ZORBAX Eclipse XDB-C18 150 mm × 4.6 mm column, with a 5-μm particle size (Agilent Technologies, Wilmington, DE, USA). The gradient applied was 30% B, 0–30 min linear to 76% B, 30–35 min to 100% B, 35–45 min isocratic 100% B (A – H_2O, B – MeCN). The column was conditioned with the initial mobile phase composition for 45 min at a flow rate of 1 mL/min. Additionally, after each olive oil sample was run, the column was washed with MeCN before conditioning.

The calibration was based on the peak areas obtained from pesticide standards prepared as solutions in methanol at seven or eight concentrations. Each solution was injected in triplicate under the same chromatographic conditions.

2.9. Validation of the HPLC method

The limits of detection (LOD) and quantification (LOQ) were calculated using the LOD = 3.3(SD/S) and LOQ = 10(SD/S) formulas, respectively, where SD is the standard deviation of the response, and S is the slope of the calibration plot (Swartz & Krull, 1997) (Table 1).

Recovery and precision (Table 2) were determined by the standard addition method in which olive oil and grapeseed oil samples were spiked with a mixture of the investigated pesticides at a concentration level over the range from 75 ng g^{-1} to 1000 ng g^{-1}. The procedure described above was applied to three 6 g portions of edible oil (sum of 18 g was used). Recovery studies were performed based on three replicates, and relative standard deviations are expressed as a percentage (% RSD) for all of the analytes, as presented in Table 2.

3. Results and discussion

3.1. Optimization of chromatographic conditions

In the first phase of the experiment, optimal stationary and mobile phases were selected for the separation and detection of analytes. Chromatographic conditions were optimized with the focus on sensitivity for the studied pesticides. The stationary phase should be particularly useful to separate the widest possible groups or classes of analytes in environmental samples.

The selection of the stationary phase and the mobile phase composition for this study was based on previous experience and published results (Tuzimski, 2009; Tuzimski & Rejczak, 2014; Tuzimski & Sobczyński, 2009). Chromatographic separation on a ZORBAX Eclipse XDB-C18 column provided satisfactory results for a wide range of analytes. The applied gradient elution allowed proper separation of the studied pesticides, as presented in Fig. 1.

3.2. Linearity

Under the conditions described above, the calibration curves of the analysed pesticides showed a satisfactory linearity and a strong correlation between concentration and peak area over the studied range with the correlation coefficient, r, $\geqslant 0.9994$ for all of the analytes (Table 1).

LOD and LOQ calculated as described above were over the range from 0.01 to 0.10 and from 0.03 to 1.08 μg mL^{-1}, respectively (Table 1). The standard calibration curves of the analytes were constructed by plotting analyte concentration against peak area.

3.3. Recovery test

Pesticides were added to untreated control samples at five levels (75, 125, 250, 500, and 1000 ng g^{-1}). For method validation, control and fortified samples were analysed under the same conditions. The reported results are the mean of three replicates at each spiked level. The recoveries and precision values obtained from the validation study for extra virgin olive oil and grapeseed oil samples are presented in Table 2.

Table 2
Average percentage recoveries and % RSD obtained by SPE/QuEChERS from fortified extra virgin olive oil and grapeseed oil samples.

Pesticide	Recovery (% RSD)									
	Olive oil Fortification level (ng/g)					Grapeseed oil Fortification level (ng/g)				
	1000	500	250	125	75	1000	500	250	125	75
Fenuron	ns	52 (7.7)	54 (12.1)	96 (17.2)	ns	39 (3.1)	38 (6.0)	46 (7.9)	38 (13.2)	57 (13.5)
Monuron	83 (14.7)	73 (12.5)	67 (14.5)	126 (20.1)	118 (19.1)	51 (6.5)	47 (8.2)	50 (6.9)	43 (11.6)	41 (14.2)
Methabenzthiazuron	71 (4.2)	68 (5.5)	65 (9.2)	101 (14.6)	92 (12.6)	65 (7.8)	66 (9.7)	59 (11.0)	58 (14.5)	61 (14.9)
Fluometuron	94 (9.4)	94 (12.6)	84 (18.7)	128 (19.8)	–	65 (7.7)	64 (11.0)	72 (13.1)	77 (17.9)	92 (15.0)
Isoproturon	61 (12.3)	59 (11.8)	45 (16.1)	72 (18.3)	58 (7.9)	41 (2.6)	35 (2.6)	36 (8.4)	34 (14.1)	36 (2.5)
Propachlor	–	–	–	–	–	38 (1.1)	39 (5.1)	47 (8.6)	45 (9.0)	41 (10.3)
Dimethomorph	ns	78 (12.0)	ns	ns	ns	36 (6.6)	33 (13.4)	ns	ns	ns
Propazine	ns	ns	71 (10.3)	123 (7.7)	ns	ns	65 (13.2)	ns	84 (7.4)	70 (9.8)
Terbuthylazine	78 (13.1)	71 (12.5)	69 (15.6)	89 (15.0)	–	64 (8.7)	59 (11.8)	65 (12.1)	63 (11.5)	65 (11.0)
Linuron	90 (4.9)	92 (5.4)	86 (10.0)	116 (13.9)	93 (6.6)	70 (5.2)	66 (7.2)	69 (1.5)	62 (1.9)	64 (4.1)
Terbutryn	21 (15.8)	20 (17.2)	19 (17.7)	22 (22.1)	18 (20.7)	27 (9.9)	20 (17.3)	21 (8.2)	17 (8.7)	19 (9.7)
Procymidone	69 (5.1)	62 (6.9)	59 (11.3)	86 (14.2)	86 (13.9)	63 (5.7)	59 (4.7)	62 (7.3)	51 (3.0)	65 (8.6)
Fenitrothion	101 (7.8)	82 (13.9)	71 (14.1)	99 (12.9)	86 (14.0)	79 (4.6)	74 (7.5)	92 (13.8)	72 (6.8)	–
Neburon	81 (9.5)	70 (8.1)	68 (9.5)	95 (14.6)	85 (11.2)	67 (5.8)	58 (4.2)	63 (8.3)	63 (8.9)	59 (11.2)
Chlorfenvinphos	–	–	–	–	–	23 (8.9)	25 (14.7)	23 (19.0)	–	–
Clofentezine	52 (8.6)	41 (9.9)	40 (14.7)	58 (14.3)	48 (6.7)	54 (7.4)	58 (8.5)	51 (8.4)	118 (9.6)	121 (13.9)
Lufenuron	61 (11.4)	56 (10.6)	47 (13.5)	68 (11.8)	47 (12.4)	51 (9.6)	47 (10.9)	52 (12.2)	42 (13.1)	75 (14.8)
Bromopropylate	15 (20.5)	–	–	–	–	–	–	–	–	–
Flufenoxuron	62 (3.9)	61 (7.8)	51 (5.0)	78 (13.8)	88 (13.7)	47 (1.7)	44 (5.8)	48 (9.1)	49 (10.3)	50 (4.5)
Trifluralin	36 (15.0)	36 (15.2)	32 (16.1)	38 (12.5)	–	35 (9.1)	36 (10.6)	48 (15.2)	–	–
α-Cypermethrin	ns	69 (9.0)	83 (11.4)	–	ns	58 (6.0)	59 (11.4)	68 (12.0)	82 (14.5)	–

ns, not studied; –, not detected.

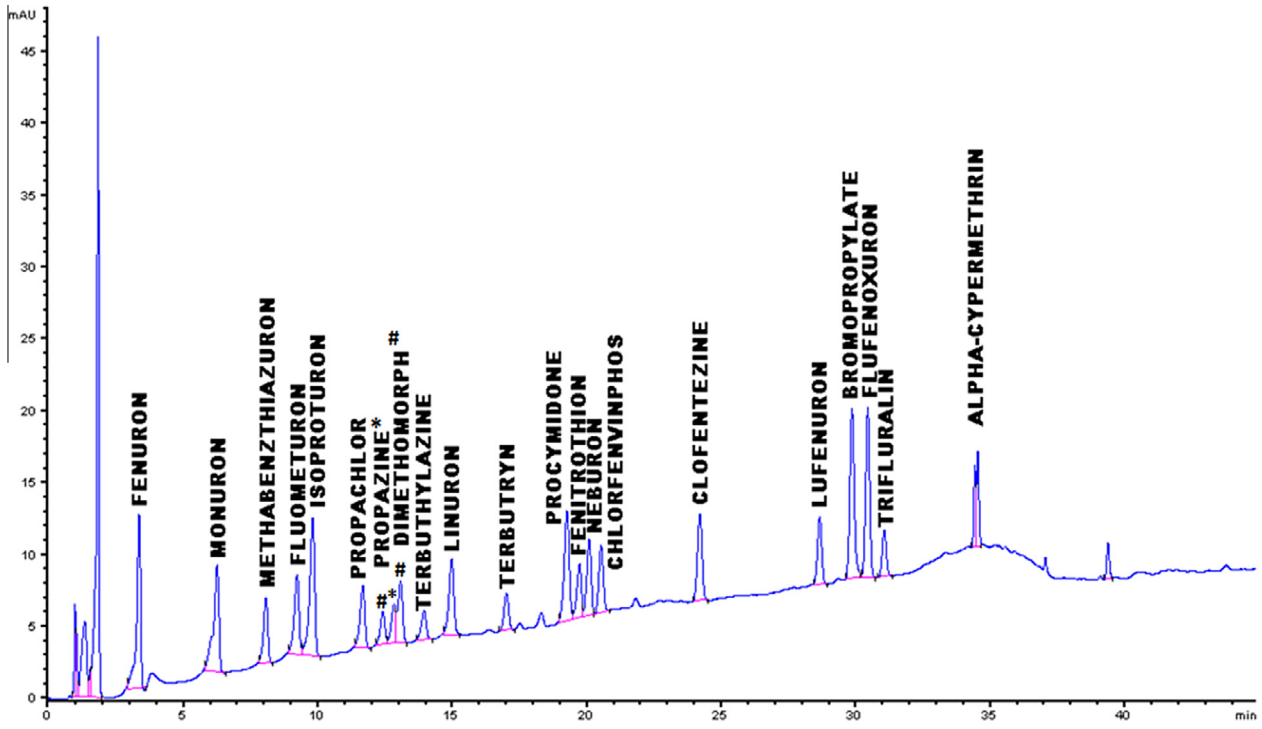

Fig. 1. Chromatogram obtained by RP-HPLC–DAD from the separated mixture of 21 pesticides at a concentration level of 0.8 μg mL^{-1}.

The recovery values for 64% of the studied analytes for extra virgin olive oil samples and 53% of the analytes for grapeseed oil samples ranged from 50% to 130%, which is in agreement with the most recent EU guidelines (Document (EU) N° SANCO/12571/2013). The relative standard deviation expressed as a percentage (% RSD) was less than 22.1% for all of the analytes. The % RSD values below 15% were observed for 67% and 86% of pesticides studied in extra virgin olive oil and grapeseed oil samples, respectively. These results demonstrate the acceptable performance of the method.

3.4. Extraction optimization

A sample preparation QuEChERS technique, which is based on the extraction with acetonitrile followed by an induced liquid–liquid partition after the addition of salts and a d-SPE clean-up step, was applied for sample extraction from extra virgin olive oils and grapeseed oil. The clean-up step is essential because it removes co-extractives found in the matrix, which might interfere in the subsequent analysis. Note that other d-SPE modifications have also

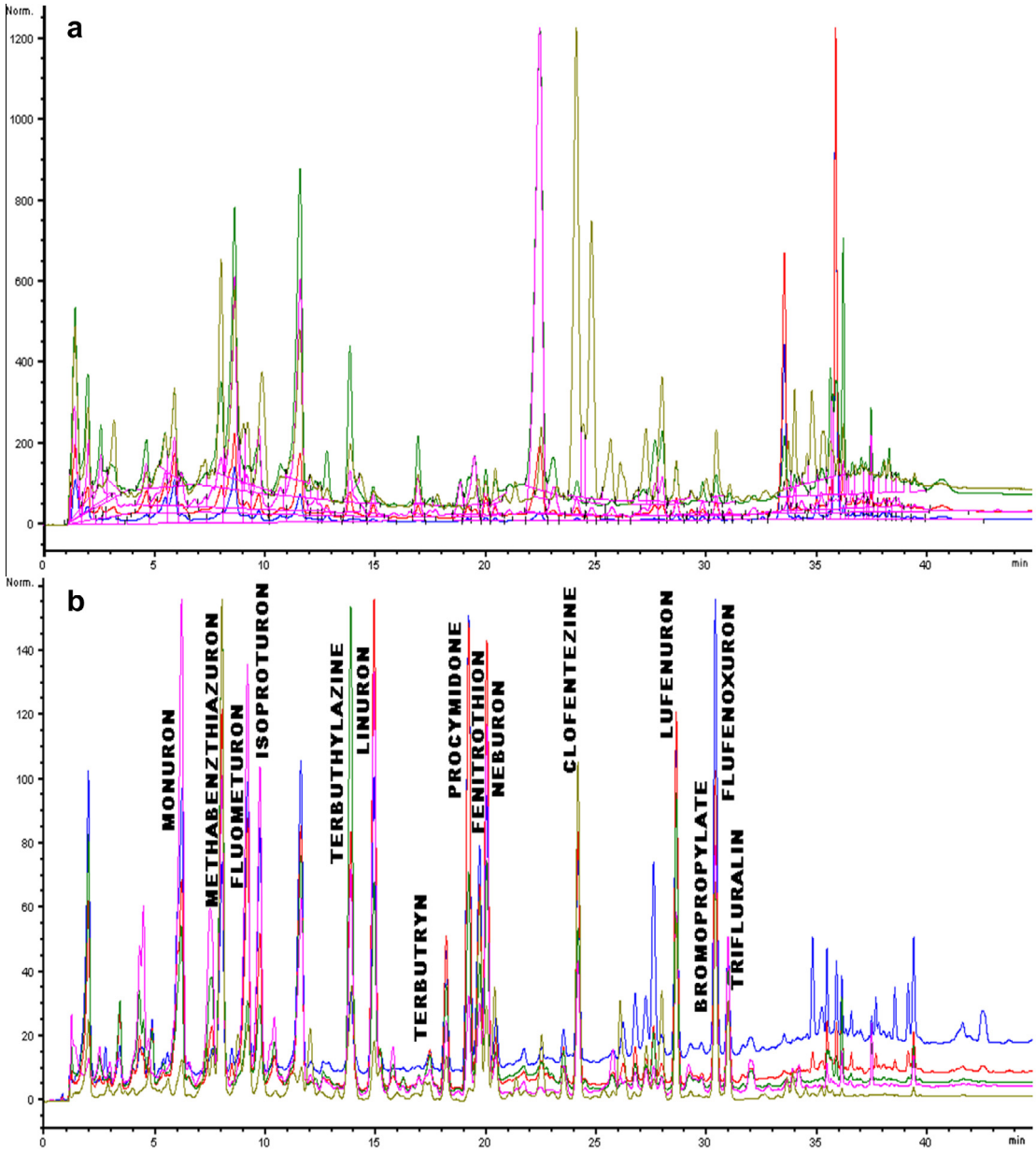

Fig. 2. Chromatograms obtained by RP-HPLC–DAD following the SPE/QuEChERS method from a spiked olive oil sample with a mixture of 17 pesticides at a concentration level of 1000 ng g^{-1}: a – only after SPE step; b – with Z-Sep sorbent for d-SPE clean-up step (propachlor and chlorfenvinphos were not detected).

been proposed through using other sorbents, such as octadecyl (C18), primary secondary amine (PSA), graphitized carbon black (GCB), chitin and their modifications, e.g., C18 + PSA (Anastassiades, Maštovská, & Lehotay, 2003; Cerqueira et al., 2014; Lehotay, de Kok, Hiemstra & van Bodegraven, 2005; Lehotay, Maštovská & Lightfield, 2005; Lehotay, Maštovská & Yun, 2005; Majors, 2007; Maštovská, Lehotay, & Anastassiades, 2005).

Z-sep is a new commercially available sorbent based on zirconium oxide, which is useful for the analysis of hydrophobic analytes in fatty matrices. Z-Sep Plus is a mixture of the two sorbents octadecyl (C18) and silica coated with zirconium dioxide with a ZrO_2/C18 ratio of 2/5 (Lozano et al., 2014). Following our previously published experiments, Z-Sep Plus was applied to the identification and quantitative analysis of pesticides from matrix components with a high lipid content in sunflower seed samples (Tuzimski & Rejczak, 2014). The d-SPE step with Z-Sep was selected to provide relatively high recoveries and clean-up efficiency (Figs. 2 and 3). The recovery experiments with five spiking levels were conducted for final method validation purposes.

At intermediate pH values, the zirconium surface contains neutral ZrOH groups, which at higher pH values, lose a proton to became ZrO^- (surface becomes negatively charged). At lower pH values, the ZrOH groups gain a proton to become $ZrOH_2^+$ (surface acquires a positive charge). Zirconia is an amphoteric oxide, and at different pH values, its surface can behave as a Brønsted acid or as Brønsted base. Zirconium dioxide has hard Lewis acid sites on its surface. These sites are present because zirconium (IV) has vacant 3d orbitals. Lewis acid sites can interact strongly with Lewis bases, such as R-SO_3^-, R-PO_3^- and R-COO^-, creating coordination bonds (Dai, Yang, & Carr, 2003; Thistlethwaite, Gee, & Wilson, 1996). Thistlethwaite et al. investigated the adsorption of oleic acid, and these authors concluded that adsorption at a low pH

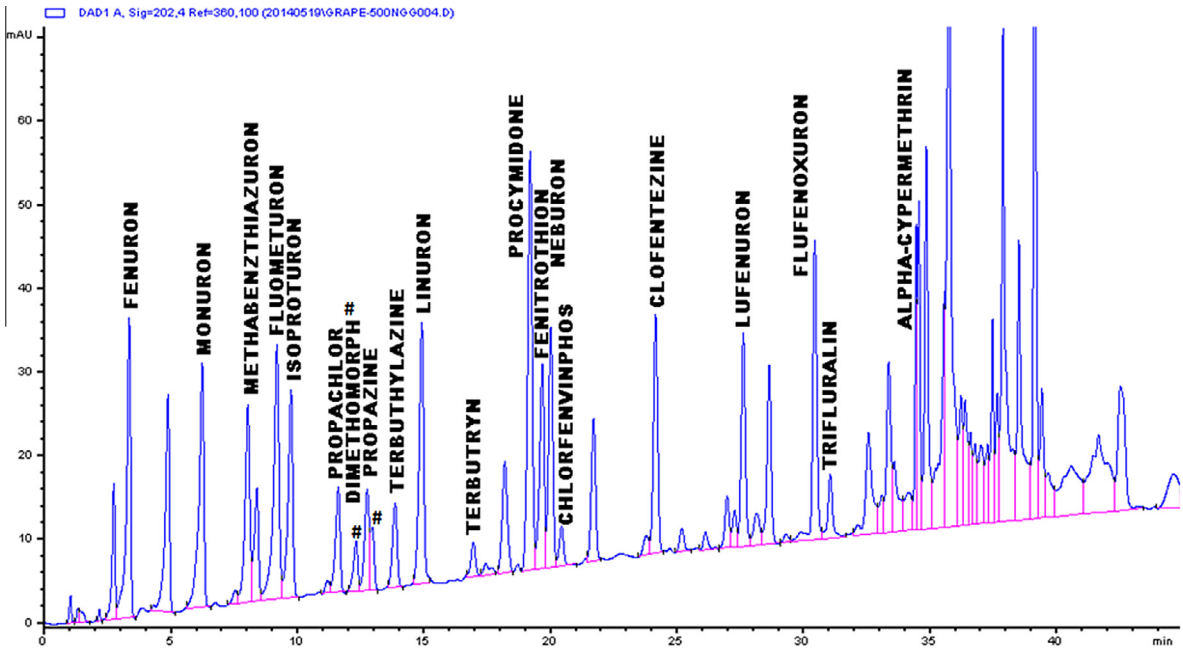

Fig. 3. Chromatogram obtained by RP-HPLC–DAD at 202 nm following the SPE/QuEChERS method (with Z-Sep sorbent for d-SPE clean-up step) from spiked grapeseed oil sample with a mixture of 21 pesticides at a concentration level of 500 ng g^{-1} (bromopropylate was not detected).

occurs due to electrostatic interactions between oleate anions and the positively charged zirconium dioxide surface (Thistlethwaite et al., 1996).

The goal of this study was to evaluate the use of Z-Sep sorbent in the d-SPE step of the proposed SPE/QuEChERS procedure for the extraction of selected analytes belonging to various classes of pesticides from edible oil samples and to determine these compounds by HPLC–DAD. Table 2 presents the recovery and RSD (%) results for the analytes at five concentration levels after treatment with the Z-Sep sorbent. From the 21 pesticides tested in this study, 16 for extra virgin olive oil samples and 14 for grapeseed oil samples provided acceptable recoveries (50–130%), especially at higher spiking levels. The termination of the experiments at the SPE step of the elaborated SPE/QuEChERS procedure precluded the correct identification of pesticides in the olive oil samples due to the high interference signal from the matrix components (Fig. 2a). The clean-up step with Z-Sep is crucial to eliminate a considerable number of impeding matrix components, and valid analyte identification and quantification (Figs. 2b and 3).

For analytes with various substituent characteristics, we observed different affinities for the zirconia sorbent. With an increase of the basic nature of the functional groups (according to Lewis theory), the affinity of the analytes for the Z-Sep sorbent, which was used for purification in the d-SPE step, increased. The strength of the interaction between the analyte and the sorbent can increase in the following series of substituents (Sigma–Aldrich, 2013): chloride < formate < acetate < sulphate < citrate < fluoride < phosphate < hydroxide. After applying the Z-Sep sorbent for the fortified samples of grapeseed oil, the average recoveries for chlorfenvinphos, which have a phosphate group in their structure, were low (ranging from 23% to 25%), while the pesticide was probably completely adsorbed on the zirconia sorbent during its extraction from the fortified olive oil samples. The average recoveries for trifluralin, which contains three fluorine atoms, were among the lowest of the pesticides tested possibly due to some of the stronger interactions with the Z-Sep sorbent. For other pesticides having planar structures with fluorine atoms, such as flufenoxuron and lufenuron, the average values of recoveries were approximately 60%.

For some analytes (e.g., fluometuron, terbuthylazine), the results of our experiments showed unacceptable indices for peak purity, which were below 99%, probably caused by matrix interferences, especially at the lowest level of fortification (75 ng/g). Average percentage recoveries (and % RSD) in these cases were not considered (in Table 2, marked as '–'). Bromopropylate was the analyte with a very low recovery (15%, only for the highest fortification level); these results may be related to its high non-polarity (log $P = 5.4$). For some more non-polar pesticides (e.g., trifluralin), we obtained lower average values of recovery. The average recovery value of propachlor in the fortified grapeseed oil samples was approximately 40%, whereas it was not determined in the extra virgin olive oil samples (Table 2).

The acceptable values of recovery for residue analysis of contaminants are between 60% and 120%, with an RSD of ±20%; however, depending on the complexity and the matrix, these values can be 50–130% with an RSD of 15% (Document (EU) N° SANCO/12571/2013; Ribani, Bottoli, Collins, Jardim, & Melo, 2004).

Considering that the use of the Z-Sep sorbent provided acceptable recovery values at the levels under evaluation, it may be recommended as a suitable sorbent for the cleaning step of the QuEChERS method for samples with high fat concentrations.

3.5. Application to natural samples of HPLC–DAD after the SPE/QuEChERS procedure

The validated method was applied to the analysis of pesticides in food products. The application of HPLC chromatography coupled with a diode array detector (DAD) allowed the determination of pesticides at concentrations of ng g^{-1} of the food samples. Alpha-cypermethrin was determined in extra virgin olive oil (Carbonell, Spain) at concentrations ranging from 17.45 to 23.05 ng g^{-1} ($n = 3$) of the product. Fenuron, dimethomorph and propazine were determined in grapeseed oil (Olitalia, Italy) at concentrations ranging from 2.24 to 3.08 ($n = 3$), from 2.95 to 3.01

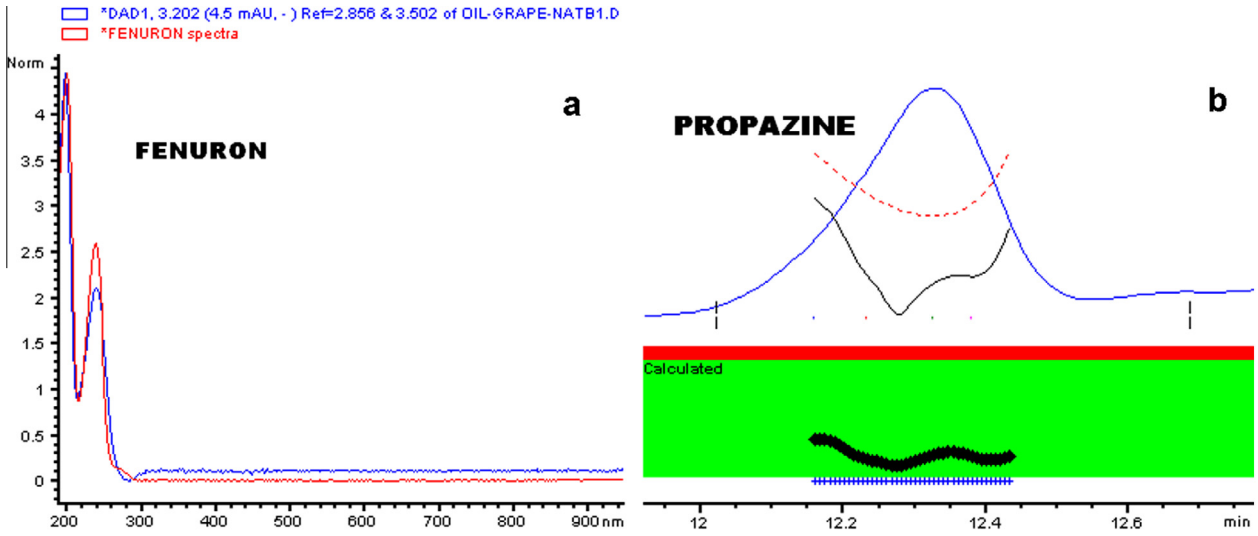

Fig. 4. Examples of: a – comparison of the UV–VIS spectrum of the fenuron standard (library) and the spectrum of the grapeseed oil sample by RP-HPLC–DAD experiments after SPE/QuEChERS (step B); b – purity of propazine peak found in grapeseed oil sample analysed by RP-HPLC–DAD after the SPE/QuEChERS procedure (step C).

($n = 2$) and from 0.46 to 1.12 ($n = 2$) ng g^{-1} of the product, respectively. In our experiments, the use of different detection wavelengths (202, 212, 222, 240 and 270 nm) allowed valid identification of analytes, even in the presence of some remaining interferences from the matrix. In the case when co-eluted matrix compounds had the same retention time as the analytes, these interferents demonstrated different UV–VIS absorbance spectra; it was, therefore, possible to identify and quantify the compounds of interest at the optimal wavelength for individual pesticides where the absorbance of matrix compounds was minimal. The exemplary UV–VIS spectra correlation for the identified fenuron in a natural grapeseed oil sample from Italy and its reference standard is presented in Fig. 4a. The peak purity for accurate pesticide determination in edible oil samples was also determined. The match factor equal to or greater than 990 was used to confirm the identity of analytes based on spectra collected and reference examples in the library. The results obtained by comparison of these spectra against each other were very close to a perfect 100% match ([(990:1000)·100%] = 99% or above). By comparing spectra, impurities with less than 1% could be identified. Therefore, the technique described offers an alternative to using a mass spectrometric detector for peak purity. The identification potential of the LC–MS or/and LC–MS/MS technique, however, is greater than that of the LC–DAD. Fig. 4b shows the peak purity for the propazine identified in the grapeseed oil sample selected from the market. In the case in which the calculated match factor is $\geqslant$990, the part below the peak is green (on a black and white printout, it is light grey) and the peak is considered pure (Fig. 4b).

4. Conclusions

For the first time, we suggest the application of methodology that relies on successive extractions of SPE/QuEChERS for the quantitative analysis of pesticide residues in grapeseed oil and extra virgin olive oils by HPLC–DAD. We also evaluated the zirconium dioxide-based sorbent in the d-SPE step of the QuEChERS technique to decrease the matrix effect from samples with a high fat content.

The method described allowed acquisition of acceptable results for the correct identification and quantitative analysis of pesticide residues in food samples. Because of the d-SPE clean-up step of the samples and the use of the Z-Sep sorbent in the elaborated procedure, we obtained high recovery values for most of the pesticides. The use of the zirconium dioxide-based sorbent (Z-Sep) enables purification of the sample and elimination of most of the interfering compounds in the matrix. The recovery values for most of the studied pesticides for extra virgin olive oil and grapeseed oil samples ranged from 50% to 130%, which were in agreement with the requirements of the European Union (Document (EU) N° SANCO/12571/2013).

The application of liquid chromatography coupled with diode array detection is less expensive solution compared with mass spectrometry (LC–MS) or tandem mass spectrometry (LC–MS/MS). As demonstrated, HPLC–DAD after the SPE/QuEChERS procedure may be successfully applied to the analysis of pesticide residues at the level of ng g^{-1} of edible oil samples.

References

Anagnostopoulos, C., & Miliadis, G. E. (2013). 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*, 1–10. http://dx.doi.org/10.1016/j.talanta.2013.03.051.

Anastassiades, M., Lehotay, S. J., et al. (2002) Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) approach for the determination of pesticide residues. 2002 European Pesticide Residues Workshop, EPRW, Rome, Book of Abstracts.

Anastassiades, M., Lehotay, S. J., Stainbaher, D., & Schenk, F. (2003a). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International, 86*, 412–431.

Anastassiades, M., Maštovská, K., & Lehotay, S. J. (2003b). Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides. *Journal of Chromatography A, 1015*(1-2), 163–184. http://dx.doi.org/10.1016/S0021-9673(03)01208-1.

AOAC. Official Method 2007.01. Pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate, 2007.

Cerqueira, M. B. R., Caldas, S. S., & Primel, E. G. (2014). New sorbent in the dispersive solid phase extraction step of quick, easy, cheap, effective, rugged, and safe for the extraction of organic contaminants in drinking water treatment sludge. *Journal of Chromatography A, 1336*, 10–22. http://dx.doi.org/10.1016/j.chroma.2014.02.002.

Chamkasen, N., Ollis, L. W., Harmon, T., Lee, S., & Mercer, G. (2013). Analysis of 136 pesticides in avocado using a modified QuEChERS method with LC–MS/MS and GC–MS/MS. *Journal of Agriculture and Food Chemistry, 61*, 2315–2329. http://dx.doi.org/10.1021/jf304191c.

Dai, J., Yang, X., & Carr, P. W. (2003). Comparison of the chromatography of octadecyl silane bonded silica and polybutadiene-coated zirconia phases based on a diverse set of cationic drugs. *Journal of Chromatography A, 1005*, 63–82. http://dx.doi.org/10.1016/S0021-9673(03)00929-4.

Document (EU) N° SANCO/12571/2013. Chemicals, contaminants, pesticides. Guidance document on analytical quality control and validation procedures

for pesticide residues analysis in food and feed. SANCO/12571/2013, Supersedes SANCO/12495/2011, Implemented by 01/01/2014.

Eitzer, B. D., Hammack, W., & Filigenzi, M. (2014). Interlaboratory comparison of a general method to screen foods for pesticides using QuEChERs extraction with high performance liquid chromatography and high resolution mass spectrometry. *Journal of Agriculture and Food Chemistry, 62*, 80–87. http://dx.doi.org/10.1021/jf405128.

EN 15662:2008. Foods of plant origin – determination of pesticide residues using GC–MS and/or LC–MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE QuEChERS-method, 2008.

Frenich, A. G., Fernández, M. M. M., Moreno, L. D., Vidal, J. L. M., & Gutiérrez, N. L. (2012). Multiresidue pesticide analysis of tuber and root commodities by QuEChERS extraction and ultra-performance liquid chromatography coupled to tandem mass spectrometry. *Journal of AOAC International, 95*, 1319–1330. http://dx.doi.org/10.5740/jaoacint.SGE_GarridoFrenich.

García-Reyes, J. F., Ferrer, C., Gomez-Ramos, M. J., Molina-Díaz, A., & Fernández-Alba, A. R. (2007). Determination of pesticide residues in olive oil and olives. *Trends in Analytical Chemistry, 26*, 239–251. http://dx.doi.org/10.1016/j.trac.2007.01.004.

Geis-Asteggiante, L., Lehotay, S. J., & Heinzen, H. (2012). Effects of temperature and purity of magnesium sulfate during extraction of pesticide residues using the QuEChERS method. *Journal of AOAC International, 95*, 1311–1318. http://dx.doi.org/10.5740/jaoacint.SGE_Geis-Asteggiante.

Gilbert-López, B., García-Reyes, J. F., Fernández-Alba, A. R., & Molina-Díaz, A. (2010). Evaluation of two sample treatment methodologies for large-scale pesticide residue analysis in olive oil by fast liquid chromatography–electrospray mass spectrometry. *Journal of Chromatography A, 1217*, 3736–3747. http://dx.doi.org/10.1016/j.chroma.2010.04.025.

Gilbert-López, B., García-Reyes, J. F., & Molina-Díaz, A. (2009). Sample treatment and determination of pesticide residues in fatty vegetable matrices: A review. *Talanta, 79*, 109–128. http://dx.doi.org/10.1016/j.talanta.2009.04.022.

Guan, W., Li, Z., Zhang, H., Hong, H., Rebeyev, N., Ye, Y., et al. (2013). Amine modified graphene as reversed-dispersive solid phase extraction materials combined with liquid chromatography–tandem mass spectrometry for pesticide multi-residue analysis in oil crops. *Journal of Chromatography A, 1286*, 1–8. http://dx.doi.org/10.1016/j.chroma.2013.02.043.

Koesukwiwat, U., Lehotay, S. J., & Leepipatpiboon, N. (2011). Fast, low-pressure gas chromatography triple quadrupole tandem mass spectrometry for analysis of 150 pesticide residues in fruits and vegetables. *Journal of Chromatography A, 1218*, 7039–7050. http://dx.doi.org/10.1016/j.chroma.2011.07.094.

Koesukwiwat, U., Lehotay, S. J., Maštovská, K., Dorweiler, K. J., & Leepipatpiboon, N. (2010). Extension of the QuEChERS method for pesticide residues in cereals to flaxseeds, peanuts and doughs. *Journal of Agriculture and Food Chemistry, 58*, 5950–5958. http://dx.doi.org/10.1021/jf902988b.

Koesukwiwat, U., Lehotay, S. J., Miao, S., & Leepipatpiboon, N. (2010). High throughput analysis of 150 pesticides in fruits and vegetables using QuEChERS and low-pressure gas chromatography–time-of-flight mass spectrometry. *Journal of Chromatography A, 1217*, 6692–6703. http://dx.doi.org/10.1016/j.chroma.2010.05.012.

Lacina, O., Zachariasova, M., Urbanova, J., Vaclavikova, M., Cajka, T., & Hajslova, J. (2012). Critical assessment of extraction methods for the simultaneous determination of pesticide residues and mycotoxins in fruits, cereals, spices and oil seeds employing ultra-high performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 1262*, 8–18. http://dx.doi.org/10.1016/j.chroma.2012.08.097.

Lehotay, S. J., de Kok, A., Hiemstra, M., & van Bodegraven (2005a). Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. *Journal of AOAC International, 88*, 595–614 (PMID: 15859089).

Lehotay, S. J., Maštovská, K., & Jong, Y. (2005b). Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrices. *Journal of AOAC International, 88*, 630–638.

Lehotay, S. J., Maštovská, K., & Lightfield, A. R. (2005c). Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. *Journal of AOAC International, 88*, 615–629.

Lehotay, S. J., Maštovská, K., & Yun, S. J. (2005d). Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. *Journal of AOAC International, 88*, 630–638.

Lehotay, S. J., Son, K. A., Kwon, H., Koesukwiwat, U., Fu, W., Maštovská, K., et al. (2010). Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *Journal of Chromatography A, 1217*, 2548–2560. http://dx.doi.org/10.1016/j.chroma.2010.01.044.

Lozano, A., Rajski, Ł., Uclés, S., Belmonte-Valles, N., Mezcua, M., & Fernández-Alba, A. R. (2014). Evaluation of zirconium dioxide-based sorbents to decrease the matrix effect in avocado and almond multiresidue pesticide analysis followed by gas chromatography tandem mass spectrometry. *Talanta, 118*, 68–83. http://dx.doi.org/10.1016/j.talanta.2013.09.053.

Majors, R. E. (2007). QuEChERS – A new sample preparation technique for multiresidue analysis of pesticides in foods and agricultural samples. *LCGC North America, 25*(5), 436–446.

Majors, R. E. (2013). *Sample preparation fundamentals for chromatography*. Canada: Agilent Technologies.

Maštovská, K., Lehotay, S. J., & Anastassiades, M. (2005). Combination of analyte protectants to overcome matrix effects in routine GC analysis of pesticide residues in food matrices. *Analytical Chemistry, 77*(24), 8129–8137. http://dx.doi.org/10.1021/ac0515576.

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/EEC, (2005). *Official Journal of the European Union, L70*, 1–16. http://ec.europa.eu/sanco_pesticides/public/index.cfm (accessed on 22.10.11).

Ribani, M., Bottoli, C. B. G., Collins, C. H., Jardim, I., & Melo, L. F. C. (2004). Validação em métodos cromatográficos e eletroforéticos. *Química Nova, 27*, 771–780.

Sapoznhnikova, Y., & Lehotay, S. J. (2013). Multi-class, multi-residue analysis of pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and novel flame retardants in fish using fast, low-pressure gas chromatography–tandem mass spectrometry. *Analytica Chimica Acta, 758*, 80–92. http://dx.doi.org/10.1016/j.aca.2012.10.034.

Savant, R. H., Banerjee, K., Utture, S. C., Patil, S. H., Dasgupta, S., Ghaste, M. S., et al. (2010). Multiresidue analysis of 50 pesticides in grape, pomegranate, and mango by gas chromatography-ion trap mass spectrometry. *Journal of Agriculture and Food Chemistry, 58*, 1447–1454. http://dx.doi.org/10.1021/jf903398f.

Swartz, M. E., & Krull, I. S. (1997). *Analytical method development and validation*. New York: Marcel Dekker.

Thistlethwaite, P. J., Gee, M. L., & Wilson, D. (1996). Diffuse reflectance infrared fourier transform spectroscopic studies of the adsorption of oleate/oleic acid onto zirconia. *Langmuir, 12*, 6487–6491. http://dx.doi.org/10.1021/la9603939.

Tuzimski, T. (2009). Application of SPE–HPLC–DAD and SPE–HPTLC–DAD to the analysis of pesticides in lake water. *Journal of Planar Chromatography – Modern TLC, 22*, 235–240. http://dx.doi.org/10.1556/JPC.22.2009.4.1.

Tuzimski, T., & Rejczak, T. (2014). Determination of pesticides in sunflower seeds by high-performance liquid chromatography coupled with a diode array detector. *Journal of AOAC International, 97*(4), 1012–1020. http://dx.doi.org/10.5740/jaoacint.SGETuzimski.

Tuzimski, T., & Sobczyński, J. (2009). Application of HPLC-DAD and TLC-DAD after SPE to the quantitative analysis of pesticides in water samples. *Journal of Liquid Chromatography & Related Technologies, 32*, 1241–1258. http://dx.doi.org/10.1080/10826070902854391.

Zheng, H.-B., Zhao, Q., Mo, J.-Z., Huang, Y.-Q., Luo, Y.-B., Yu, Q.-W., et al. (2013). Quick, easy, cheap, effective, rugged and safe method with magnetic graphitized carbon black and primary secondary amine as adsorbent and its application in pesticide residue analysis. *Journal of Chromatography A, 1300*, 127–133. http://dx.doi.org/10.1016/j.chroma.2013.04.040.