ALLEGATO A
VERBALE 21/12/2021

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| 1 | Linearità della risposta strumentale di un metodo analitico: definizione, allestimento curva, scelta del tipo di retta, parametri valutati. | Quali caratteristiche deve avere, in base alla normativa di riferimento, un metodo di screening per ricerca di sostanze anabolizzanti? |
| 2 | La precisione di un metodo analitico: definizione, tipi di precisione, allestimento prove, parametri valutati. | Significato del termine CCalfa, campo di applicazione, normativa di riferimento e differenze nel calcolo tra sostanze di Cat A e Cat B. |
| 3 | Standard degli analiti ricercati: caratteristiche in base allo scopo del metodo, certificazione degli standard. Preparazione delle soluzioni madri di standard e verifica della stabilità. | Quali caratteristiche deve avere, in base alla normativa di riferimento, un metodo di conferma per ricerca di farmaci non autorizzati? |
| 4 | Recupero di un metodo analitico: definizione, allestimento prove, parametri valutati. | Significato del termine CCbeta e campo di applicazione per farmaci di Cat A e Cat B. |
| 5 | Determinazione quantitativa di una micotossina in alimenti: metodi di quantificazione, criteri di identificazione e di prestazione. | Descrivere l'indice di prestazione più importante per la validazione dei metodi di screening e indicare le modalità di valutazione |
| 6 | Approcci alla stima dell'incertezza di misura associata al risultato di una misura e suo utilizzo nella valutazione della conformità di un campione per una micotossina | Caratteristiche dello standard interno nei metodi di screening e differenze nella scelta quando invece esso si utilizza in metodi quantitativi in spettrometria di massa. |
| 7 | Quali caratteristiche deve avere un materiale di riferimento e a cosa serve? | Quali caratteristiche deve avere uno standard interno utilizzato per scopi quantitativi? |

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| 8 | Strumenti per assicurare la qualità dei risultati nelle analisi di conferma per ricerca di residui di sostanze vietate o contaminanti in alimenti. | Validazione secondaria di un metodo normato. |
| 9 | Criteri di rendimento dei metodi analitici destinati alla ricerca di residui di farmaci o contaminanti in alimenti | Costruzione della curva di taratura con il metodo dello standard interno |
| 10 | Modelli di stima dell'incertezza di misura associata al risultato di una prova. | Costruzione della curva di taratura con il metodo delle aggiunte standard |
| 11 | Validazione primaria di un metodo interno sviluppato dal laboratorio: pianificazione e svolgimento | Come si assicura la qualità di un risultato nelle analisi di routine di screening? |
| 12 | Accuratezza di un metodo analitico: cosa rappresenta e come si esprime. | Come si controlla il mantenimento delle prestazioni di un metodo di analisi durante la sua applicazione in routine? |
| 13 | Come si assicura la qualità di un risultato nelle analisi di routine di conferma? | Rivelatori per HPLC: differenze nelle caratteristiche e applicazioni tra DAD, FLD E MS/MS |
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| 15 | Criteri per l'identificazione di un analita in HPLC-DAD e FLD nei metodi di conferma | La norma 17025: requisiti dell'operatore addetto alle prove |
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| 19 | Il campione ufficiale legale: definizione e caratteristiche. | Come si costruisce una carta di controllo e a cosa serve. |
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# 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

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ABSTRACT

The aim of this research was to adapt the QuEChERS method for routine pesticide multiresidue analysis in edible vegetable oil samples using gas chromatography coupled to tandem mass spectrometry (GC–MS/MS). Several clean-up approaches were tested: (a) D-SPE with Enhanced Matrix Removal-Lipid (EMR-Lipid™); (b) D-SPE with PSA; (c) D-SPE with Z-Sep; (d) SPE with Z-Sep. Clean-up methods were evaluated in terms of fat removal from the extracts, recoveries and extraction precision for 213 pesticides in different matrices (soybean, sunflower and extra-virgin olive oil). The QuEChERS protocol with EMR-Lipid d-SPE provided the best reduction of co-extracted matrix compounds with the highest number of pesticides exhibiting mean recoveries in the 70–120% range, and the lowest relative standard deviations values (4% on average). A simple and rapid (only 5 min) freeze-out step with dry ice ($CO_2$ at −76 °C) prior to d-SPE clean-up ensured much better removal of co-extracted matrix compounds in compliance of the necessity in routine analysis. Procedural Standard Calibration was established in order to compensate for recovery losses of certain pesticides and possible matrix effects. Limits of quantification were 10 μg kg$^{-1}$ for the majority of the pesticides. The modified methodology was applied for the analysis of different 17 oil samples. Fourteen pesticides were detected with values lower than MRLs and their concentration ranged between 10.2 and 156.0 μg kg$^{-1}$.



## 1. Introduction

Nowadays, the olive crop as well as the soybean and the sunflower crops demand a wide range of insecticides (organophosphorus, carbamate, organochlorine, pyrethroid and other chemical classes) and fungicides (phthalimides, triazoles, imidazoles, sulfamides and others chemical classes) consumption. Herbicides (sulfonylurea and diphenyl ethers) is another type of pesticide commonly used in these groves. Thus, pesticide residues may occur in the final vegetable oil products. According to the European Food Safety Authority (EFSA) [1], out of the 794 samples of olive oil analysed in 2012, 175 samples (22%) contained one or several pesticides in measurable concentrations. Residues above the MRL were detected for pendimethalin (0.2%), terbuthylazine (1%), endosulfan (RD) (0.2%), famoxadone (0.2%) and fenthion (0.5%). Since olive oil was not included in other EU-coordinated monitoring programs, no comparison of the 2012 results with recent years is possible. However, pesticide residues were reported by different authors between 2013 and 2016 [2–4]. The results showed that the incidence and levels of pesticides were higher in virgin olive oil than in refined olive oil. Pesticide residues were also detected in soybean [5,6] and sunflower oil [7].

Regardless of the pesticide-residue determination in edible oils, extraction and clean-up remains the main limiting step. A compilation of applications involving additional clean-up steps after solvent extraction when dealing with edible oil samples is given in several reviews [8–11], most of them related to the selective determination of pesticide residues in edible oil, but only few of these protocols were proposed for a wide-scope multiresidue analysis of pesticides in this complicated matrix [3,12–15].

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Mass spectrometric techniques (i.e. tandem mass spectrometry using triple quadrupole or ion-trap instruments) in combination with gas chromatography (GC) or liquid chromatography (LC) are the techniques of choice for pesticide residue analysis in edible oil due to their high selectivity, sensitivity and throughput. The development of multiresidue methods for the determination of pesticides in edible oil samples at low levels is yet a challenging issue to which much effort in separation of lipid material from extracts has being applied. An exhaustive clean-up of the sample extract is necessary in order to avoid high amount of fat residues in the final extract, which would decrease the column lifetime and the maintenance of the instrument in working conditions. Since lipids deposits on the source, the analyte sensitivity is highly reduced too due to ion suppression. Difficulty it is focusing on remove interfering lipids without losing certain analytes considering that many of the target pesticides are fat-soluble non-polar compounds (e.g., organochlorine, pyrethroids) and they tend to remain in the fat. Liquid partitioning between the oil matrix dissolved in petroleum ether or *n*-hexane saturated with acetonitrile was one of the most reported methods for the isolation of pesticides in edible oils [10,14,16–18]. In these procedures pesticides are partitioned into the polar acetonitrile layer while the lipids are removed in the non-polar petroleum ether or *n*-hexane phase. Usually, liquid partitioning has been used combined with GPC [10,16,17] or SPE purification using florisil cartridges [14,18]. However, these procedures are laborious, time-consuming and require large amounts of potentially hazardous solvents. MSPD [14,18] has often been combined with liquid partitioning overcoming these pitfalls with satisfactory results. Current developments involve the use of extraction methods based on modifications to the QuEChERS procedure [3,4,12,14,15,19] as originally reported by Anastassiades et al. [20]. These methods result in advantages such as low solvent consumption, simplicity, flexible approach and high workflow. They involve initial liquid-liquid partition with acetonitrile and then cleaning up by dispersive-solid-phase extraction (d-SPE) in which the extract is mixed with different sorbents combination (PSA + C18 + GCB) and anhydrous $MgSO_4$. Additionally, a freezing out step prior to d-SPE has been used for further clean-up of the edible oil extract. Anagnostopoulos et al. [3] proposed a method for 102 pesticides in olive oil and olives by gas and liquid chromatography coupled to tandem mass spectrometry (GC–MS/MS and LC–MS/MS) using this simple combination. Although all analytical parameters evaluated were excellent, the main drawback of this method was the significant matrix effect for most compounds.

Until now, apart from GPC, no sample preparation has been able to eliminate matrix effect which is caused by co-eluting compounds influencing ionization and, thus, signal intensity [3,14,15,18,19]. In this sense, reported multiresidue methods for pesticides in edible oils by gas and liquid chromatography/mass spectrometry show a significant or strong matrix effect for most compounds which hampers sensitivity. Recently, the use of zirconia sorbent materials (Z-Sep, Z-Sep* and Yttria-stabilized zirconium dioxide nanoparticles) for removal of lipids from fatty samples improved matrix clean-up compared to PSA, C18 and GCB sorbents [21–24], but also resulted in more analyte loss, especially for hydroxyl and carboxylic acid-containing compounds. Preliminary results with the novel sorbent material Agilent Bond Elut Enhanced Matrix Removal-Lipid (EMR-Lipid) are promising for highly selective lipid removal without unwanted analyte retention [25–29]. Application studies involving QuEChERS extraction followed by EMR-Lipid dSPE and polish salts indicate that this new product delivers fast, effective and robust sample preparation with the most complete matrix removal available for multiresidue analysis of pesticides in avocado by GC–MS/MS [27] and LC–MS/MS [28]. The performance of EMR-Lipid has also been tested for other representative high lipid content samples including bovine liver [25] and salmon [29]. Effective clean-up of EMR-Lipid and better precision results were obtained compared to alternative QuEChERS procedures.

The objective of this study was the evaluation and development of a sensitive, reliable and robust multiresidue analytical method, based on QuEChERS methodology followed by GC–MS/MS for the simultaneous analysis of an extended list of 213 pesticides in edible oils. Several clean-up methods were evaluated concentrating on efficient clean-up and the highest number of pesticides satisfying the recovery and precision criteria. The tested methods were: modified QuEChERS using d-SPE with EMR-Lipid (a), PSA (b), Z-Sep (c) as well as modified QuEChERS using SPE with Z-Sep (d) and EMR-Lipid (e). A simple and rapid freeze-out step with dry ice ($CO_2$ at −76 °C) for a previous removal of lipids were done before the d-SPE or the SPE clean-up.

## 2. Experimental

### 2.1. Reagents and materials

All pesticide standards of high purity were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Riedel-de Haën (Selze, Germany) and were stored at −30 °C. Stock standard solutions of each pesticide were prepared in acetonitrile and ethyl acetate at concentrations of 1000–2000 mg $L^{-1}$ and were stored in amber screw-capped glass vials in the dark at −20 °C. Individual standard solutions for optimisation and three standard-mix solutions for calibration were prepared from the stock standards.

Ultra-gradient HPLC-grade acetonitrile was obtained from Sigma-Aldrich (Steinheim, Germany). Trisodium citrate dihydrate was purchased from Fluka (Steinheim, Germany). Sodium chloride was purchased from J.T. Baker (Deventer, The Netherlands). Disodium hydrogencitrate sesquihydrate was obtained from Sigma-Aldrich (Steinheim, Germany). Anhydrous magnesium sulphate was supplied by Panreac (Barcelona, Spain). EMR-Lipid was purchased from Agilent Technologies (Santa Clara, CA, USA). PSA and Z-Sep were obtained from Supelco (Bellefonte, PA). A Milli-Q-Plus ultra-pure water system from Milli-pore (Milford, MA, USA) was used throughout the study to obtain the ultra-pure grade water used during the analyses. Formic acid (98% purity) was purchased from Fluka (Buchs, Switzerland). Dry ice was supplied from technical services (University of Almería).

### 2.2. Equipment

For GC analysis, an Agilent 7000GC (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent 7693B autosampler, 7890A GC system, and an Agilent 7000 series GC–MS/MS triple quadrupole system (Agilent Technologies) were used. Data acquisition and processing were developed using Agilent MassHunter QQQ Quantitative Analysis B.05.00 software. Analyses on GC–MS/MS were performed on an Agilent Ultra Inert GC column HP-5MS UI (15 m long × 0.25 mm i.d. × 0.25 μm film thickness). The samples were injected using a multimode injector inlet in cold splitless mode through an ultra-inert inlet liner with a glass wool frit from Agilent: the injection volume was 2 μL. The injector temperature was kept at 80 °C during the solvent evaporation stage (0.1 min) and then ramped up to 300 °C at 600 °C $min^{-1}$. This temperature was maintained for 20 min. Helium (99.999% purity) was used as the carrier and quenching gas, and nitrogen (99.999% purity) as the collision gas. The oven temperature program was as follows: 70 °C for 1 min, up to 150 °C at 50 °C $min^{-1}$, then up to 200 °C at 6 °C $min^{-1}$ and finally up to 280 °C at 16 °C $min^{-1}$, and then maintained for 4.07 min. The total run time was 20 min with 3 additional minutes for backflushing at 280 °C; the pressure was maintained at 60 psi. The system worked at constant pressure (14.1 psi) with

the setting of the retention time lock employing trifluralin as the locking compound at 5.82 min. Both the transfer line and the ion source were kept at 280 °C. The ion source and quadrupole analyser temperatures were fixed at 280 °C and 150 °C, respectively. The collision gas flow was 1.5 mL $min^{-1}$ and the quenching gas flow was 2.25 mL $min^{-1}$.

For the prior screening, an Agilent 7890A gas chromatograph was used. The samples were injected using a multimode injector inlet in a splitless mode with an ultra-inert inlet liner, with a glass wool frit obtained from Agilent. The injection volume was 2 μL and was carried out at 280 °C. Helium (99.999% purity) was used as the carrier gas. The GC separation was performed using two fused silica HP-5MS UI capillary column of 15 m × 0.25 mm inner diameter and a film thickness of 0.25 μm (from Agilent) connected by a capillary flow technology (CFT) union. The oven temperature was programmed as follows: 60 °C for 1 min; 40 °C $min^{-1}$ to 120 °C, and finally up to 310 °C at 5 °C $min^{-1}$. The total run time was 40.5 min with two additional minutes for backflushing at 310 °C. The gas chromatography system was connected to a quadrupole time-of-flight (QTOF) mass spectrometer Agilent 7200 (Agilent Technologies, Santa Clara, USA), operating in electron-impact ionization (EI) mode (70 eV). The ion source and quadrupole analyser temperatures were set at 280 and 150 °C, respectively. TOF-MS was operated at 4 GHz (12 000 FWHM), with acquisition over the mass range of *m/z* 45–550.

### 2.3. Optimization of GC–MS/MS parameters

For the optimisation of the MS parameters, all pesticides were monitored in full scan mode in the 50–550 *m/z* range, selecting the precursor ion (PI). In the PI experiment, the PI was fragmented and the best product ions were selected. Finally, the CE was optimized for each transition in an SRM experiment. For the identification of analytes, the EU guidelines for GC–MS/MS analysis were considered (Document N° SANTE/11945/2015) [30]. The transitions obtained and collision energies chosen are shown in Table S1 (in the Supplementary material section). The most intense transition was selected as the quantifier transition and the second most intense as the qualifier transition. A thirty-nine time-segment method was created to obtain adequate sensitivity and S/N ratio. The solvent delay was 2 min.

### 2.4. Spiking procedure

For recovery studies, the organic extra virgin olive, sunflower and soybean oil samples obtained from a green store from Almería (South of Spain) were spiked with the standard solution at the appropriate levels. A prior analysis of the samples was performed in order to ensure that they did not contain any of the target compounds, and that samples were selected as blank for spiking, calibration curves and recovery studies. The spiked sample was stirred for 60 min to let the ethyl acetate evaporate before sample extraction and d-SPE clean-up. The final spiking concentration levels in the recovery study samples were 10 and 20 μg $kg^{-1}$.

### 2.5. Sample preparation procedure

For the extraction, 15 g of sample was weighed in a 50 mL PTFE centrifuge tube. Then, 15 mL of acetonitrile and 15 μL of a mix of surrogate standards with 10 mg $L^{-1}$ – triphenyl phosphate (TPP), dichlorvos-d6, malathion-d10 and carbendazim-d3- were added and the samples were shaken in an automatic axial extractor (AGYTAX®, Cirta Lab. S.L., Spain) for 4 min. Afterwards, 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquihydrate were added and the samples were again shaken in the automatic axial extractor for 4 min. The extract was then centrifuged (2860 × g) for 5 min. For the freezing-out clean-up, an 10 mL aliquot of the supernatant was transferred to a 15 mL PTFE centrifuge tube and placed in a polystyrene box filled with dry ice ($CO_2$ at −76 °C) for 5 min. 5 mL of the extract were then separated from the precipitate using a Pasteur pipette and these were used for the following clean-up methodologies: (a) D-SPE with EMR-Lipid. 5 mL of water had been added to the EMR-Lipid d-SPE tube prior to addition of the freezing-out extract. Subsequently, the mixture was vigorously shaken by vortex for 60 s to disperse sample and then centrifuged (2860 × g) for 5 min. After that, a 5 mL aliquot of the above extract was transferred to a 15 mL EMR-Lipid polish tube containing 2 g salts (1:4, NaCl:$MgSO_4$). The contents in the tube were vortexed for 60 s and centrifuged at 2860 × g for 5 min; (b) D-SPE with PSA (125 mg) + $MgSO_4$ (750 mg). The contents in the tube were vortexed for 60 s and centrifuged at 2860 × g for 5 min; (c) D-SPE with Z-Sep (125 mg) + $MgSO_4$ (750 mg). The contents in the tube were vortexed for 60 s and centrifuged at 2860 × g for 5 min; (d) SPE with Z-Sep (45 mg). 1 mL aliquot of the extract was percolated through a SPE cartridge containing 45 mg of Z-Sep at a flow rate of 0.8 mL $min^{-1}$ in the extraction manifold system at a pressure of 1.09 psig for the vacuum system (the SPE cartridge had been preconditioned prior to percolation with 1 mL of acetonitrile). Finally, a 2 mL aliquot (1 mL for approach (d)) was transferred into a glass test tube and acidified by adding 20 μL of 5% formic acid in acetonitrile (v/v) to stabilize base-sensitive pesticides. In (b)–(d) strategies, 10 g of sample and 10 mL of acetonitrile were used for the extraction. In these cases, only 2.5 mL of the extract were obtained after using a 5 mL aliquot of acetonitrile extract in the freezing-out step. Prior to injection into the GC–MS/MS system, a 50 μL aliquot was evaporated under a gentle nitrogen stream and reconstituted with 50 μL ethyl acetate and 2 μL lindane-d6 (1.25 mg $L^{-1}$), which were added to each vial as the injection control standard. With this treatment, 1 mL of sample extract represents 1 g of sample. The detailed workflow for the best procedure (approach a) is shown in Fig. 1.

### 2.6. Method validation

A validation study was performed in terms of apparent recovery, linearity, limit of quantitation, matrix effects, as well as intra-day and inter-day precision.

The recoveries and precision of the extraction method were determined as the average of five spiked matrix blanks analyzed at concentration levels of 10 and 20 μg $kg^{-1}$.

The LOQ was set as the lowest spiking level that can be quantified with acceptable accuracy and precision, as described in Document No. SANTE/11945/2015 [30].

In order to demonstrate linearity, five sets of calibration curves with six concentration points (from 10 to 500 μg $kg^{-1}$ in the sample) were prepared by spiking the analytes before the extraction procedure to obtain a more realistic concentration in the final extracts. Procedural Standard Calibration [30] was applied to compensate analyte losses during sample preparation and variability in the matrix effects.

The repeatability of the method was estimated by determining the inter- and intra-day relative standard deviation (RSD, %) by the repeated analysis (n = 5) of a spiked oil sample at the 10 and 20 μg $kg^{-1}$ levels, from run-to-run over 1 and 5 days, respectively.

### 2.7. Studied pesticides

The pesticides included in this study were selected based on their relevance in terms of high probability of being found in edible oil samples. Most of the studied compounds are low- and medium-polarity pesticides (pKow > 3) which facilitates the contamination of edible oil matrices. Various studies revealed the presence of

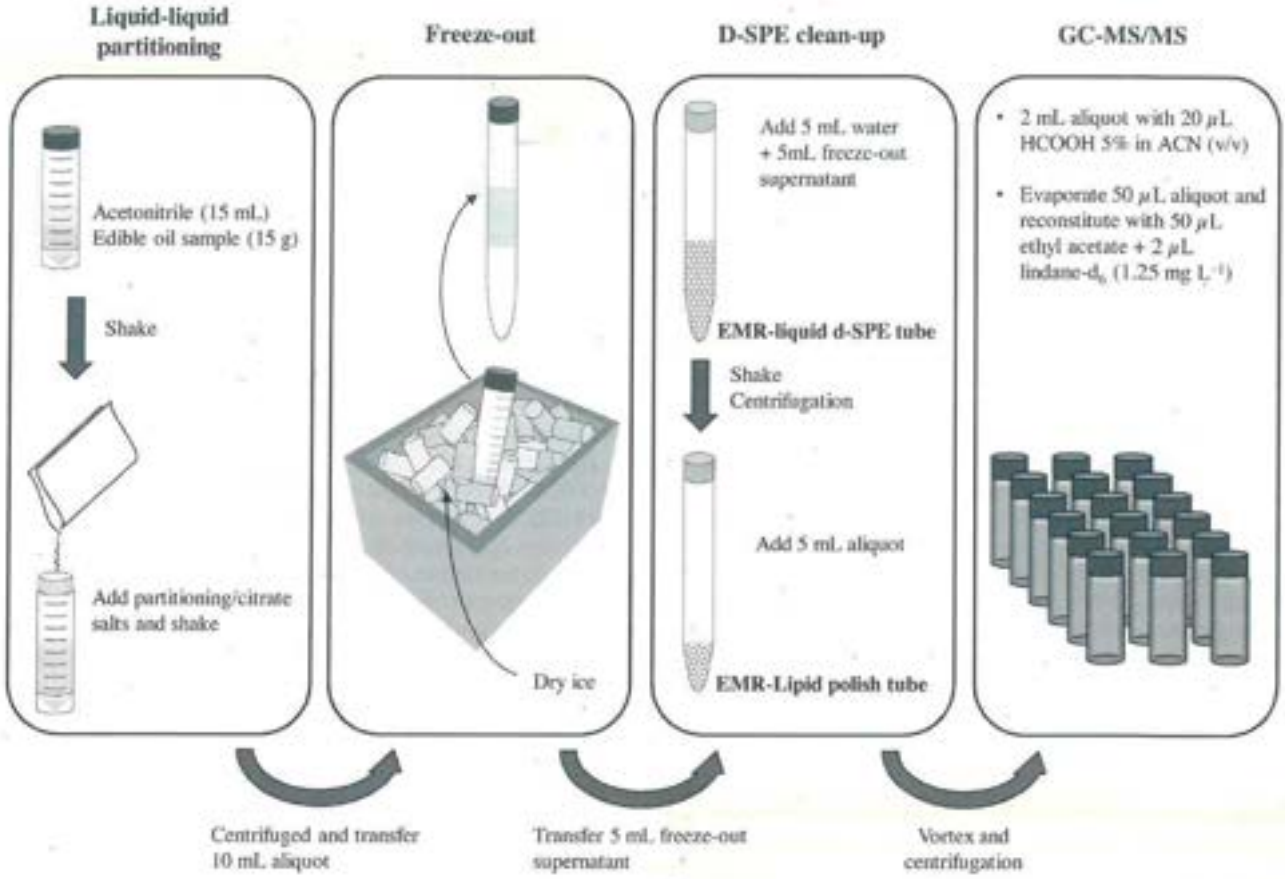


**Fig. 1.** Schematic diagram of the proposed sample preparation strategy based on QuEChERS method (approach a).

many of these pesticides in edible vegetable oils [1–7] at levels, in certain cases, above MRLs [1].

## 3. Result and discussion

### 3.1. Method selection

The new clean-up sorbent, Agilent Bond Elut EMR-Lipid, was applied in soybean, sunflower and extra virgin olive oil extracts and efficiency of the dispersive-solid-phase extraction (d-SPE) clean-up step as well as pesticides extraction effectiveness were evaluated by comparison with other sorbents (i.e. PSA and Z-Sep) previously used for d-SPE or SPE of this matrix [2,3,9,11,12,15,22].

The extraction/clean-up procedures were based on the QuEChERS methodology followed by analysis with a GC-EI-TOF-MS and GC–MS/MS. The extraction step involved liquid-liquid extraction of 10 g edible oil with 10 mL of acetonitrile followed by salting-out with citrate buffer, magnesium sulphate and sodium chloride. The following step was to discard in the extract an amount of matrix constituents with limited solubility in acetonitrile by precipitation at low-temperature (freezing-out clean-up). Hereafter, (a)–(d) clean-up strategies were checked.

For the freezing-out clean-up with dry ice (CO2 at −76 °C), an aliquot of the acetonitrile phase (5 mL) was transferred into a centrifuge tube and placed in a polystyrene box filled with 1.5 kg of dry ice. The freezing time study revealed that a homogenous fat precipitate in the bottom of the tube was achieved within 5 min and longer freezing times resulted in the freezing of the whole extract. Then, an aliquot of the supernatant solution (2.5 mL) was transferred into a centrifugation tube for further clean-up by d-SPE or SPE. In the case of d-SPE with EMR-Lipid (a), it was necessary to increase the amount of edible oil sample and acetonitrile to 15 g and 15 mL, respectively, because manufacturer recommends a volume of 5 mL for the EMR-Lipid dSPE tube which could be obtained after using a 10 mL aliquot of acetonitrile extract in the freezing-out step. Highly lipophilic compounds in edible oils such as triglycerides, diglycerides, monoglycerides and free fatty acids are expected to be removed to a large extent by freezing-out. The effectiveness of freezing-out clean-up as an additional step to the d-SPE can be assessed by evaluating the amounts of matrix compounds in the final extract using a GC-EI-TOF-MS working in full-scan mode. As can be seen in Fig. 2, the Total Ion Chromatograms (TICs) obtained for the same extra virgin olive oil sample showed that freezing-out plus d-SPE with EMR-Lipid ensured better clean-up (61% cleaner) than d-SPE (EMR-Lipid) only, especially in the case of compounds eluting from 23 to 40 min due to the presence of highly non-polar compounds in this part of the chromatogram.

Regarding the evaluation of clean-up sorbents, our main criterion was to find one that gives an efficient clean-up of the oil extract with the major number of pesticides having recoveries in the 70–120% range as well as giving the lowest average RSD values. SPE with Z-Sep (d) and d-SPE with EMR-Lipid (a) were the most effective clean-up procedures (Fig. 3), obtaining 43% and 34% reduction, respectively, in the amounts of matrix compounds in the final extract compared to d-SPE with Z-Sep (c). The advantage of these two procedures over d-SPE with Z-Sep (c) and d-SPE with PSA (b) is clearly visible from 34 to 40 min (Fig. 3B). Although Agilent Technologies recommends EMR-Lipid sorbent for d-SPE procedures (a), we tried SPE with 45 mg EMR-Lipid and, in this case, it was technically impossible to pass the acetonitrile phase (5 mL) through the cartridge even when the sorbent was previously hydrated with 5 mL of ultrapure water. Since no available information from Agi-

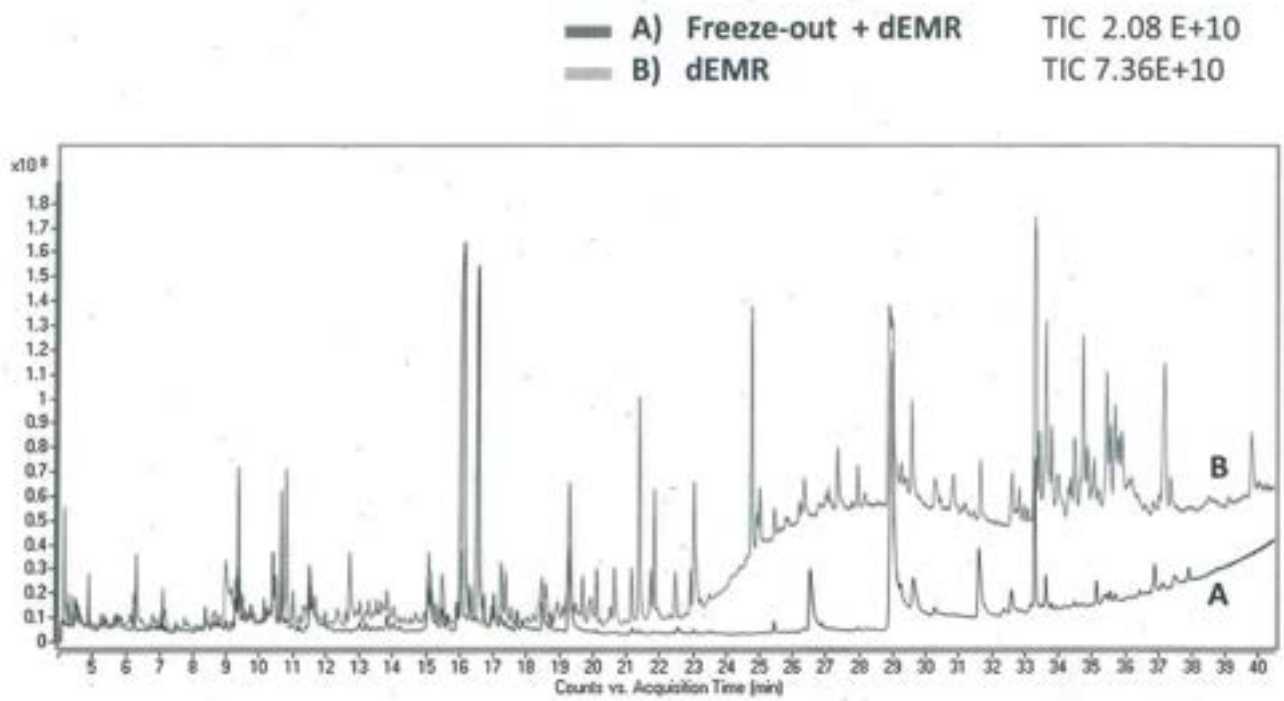


**Fig. 2.** GC-EI-TOF-MS full-scan chromatograms of blank olive oil extracts obtained using QuEChERS methodology with freezing-out followed by d-SPE (EMR-Lipid) (A) and d-SPE (EMR-Lipid) without freezing-out (B).

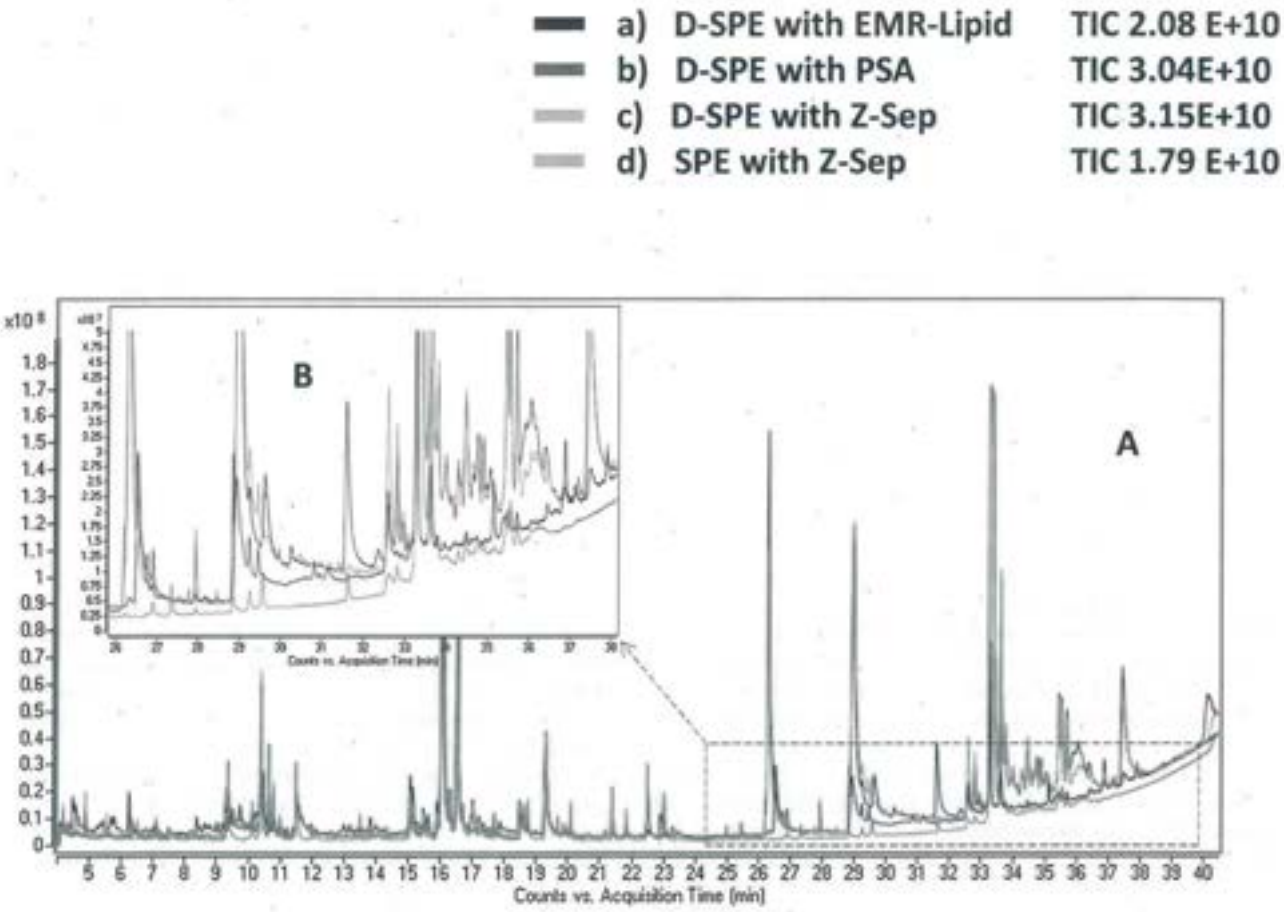


**Fig. 3.** GC-EI-TOF-MS full-scan chromatograms of blank olive oil extract obtained using QuEChERS methodology with freezing-out followed different d-SPE or SPE clean-up procedures (a, b, c and d).

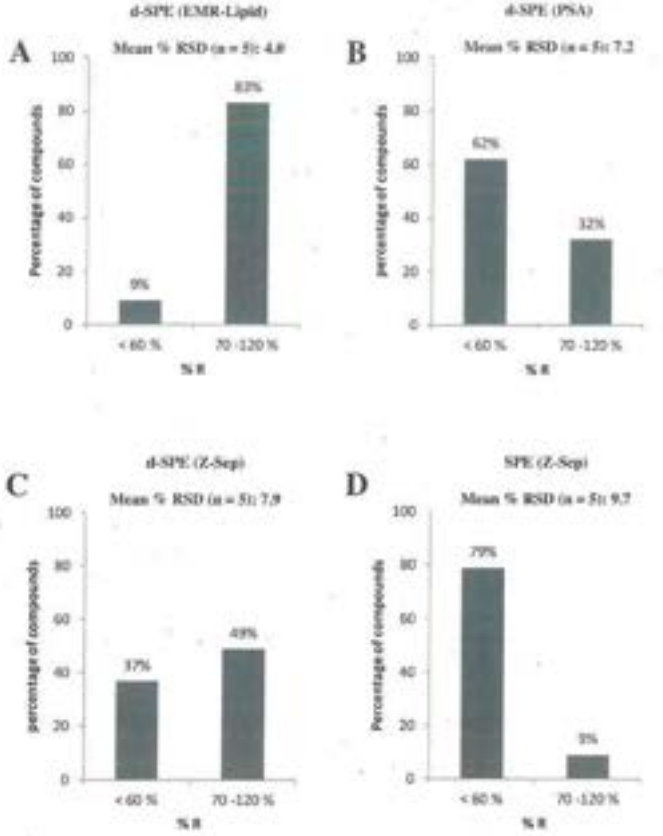


**Fig. 4.** Percentage of the total number of evaluated pesticides with recoveries from different ranges and mean% RSD (n = 5) in olive oil samples. Modified QuEChERS with freezing-out followed by d-SPE (EMR-Lipid) (A), d-SPE (PSA) (B), d-SPE (Z-Sep) (C) and SPE (Z-Sep) (D).

lent about characteristics of this new material, we cannot conclude the reason for the difficulty to be used as conventional SPE.

Recovery and precision studies carried out at a concentration level of 50 $\mu g\,kg^{-1}$ gave considerably better results for extracts cleaned with d-SPE (EMR-Lipid) than in the case of cleaning by SPE with Z-Sep (see Fig. 4). Out of the 213 pesticides, QuEChERS with d-SPE (EMR-Lipid) ensured recoveries in the 70–120% range and mean RSD equal to 4.0% for 177 pesticides whereas extracts cleaned with SPE (Z-Sep) resulted in 19 pesticides and a mean RSD equal to 9.7% (see Fig. 4A and D). A similar trend can be also observed in the case of d-SPE (Z-Sep), although extraction efficiency was better than that exhibited by the SPE (Z-Sep) alternative method tested.

It was noticed generally that azole pesticides (flusilazole, myclobutanil, penconazole, propiconazole, tetraconazole, fenbuconazole, epoxiconazole, cyproconazole, diclobutrazole, tebuconazole, paclobutrazol, metconazole, hexaconazole and flutriafol) did not show recovery values at the studied concentration level when SPE clean-up with Z-Sep was used, while they are completely recovered using d-SPE (EMR-Lipid) protocol. Z-Sep sorbent is a mixture of two sorbents, C18 and silica coated with $ZrO_2$, with a $ZrO_2$/C18 ratio of 2/5 [21]. Zr atoms have vacant 3d orbitals so they are electron acceptors (Lewis acids). This fact explains adsorption of azole pesticides which are nitrogen heterocyclic compounds with two double bonds and one heteroatom is not part of a double bond, thus contain atoms donors of electroms that could interact with the $ZrO_2$ sorbent [21]. The strength of the interaction between the azole pesticides and this sorbent can be increased for cyproconazole, diclobutrazole, tebuconazole, paclobutrazol, metconazole, hexaconazole and flutriafol due to the presence of a hydroxyl functional group in their molecules. Analytes with hydroxyl (fenhexamid, nuarimol, orto-phenylphenol and fenarimol), hydroxyacetate (chlorobenzilate and bromopropylate), phosphate (paraoxon-methyl and dichlorvos), phosphorodithioate (disulfoton and disulfoton-sulfoxide) and methoxy (metalaxyl, terbumeton, prometon, pyrifenox and secbumeton) substituents could not be recovered, possibly due to their capacity to interact strongly with the $ZrO_2$ sorbent according to Lewis theory [21]. The same occurred for other pesticides also have Lewis base sites in their structure such as piperidine (fenpropidin), morpholine (fenpropimorph) and diamine (ametryn) groups. A similar behavior based on the electrostatic interaction between the above electron donors functional groups and the amphoteric zirconium dioxide was found by Tuzimski et al. [2] for the d-SPE clean-up with Z-Sep developed for pesticide analysis in edible oils. For other pesticides having planar structures with chloride atoms, such as chlorothalonil, a very low recovery was also obtained. Table S2 in the Supplementary data presents the recoveries and RSD values obtained in extra virgin oil with the tested clean-up procedures (experiments (a)–(d)).

Based on the results of our experiments, it was found that QuEChERS with d-SPE (EMR-Lipid) would ensure efficient and robust clean-up while maintaining quantitative recovery for most of the target pesticides (83% of pesticides). In addition, compared with SPE procedure, other advantages of d-SPE are its simplicity, lesser disposable materials and volume of organic solvent consumed and, the large number of samples that can be processed per hour. For this reason, validation experiments were carried out with d-SPE (EMR-Lipid) procedure.

### 3.2. Method validation

#### 3.2.1. Recovery studies

For recovery studies, two fortification levels were selected: 10 $\mu g\,kg^{-1}$ and 20 $\mu g\,kg^{-1}$. All recovery experiments were performed five times at each level, as suggested by SANTE [30]. In olive oil (extra virgin quality), at the 20 $\mu g\,kg^{-1}$ level, 171 pesticides had recoveries in the 70–120% range; 23 had recoveries below 60% and the recovery of 3 analyte (fenitrothion, fipronil, mevinphos) was above 120% (Table 1). At the 10 $\mu g\,kg^{-1}$ level, 162 pesticides had recoveries in the 70–120% range, 23 were extracted with low recoveries or were not detected and, likewise at this level, mevinphos had an recovery higher than 120%. Low recoveries for ethoxyquin were obtained as a result of their degradation [23]. In the case of quinoxyfen, 4,4-DDE, endosulfan a, endosulfan b, heptachlor, dieldrin, quintozene, 4,4-DDD, 2,4-DDE, 2,4-DDT and 4,4-DDT recoveries within the 40–60% range were obtained at both concentration levels. Different authors have also reported poor recoveries when several extraction and clean-up methods were used in high fat content matrices for these pesticides without an unambiguous reason of this fact [21,31,32]. With respect to the sunflower oil matrix, a similar situation occurred when compared with the extra virgin olive oil matrix. Results obtained in soybean oil matrix showed that at 20 $\mu g\,kg^{-1}$, 160 pesticides had recoveries within the 70–120% range and at 10 $\mu g\,kg^{-1}$, there were 136 pesticides (Table 1). The pesticides with the lowest recoveries were aldrin, flonicamid, HCB, paclobutrazol, paraoxon methyl, pentachloroaniline and 3-chloroaniline.

Hence, the proposed modified QuEChERS method using a freeze-out step followed by EMR-Lipid d-SPE sorbent provided recoveries ranging from 70 to 120% for 76% of studied pesticides in extra virgin olive and sunflower oil; and 64% of studied pesticides in soybean oil (at the 10 $\mu g\,kg^{-1}$ spiking level). At this point we have to consider the large scope of pesticides presented and therefore, the difficulties to get good recoveries for all compounds by Matrix Matched Standard Calibration. To compensate this loss of analyte, we decided to use Procedural Standard Calibration providing the

**Table 1**
Recoveries and relative standard deviation of the proposed extraction/clean-up method in the four matrices, consisting of Freeze-out followed by d-SPE with EMR-Lipid.

| Compound | Recovery (% RSD) Olive oil 1[a] | | Olive oil 2[b] | | Sunflower oil | | Soybean oil | |
|---|---|---|---|---|---|---|---|---|
| | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ |
| 2,4-DDE | 49 (3) | 42 (3) | 45 (4) | 44 (9) | 56 (15) | 40 (7) | 28 (5) | 18 (5) |
| 2,4-DDT+4,4-DDD | 46 (6) | 51 (2) | 43 (7) | 50 (4) | 35 (9) | 43 (5) | 34 (8) | 25 (6) |
| 3,5-Dichloroaniline | 70 (5) | 71 (3) | 67 (3) | 73 (3) | 88 (3) | 79 (2) | 65 (5) | 69 (5) |
| 3-Chloroaniline | 54 (20) | 52 (19) | 55 (5) | 52 (5) | 64 (5) | 67 (4) | 31 (5) | 47 (6) |
| 4,4-DDE | 44 (14) | 45 (7) | 41 (5) | 42 (6) | 56 (7) | 57 (3) | 59 (9) | 44 (9) |
| 4,4-DDT | 49 (8) | 42 (2) | 54 (8) | 40 (5) | 56 (5) | 61 (5) | 58 (7) | 52 (7) |
| Acrinathrin | 98 (5) | 108 (3) | 92 (1) | 99 (4) | 86 (5) | 89 (6) | 79 (6) | 103 (15) |
| Alachlor | 87 (6) | 93 (3) | 85 (6) | 78 (5) | 90 (4) | 75 (4) | 73 (4) | 71 (8) |
| Aldrin | 28 (10) | 34 (11) | 33 (3) | 31 (6) | 30 (5) | 43 (6) | 7 (35) | 11 (26) |
| Ametryn | 75 (4) | 65 (4) | 72 (5) | 69 (4) | 75 (7) | 71 (6) | 67 (8) | 69 (6) |
| Anthraquinone | 62 (3) | 62 (2) | 74 (6) | 77 (5) | 86 (4) | 94 (7) | 70 (7) | 68 (8) |
| Atrazine | 76 (7) | 78 (4) | 88 (7) | 76 (4) | 93 (6) | 66 (6) | 74 (3) | 69 (6) |
| Azoxystrobin | 118 (3) | 118 (3) | 117 (8) | 105 (3) | 94 (5) | 98 (5) | 97 (4) | 119 (5) |
| Benalaxyl | 104 (4) | 107 (2) | 97 (9) | 100 (4) | 87 (6) | 103 (5) | 104 (6) | 87 (4) |
| Bifenox | 74 (5) | 81 (5) | 76 (6) | 84 (3) | 89 (7) | 75 (6) | 89 (4) | 85 (5) |
| Bifenthrin | 65 (4) | 68 (3) | 65 (5) | 61 (2) | 73 (5) | 66 (9) | 67 (7) | 65 (4) |
| Biphenyl | 67 (6) | 70 (6) | 64 (6) | 72 (6) | 74 (7) | 79 (4) | 31 (26) | 14 (23) |
| Bixafen | 110 (2) | 107 (2) | 101 (5) | 98 (4) | 95 (3) | 102 (6) | 60 (18) | 119 (6) |
| Boscalid | 86 (3) | 89 (2) | 93 (5) | 90 (7) | 92 (6) | 89 (5) | 92 (6) | 88 (7) |
| Bromopropylate | 72 (3) | 73 (3) | 76 (6) | 75 (5) | 66 (9) | 64 (5) | 82 (5) | 93 (8) |
| Bupirimate | 94 (6) | 97 (6) | 98 (3) | 99 (5) | 85 (5) | 101 (6) | 78 (7) | 78 (8) |
| Buprofezin | 92 (2) | 99 (2) | 84 (5) | 76 (7) | 96 (5) | 87 (2) | 92 (2) | 90 (5) |
| Butralin | 75 (6) | 78 (5) | 77 (5) | 91 (9) | 103 (6) | 87 (9) | 47 (5) | 57 (5) |
| Butylate | 61 (4) | 67 (4) | 74 (2) | 83 (1) | 77 (4) | 79 (7) | 77 (9) | 60 (5) |
| Cadusafos | 74 (3) | 71 (3) | 64 (6) | 70 (5) | 88 (5) | 77 (3) | 66 (6) | 68 (5) |
| Captan | 71 (7) | 72 (6) | 70 (4) | 75 (7) | 84 (6) | 73 (7) | - | 63 (17) |
| Carbofuran | 82 (3) | 94 (2) | 105 (5) | 92 (3) | 78 (5) | 90 (4) | 105 (5) | 95 (6) |
| Carbophenothion | 63 (3) | 66 (3) | 75 (2) | 73 (4) | 83 (7) | 95 (5) | 73 (15) | 76 (5) |
| Carbosulfan | 73 (3) | 71 (3) | 73 (4) | 72 (4) | 85 (5) | 102 (5) | - | 66 (27) |
| Chinomethionat | 24 (3) | 24 (2) | 47 (4) | 56 (15) | 34 (15) | 35 (9) | 16 (6) | 18 (4) |
| Chlorbromuron | 76 (4) | 77 (4) | 71 (6) | 77 (4) | 84 (5) | 81 (4) | 67 (5) | 73 (2) |
| Chlordane | 61 (10) | 62 (10) | 64 (8) | 63 (5) | 64 (6) | 65 (5) | - | 55 (9) |
| Chlorfenapyr | 85 (3) | 90 (3) | 80 (5) | 89 (5) | 100 (7) | 85 (1) | 60 (18) | 71 (6) |
| Chlorfenvinphos | 95 (5) | 100 (3) | 97 (3) | 91 (4) | 97 (5) | 101 (4) | 84 (4) | 94 (8) |
| Chlorobenzilate | 72 (2) | 74 (2) | 86 (5) | 85 (3) | 77 (6) | 93 (5) | 69 (6) | 77 (7) |
| Chlorothalonil | 77 (2) | 78 (1) | 79 (2) | 81 (4) | 90 (7) | 82 (4) | 82 (4) | 84 (8) |
| Chlorpropham | 67 (4) | 73 (2) | 74 (4) | 79 (7) | 81 (6) | 67 (3) | 63 (20) | 66 (4) |
| Chlorpyrifos | 74 (5) | 72 (3) | 88 (1) | 79 (1) | 77 (7) | 70 (6) | 84 (6) | 88 (8) |
| Chlorpyrifos Methyl | 74 (2) | 71 (2) | 74 (4) | 81 (5) | 65 (4) | 74 (3) | 62 (7) | 73 (9) |
| Chlorthal-Dimethyl | 63 (3) | 73 (2) | 80 (6) | 81 (5) | 74 (5) | 90 (2) | 74 (4) | 68 (6) |
| Chlozolinate | 99 (1) | 109 (1) | 102 (3) | 111 (4) | 94 (6) | 96 (4) | 87 (8) | 102 (6) |
| Coumaphos | 95 (2) | 99 (1) | 97 (5) | 104 (6) | 87 (5) | 102 (5) | 69 (18) | 117 (28) |
| Cyfluthrin | 76 (5) | 80 (2) | 72 (4) | 75 (3) | 78 (15) | 102 (6) | 72 (5) | 78 (6) |
| Cypermethrin | 87 (3) | 87 (2) | 83 (5) | 75 (7) | 101 (7) | 91 (5) | 75 (6) | 75 (4) |
| Cyproconazole | 79 (3) | 75 (4) | 74 (9) | 68 (5) | 75 (5) | 76 (7) | 60 (9) | 79 (6) |
| Cyprodinil | 39 (3) | 43 (7) | 28 (5) | 40 (19) | 53 (9) | 47 (19) | 42 (5) | 36 (9) |
| DMST | 116 (3) | 119 (5) | 91 (8) | 85 (4) | 89 (7) | 76 (4) | 73 (31) | 105 (9) |
| Deltamethrin | 69 (4) | 76 (3) | 64 (9) | 79 (6) | 71 (6) | 87 (7) | 62 (6) | 86 (5) |
| Desmethyl-pirimicarb | - | - | 45 (5) | 43 (10) | 25 (5) | 54 (7) | 48 (19) | 52 (5) |
| Diazinon | 78 (4) | 80 (3) | 71 (1) | 82 (6) | 94 (5) | 102 (4) | 77 (4) | 73 (3) |
| Dichlofluanid | 87 (5) | 102 (3) | 93 (2) | 103 (6) | 98 (10) | 103 (5) | 81 (4) | 88 (6) |
| Dichlorvos | 88 (3) | 98 (4) | 89 (5) | 98 (4) | 85 (5) | 81 (4) | 65 (4) | 84 (8) |
| Diclobutrazole | 71 (3) | 73 (3) | 97 (1) | 82 (4) | 86 (7) | 92 (5) | 74 (6) | 83 (4) |
| Dicloran | 61 (4) | 71 (3) | 84 (3) | 82 (6) | 89 (6) | 100 (4) | 62 (5) | 76 (9) |
| Dicofol | 33 (3) | 40 (3) | 21 (5) | 24 (6) | 38 (5) | 36 (5) | 30 (5) | 31 (9) |
| Dieldrin | 47 (6) | 43 (4) | 44 (4) | 40 (23) | 55 (4) | 50 (7) | 27 (6) | 24 (8) |
| Diethofencarb | 75 (6) | 70 (3) | 72 (7) | 65 (5) | 84 (3) | 79 (4) | - | 98 (5) |
| Dimethenamid | 82 (4) | 91 (1) | 74 (3) | 73 (5) | 88 (4) | 78 (2) | 75 (5) | 76 (4) |
| Dimethipin | 114 (2) | 119 (3) | 106 (6) | 84 (2) | 104 (6) | 103 (7) | 112 (8) | 104 (3) |
| Diphenylamine | 69 (3) | 79 (4) | 95 (3) | 74 (4) | 87 (5) | 95 (1) | 81 (5) | 88 (6) |
| Disulfoton | 74 (5) | 71 (3) | 86 (8) | 73 (9) | 83 (5) | 88 (3) | 77 (6) | 74 (2) |
| Disulfoton-sulfoxide | 113 (3) | 119 (3) | 106 (7) | 106 (6) | 98 (5) | 90 (4) | 105 (3) | 99 (4) |
| Dodemorph | 69 (7) | 71 (5) | 66 (3) | 70 (6) | 75 (6) | 67 (2) | 83 (7) | 76 (1) |
| EPN | 78 (12) | 86 (7) | 75 (7) | 80 (7) | 88 (7) | 95 (4) | 78 (5) | 76 (6) |
| Endosulfan Alpha | 40 (4) | 48 (3) | 35 (6) | 46 (5) | 29 (9) | 54 (5) | 61 (6) | 59 (6) |
| Endosulfan Beta | 40 (5) | 46 (3) | 35 (5) | 39 (8) | 49 (5) | 58 (15) | 49 (9) | 38 (4) |
| Endosulfan Sulfate | 81 (5) | 88 (5) | 81 (4) | 85 (4) | 98 (4) | 73 (5) | 75 (5) | 72 (9) |
| Endrin | 41 (3) | 40 (2) | 37 (5) | 53 (22) | 45 (9) | 57 (4) | 32 (5) | 27 (6) |
| Epoxiconazole | 95 (8) | 98 (6) | 90 (5) | 89 (3) | 104 (7) | 97 (8) | 78 (8) | 92 (5) |
| Ethion | 84 (3) | 93 (4) | 80 (9) | 92 (6) | 86 (7) | 96 (7) | 73 (4) | 71 (3) |
| Ethofumesate | 115 (4) | 114 (1) | 119 (5) | 103 (7) | 96 (6) | 75 (7) | 104 (2) | 98 (5) |
| Ethoprophos | 79 (3) | 85 (2) | 87 (4) | 85 (5) | 90 (4) | 81 (6) | 67 (19) | 70 (6) |
| Ethoxyquin | - | 24 (9) | - | 18 (24) | - | - | - | 4 (15) |

Table 1 (Continued)

| Compound | Recovery (% RSD) Olive oil 1[a] | | Olive oil 2[b] | | Sunflower oil | | Soybean oil | |
|---|---|---|---|---|---|---|---|---|
| | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ |
| Etofenprox | 57 (2) | 53 (2) | 46 (9) | 50 (5) | 57 (5) | 64 (4) | 33 (5) | 35 (4) |
| Etrimfos | 77 (2) | 81 (3) | 86 (4) | 85 (7) | 89 (5) | 92 (3) | 78 (4) | 72 (8) |
| Fenamidone | 91 (3) | 102 (1) | 79 (5) | 94 (4) | 94 (4) | 101 (1) | 82 (6) | 93 (1) |
| Fenarimol | 72 (2) | 65 (1) | 70 (6) | 61 (6) | 87 (5) | 79 (4) | 64 (7) | 75 (3) |
| Fenazaquin | 67 (3) | 68 (2) | 73 (4) | 87 (5) | 76 (2) | 70 (5) | 70 (6) | 84 (4) |
| Fenbuconazole | 90 (3) | 94 (2) | 95 (4) | 97 (5) | 96 (7) | 88 (5) | 82 (6) | 91 (5) |
| Fenchlorphos | 65 (3) | 60 (4) | 93 (5) | 95 (3) | 76 (4) | 90 (8) | 76 (7) | 72 (6) |
| Fenhexamid | 74 (3) | 78 (1) | 76 (4) | 79 (5) | 82 (5) | 76 (6) | 91 (5) | 89 (6) |
| Fenitrothion | 103 (3) | 122 (5) | 99 (3) | 87 (6) | 101 (5) | 93 (7) | 74 (5) | 87 (8) |
| Fenpropathrin | 79 (5) | 77 (5) | 76 (15) | 71 (5) | 84 (6) | 81 (7) | 66 (8) | 70 (6) |
| Fenpropidin | 91 (4) | 94 (2) | 90 (4) | 92 (1) | 83 (5) | 92 (1) | 95 (6) | 96 (6) |
| Fenpropimorph | 78 (2) | 76 (4) | 74 (3) | 75 (5) | 69 (2) | 74 (1) | 96 (9) | 95 (7) |
| Fention | 73 (7) | 79 (3) | 78 (2) | 73 (8) | 89 (5) | 73 (3) | 72 (5) | 74 (3) |
| Fenvalerate/Esfenvalerate RR/SS | 65 (11) | 71 (4) | 61 (5) | 65 (9) | 79 (7) | 69 (5) | 59 (5) | 75 (15) |
| Fenvalerate/Esfenvalerate RS/SR | 72 (5) | 73 (2) | 70 (7) | 73 (8) | 99 (4) | 76 (7) | 65 (6) | 77 (3) |
| Fipronil | 119 (7) | 122 (5) | 102 (6) | 114 (4) | 93 (6) | 90 (8) | 112 (6) | 112 (4) |
| Fipronil Desulfinil | 109 (3) | 116 (2) | 115 (6) | 102 (5) | 95 (5) | 97 (5) | 110 (8) | 109 (4) |
| Fipronil Sulfone | 116 (5) | 119 (4) | 98 (1) | 97 (7) | 103 (7) | 105 (4) | 101 (19) | 106 (3) |
| Flamprop-Isopropyl | 101 (3) | 102 (2) | 113 (5) | 101 (6) | 97 (6) | 96 (5) | 93 (4) | 92 (6) |
| Flamprop-Methyl | 100 (4) | 107 (3) | 102 (5) | 104 (5) | 97 (4) | 95 (7) | 99 (8) | 97 (6) |
| Flonicamid | 96 (3) | 85 (3) | 93 (4) | 84 (5) | 111 (9) | 96 (3) | – | – |
| Fluacrypyrim | 118 (3) | 119 (2) | 97 (5) | 102 (9) | 84 (8) | 92 (4) | 108 (3) | 109 (5) |
| Fluazifop-*p*-butyl | 100 (4) | 106 (6) | 103 (4) | 99 (1) | 98 (4) | 102 (2) | 83 (5) | 82 (4) |
| Flucythrinate | 103 (3) | 101 (1) | 85 (6) | 94 (8) | 102 (7) | 98 (7) | 79 (15) | 110 (6) |
| Fludioxonil | 98 (3) | 101 (2) | 102 (3) | 96 (4) | 89 (8) | 76 (5) | 85 (3) | 91 (4) |
| Fluopicolide | 96 (2) | 100 (3) | 95 (5) | 103 (4) | 85 (7) | 77 (4) | 90 (2) | 92 (2) |
| Fluopyram | 110 (2) | 119 (2) | 107 (2) | 83 (5) | 97 (4) | 83 (9) | 105 (7) | 114 (4) |
| Fluquinconazole | 75 (2) | 81 (1) | 96 (3) | 88 (5) | 95 (4) | 85 (3) | 76 (6) | 77 (4) |
| Flusilazol | 86 (4) | 91 (3) | 84 (6) | 101 (6) | 96 (3) | 102 (2) | 78 (5) | 86 (8) |
| Flutolanil | 108 (3) | 111 (3) | 100 (4) | 95 (5) | 102 (5) | 97 (5) | 100 (4) | 107 (4) |
| Flutriafol | 75 (3) | 77 (2) | 79 (8) | 90 (7) | 96 (4) | 97 (2) | 63 (5) | 71 (4) |
| Fluvalinate-tau | 73 (2) | 78 (2) | 98 (4) | 76 (3) | 89 (3) | 85 (6) | 63 (4) | 89 (4) |
| Folpet | 70 (6) | 81 (3) | 87 (3) | 89 (6) | 90 (3) | 85 (5) | 116 (5) | 94 (7) |
| Fonofos | 65 (7) | 68 (2) | 66 (22) | 76 (5) | 72 (9) | 77 (5) | 85 (5) | 74 (4) |
| Formothion | 112 (8) | 111 (3) | 100 (4) | 106 (3) | 98 (4) | 91 (3) | 93 (6) | 120 (4) |
| Fosthiazate | 110 (4) | 119 (1) | 107 (3) | 95 (3) | 86 (7) | 106 (5) | 82 (4) | 119 (5) |
| HCB | 17 (6) | 17 (3) | 26 (4) | 22 (6) | 32 (8) | 20 (4) | 4 (45) | 5 (3) |
| HCH-alpha | 66 (3) | 62 (4) | 78 (7) | 65 (4) | 79 (3) | 68 (9) | 63 (5) | 68 (6) |
| HCH-beta | 67 (3) | 71 (3) | 68 (6) | 73 (4) | 79 (4) | 89 (5) | 69 (8) | 57 (9) |
| Heptachlor | 42 (8) | 44 (4) | 45 (3) | 50 (4) | 63 (3) | 59 (25) | 57 (9) | 43 (8) |
| Heptachlorepoxide-cis | 70 (3) | 69 (5) | 62 (6) | 65 (3) | 74 (7) | 76 (6) | 71 (9) | 72 (5) |
| Heptachlorepoxide-trans | 67 (11) | 71 (4) | 64 (3) | 83 (5) | 79 (6) | 85 (7) | 85 (4) | 74 (6) |
| Heptenophos | 109 (5) | 115 (3) | 90 (5) | 109 (5) | 89 (8) | 97 (7) | 88 (6) | 88 (5) |
| Hexaconazole | 71 (6) | 75 (7) | 70 (5) | 73 (5) | 66 (5) | 95 (3) | 65 (8) | 63 (3) |
| Indoxacarb | 113 (3) | 107 (2) | 111 (4) | 95 (3) | 103 (3) | 96 (2) | 113 (8) | 114 (9) |
| Iprodione | 88 (6) | 91 (3) | 96 (1) | 94 (4) | 93 (6) | 85 (5) | 76 (9) | 104 (5) |
| Iprovalicarb | 89 (6) | 111 (4) | 90 (2) | 94 (3) | 97 (6) | 98 (3) | 78 (6) | 87 (2) |
| Isazofos | 99 (5) | 108 (3) | 99 (4) | 83 (6) | 101 (7) | 102 (4) | 83 (5) | 91 (7) |
| Isocarbophos | 91 (5) | 98 (5) | 94 (1) | 72 (5) | 83 (1) | 89 (2) | 88 (9) | 117 (3) |
| Isofenfos Ethyl | 90 (4) | 95 (3) | 94 (5) | 96 (3) | 98 (4) | 113 (3) | 81 (7) | 88 (4) |
| Isofenfos Methyl | 91 (5) | 99 (2) | 90 (6) | 96 (5) | 102 (5) | 97 (3) | 81 (6) | 74 (5) |
| Isoprothiolane | 90 (5) | 96 (3) | 75 (4) | 90 (3) | 111 (4) | 85 (3) | 84 (5) | 85 (7) |
| Isopyrazam | 96 (5) | 98 (3) | 93 (6) | 82 (4) | 86 (5) | 102 (3) | 84 (5) | 111 (3) |
| Kresoxim Methyl | 97 (8) | 114 (4) | 99 (4) | 103 (7) | 96 (3) | 87 (3) | 95 (7) | 97 (9) |
| Lambda-Cyhalothrin | 88 (9) | 94 (3) | 89 (3) | 97 (5) | 101 (4) | 103 (1) | 75 (4) | 91 (6) |
| Lindane | 78 (3) | 71 (3) | 78 (4) | 67 (6) | 90 (3) | 79 (2) | 73 (7) | 79 (8) |
| Malaoxon | 112 (8) | 114 (2) | 111 (7) | 109 (4) | 99 (5) | 86 (6) | 29 (4) | 120 (19) |
| Malathion | 112 (6) | 117 (3) | 92 (3) | 119 (5) | 83 (3) | 84 (4) | 102 (7) | 116 (5) |
| Mecarbam | 104 (8) | 112 (5) | 91 (4) | 87 (9) | 88 (6) | 93 (3) | 111 (5) | 111 (3) |
| Mepanypirim | 79 (8) | 74 (5) | 80 (3) | 95 (3) | 91 (5) | 99 (5) | 80 (3) | 104 (4) |
| Merphos | 49 (7) | 37 (9) | 55 (4) | 45 (3) | 58 (3) | 61 (8) | – | 57 (6) |
| Metalaxyl | 103 (6) | 100 (6) | 96 (4) | 98 (5) | 109 (5) | 112 (4) | 62 (20) | 79 (9) |
| Metazachlor | 101 (5) | 104 (1) | 109 (6) | 108 (4) | 83 (7) | 92 (6) | 91 (4) | 85 (8) |
| Metconazole | 75 (2) | 71 (3) | 71 (5) | 70 (7) | 87 (2) | 76 (3) | 95 (8) | 92 (7) |
| Methidathion | 105 (8) | 112 (4) | 109 (3) | 96 (3) | 93 (3) | 87 (2) | 73 (6) | 78 (5) |
| Methiocarb | 78 (7) | 85 (2) | 83 (7) | 90 (7) | 88 (7) | 91 (3) | 84 (7) | 105 (8) |
| Methiocarb sulfone | 118 (25) | 78 (2) | 106 (3) | 95 (4) | 101 (6) | 92 (7) | 76 (20) | 89 (3) |
| Methoxychlor | 67 (6) | 71 (3) | 52 (5) | 64 (7) | 72 (5) | 77 (6) | 65 (9) | 79 (5) |
| Metolachlor | 84 (6) | 88 (3) | 87 (5) | 100 (3) | 95 (3) | 90 (5) | 78 (7) | 71 (8) |
| Mevinphos | 121 (9) | 118 (2) | 107 (2) | 122 (7) | 98 (6) | 85 (3) | 74 (7) | 99 (5) |
| Molinate | 77 (8) | 73 (4) | 78 (6) | 75 (5) | 85 (5) | 93 (3) | 86 (8) | 63 (7) |
| Myclobutanil | 92 (5) | 98 (2) | 76 (5) | 95 (6) | 105 (1) | 99 (4) | 67 (4) | 79 (6) |
| Napropamide | 85 (7) | 93 (1) | 86 (8) | 91 (5) | 87 (6) | 94 (8) | 80 (7) | 77 (9) |
| Nuarimol | 73 (6) | 77 (3) | 70 (6) | 79 (4) | 90 (5) | 78 (1) | 79 (5) | 69 (9) |

Table 1 (Continued)

| Compound | Recovery (% RSD) Olive oil 1[a] | | Olive oil 2[b] | | Sunflower oil | | Soybean oil | |
|---|---|---|---|---|---|---|---|---|
| | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ | 10 μg kg$^{-1}$ | 20 μg kg$^{-1}$ |
| Ofurace | 116 (5) | 115 (2) | 96 (3) | 83 (2) | 110 (4) | 86 (3) | 103 (4) | 117 (3) |
| Ortophenylphenol | 74 (4) | 85 (4) | 71 (3) | 78 (3) | 79 (6) | 93 (7) | 109 (4) | 111 (4) |
| Oxadixyl | 112 (3) | 117 (2) | 110 (6) | 120 (5) | 96 (4) | 89 (3) | 109 (7) | 100 (5) |
| Paclobutrazol | 79 (6) | 85 (5) | 89 (3) | 87 (3) | 93 (2) | 75 (2) | - | - |
| Paraoxon methyl | 114 (4) | 118 (5) | 97 (3) | 85 (1) | 76 (5) | 89 (5) | - | - |
| Parathion Ethyl | 92 (8) | 111 (5) | 90 (4) | 103 (3) | 85 (3) | 83 (7) | 87 (7) | 86 (1) |
| Parathion Methyl | 105 (5) | 110 (4) | 115 (7) | 102 (4) | 86 (5) | 73 (4) | 83 (3) | 87 (3) |
| Pebulate | 66 (6) | 61 (3) | 67 (6) | 75 (5) | 94 (4) | 57 (5) | 76 (5) | 81 (5) |
| Pencoonazole | 78 (6) | 76 (5) | 70 (7) | 86 (1) | 84 (3) | 69 (6) | 73 (5) | 86 (8) |
| Pendimethalin | 69 (11) | 66 (6) | 70 (5) | 66 (9) | 76 (3) | 79 (1) | 65 (6) | 79 (3) |
| Pentachloroaniline | 58 (4) | 51 (2) | 55 (3) | 64 (1) | 76 (4) | 60 (5) | 22 (3) | 17 (5) |
| Permethrin | 32 (2) | 65 (3) | 32 (4) | 63 (2) | 65 (4) | 67 (6) | 56 (6) | 34 (3) |
| Phenothrin | 76 (8) | 74 (2) | 74 (3) | 71 (5) | 86 (7) | 97 (3) | 76 (8) | 85 (5) |
| Phenthoate | 100 (3) | 102 (3) | 105 (2) | 97 (1) | 95 (4) | 79 (2) | 81 (3) | 90 (7) |
| Phorate | 71 (7) | 79 (4) | 83 (3) | 84 (2) | 90 (3) | 75 (4) | 82 (5) | 94 (3) |
| Phorate sulfone | 106 (6) | 112 (2) | 95 (11) | 103 (2) | 102 (3) | 94 (5) | 98 (4) | 112 (4) |
| Phosmet | 106 (4) | 101 (2) | 97 (5) | 95 (4) | 98 (5) | 90 (1) | 117 (7) | 120 (5) |
| Phthalimide | 97 (2) | 101 (5) | 104 (3) | 86 (6) | 85 (4) | 109 (3) | 80 (4) | 76 (6) |
| Picolinafen | 71 (4) | 75 (1) | 70 (8) | 73 (4) | 92 (8) | 76 (5) | 75 (2) | 78 (1) |
| Picoxystrobin | 117 (8) | 101 (3) | 100 (3) | 93 (6) | 98 (6) | 103 (7) | 109 (2) | 103 (3) |
| Pirimicarb | 99 (4) | 105 (3) | 97 (5) | 83 (7) | 87 (3) | 94 (2) | 85 (7) | 84 (6) |
| Pirimiphos-Methyl | 73 (2) | 75 (1) | 92 (2) | 71 (3) | 94 (4) | 96 (6) | 82 (8) | 79 (2) |
| Procimidone | 82 (5) | 88 (2) | 66 (2) | 80 (1) | 88 (3) | 79 (1) | 74 (3) | 78 (6) |
| Profenofos | 77 (4) | 73 (2) | 97 (5) | 79 (4) | 87 (2) | 90 (1) | 64 (5) | 86 (3) |
| Prometon | 78 (9) | 78 (4) | 74 (6) | 65 (2) | 76 (4) | 87 (5) | 78 (7) | 74 (8) |
| Prometryn | 76 (3) | 75 (3) | 70 (7) | 73 (5) | 79 (3) | 80 (7) | 85 (3) | 82 (7) |
| Propaphos | 88 (4) | 89 (3) | 82 (2) | 75 (1) | 95 (8) | 87 (6) | 81 (4) | 88 (3) |
| Propargite | 71 (6) | 79 (1) | 72 (9) | 87 (3) | 89 (4) | 78 (9) | 72 (3) | 76 (8) |
| Propazine | 71 (5) | 71 (4) | 75 (6) | 86 (6) | 76 (3) | 84 (5) | 68 (5) | 73 (4) |
| Propiconazole | 72 (6) | 79 (3) | 72 (8) | 72 (4) | 87 (2) | 76 (3) | 78 (6) | 82 (2) |
| Propyzamide | 80 (8) | 83 (3) | 74 (6) | 77 (4) | 75 (5) | 92 (6) | 73 (4) | 73 (2) |
| Prosulfocarb | 66 (3) | 74 (4) | 65 (5) | 71 (7) | 69 (4) | 68 (3) | 55 (3) | 47 (8) |
| Prothiophos | 60 (8) | 63 (4) | 75 (3) | 84 (8) | 67 (6) | 69 (3) | 61 (5) | 62 (4) |
| Pyraclostrobin | 93 (5) | 102 (3) | 98 (5) | 99 (4) | 87 (6) | 84 (5) | 69 (5) | 118 (9) |
| Pyrazofos | 90 (5) | 95 (3) | 93 (6) | 94 (3) | 90 (6) | 87 (5) | 71 (19) | 107 (4) |
| Pyridaben | 61 (6) | 72 (2) | 44 (8) | 62 (6) | 79 (5) | 89 (4) | 69 (7) | 68 (8) |
| Pyrifenox | 70 (4) | 69 (2) | 65 (7) | 74 (4) | 75 (6) | 57 (1) | 64 (9) | 71 (3) |
| Pyrimethanil | 79 (3) | 85 (1) | 67 (4) | 81 (4) | 86 (7) | 84 (6) | 40 (4) | 43 (6) |
| Pyriproxyfen | 85 (9) | 80 (2) | 80 (5) | 83 (2) | 77 (9) | 71 (7) | 61 (5) | 64 (1) |
| Quinalphos | 77 (3) | 83 (3) | 72 (6) | 77 (5) | 76 (6) | 70 (5) | 77 (7) | 79 (2) |
| Quinoxyfen | 40 (2) | 46 (4) | 54 (5) | 55 (7) | 57 (6) | 51 (2) | 58 (4) | 24 (5) |
| Quintozene | 47 (29) | 50 (3) | 41 (4) | 48 (3) | 58 (5) | 57 (9) | 47 (6) | 38 (8) |
| Secbumeton | 77 (3) | 75 (4) | 75 (5) | 72 (6) | 79 (8) | 80 (6) | 83 (3) | 75 (8) |
| Spirodiclofen | 87 (9) | 85 (3) | 88 (3) | 86 (3) | 86 (7) | 75 (5) | 83 (5) | 66 (3) |
| Spiromesifen | 78 (5) | 83 (2) | 85 (4) | 77 (4) | 64 (3) | 66 (3) | 76 (8) | 79 (5) |
| Sulfotep | 98 (5) | 101 (2) | 95 (5) | 103 (6) | 85 (2) | 103 (1) | 77 (9) | 82 (7) |
| Sulprofos | 73 (8) | 77 (3) | 71 (3) | 76 (4) | 89 (4) | 73 (5) | 63 (3) | 67 (6) |
| Tebuconazole | 71 (3) | 71 (3) | 75 (7) | 94 (8) | 99 (6) | 65 (6) | 79 (4) | 85 (3) |
| Tebufenpyrad | 70 (4) | 72 (3) | 75 (9) | 79 (5) | 83 (4) | 90 (6) | 67 (3) | 82 (5) |
| Tecnazene | 60 (3) | 63 (2) | 76 (5) | 82 (6) | 75 (3) | 78 (7) | 69 (4) | 82 (5) |
| Tefluthrin | 79 (3) | 74 (4) | 66 (7) | 64 (6) | 80 (5) | 75 (2) | 70 (5) | 73 (5) |
| Terbufos | 70 (5) | 75 (2) | 67 (9) | 71 (6) | 78 (4) | 76 (4) | 58 (6) | 67 (3) |
| Terbumeton | 73 (3) | 74 (3) | 62 (2) | 69 (4) | 70 (2) | 87 (3) | 79 (5) | 81 (3) |
| Terbutryn | 68 (3) | 73 (6) | 68 (1) | 71 (5) | 76 (5) | 79 (3) | 94 (8) | 71 (5) |
| Tetrachlorvinphos | 100 (6) | 102 (2) | 98 (5) | 100 (9) | 102 (6) | 87 (4) | 55 (5) | 88 (7) |
| Tetraconazole | 103 (8) | 102 (2) | 101 (3) | 83 (3) | 97 (5) | 93 (5) | 81 (2) | 107 (4) |
| Tetradifon | 69 (6) | 73 (5) | 73 (5) | 91 (2) | 84 (7) | 76 (4) | 99 (4) | 100 (5) |
| Tetrahydrophthalimide | 99 (6) | 110 (4) | 102 (1) | 101 (2) | 95 (6) | 92 (3) | 88 (5) | 85 (5) |
| Tetramethrin | 80 (4) | 90 (3) | 85 (4) | 84 (4) | 96 (4) | 88 (7) | 70 (9) | 82 (7) |
| Tolclofos Methyl | 74 (5) | 72 (1) | 93 (5) | 87 (1) | 90 (4) | 81 (8) | 61 (4) | 80 (6) |
| Tolylfluanide | 97 (11) | 96 (3) | 102 (2) | 103 (4) | 88 (5) | 79 (5) | 86 (1) | 85 (6) |
| Triadimefon | 90 (7) | 95 (2) | 94 (1) | 99 (2) | 102 (3) | 111 (4) | 82 (2) | 85 (5) |
| Triazophos | 106 (8) | 111 (1) | 103 (3) | 97 (4) | 98 (6) | 84 (7) | 83 (3) | 85 (3) |
| Trifloxystrobin | 108 (7) | 114 (3) | 112 (7) | 109 (2) | 90 (7) | 92 (3) | 94 (19) | 113 (6) |
| Trifluralin | 99 (6) | 85 (3) | 102 (4) | 82 (4) | 97 (5) | 79 (3) | 91 (3) | 107 (6) |
| Vinclozolin | 84 (7) | 91 (4) | 76 (3) | 78 (1) | 75 (7) | 83 (8) | 73 (6) | 79 (4) |
| Average | 81 (5) | 84 (3) | 80 (5) | 81 (5) | 83 (5) | 82 (5) | 75 (7) | 79(6) |

[a] Organic extra virgin olive oil (Type 1).
[b] Organic extra virgin olive oil (Type 2).

quantitative determination of pesticides with extraction recoveries below 70%. Procedural standards were prepared by spiking a series of blank samples with different amounts of analytes, prior to extraction and these procedural standards were then analysed in exactly the same way as the samples. Thus, Procedural Standard Calibration enables a correction of recoveries for all pesticides. A number of requirements were fulfilled for a successful application of this approach: i) Good linear relation in the 10–500 $\mu g\,kg^{-1}$ concentration range ($r^2 > 0.99$ in all instances for all target analytes); ii) Precise quantification (RSD $\leq$ 5% for approximately 90% of the targeted compounds) of pesticides in oil samples were obtained; and iii) At 10 $\mu g\,kg^{-1}$ level, even in cases with low recoveries, detector signals of analyte were within the linearity range and the signal-to-noise of the quantitative transition was equal to, or higher than, 10.

Taking into account the good precision, the proposed method can be regarded as a strong alternative method to perform the determination of a large number of pesticides in routine analysis.

### 3.2.2. Limits of quantitation

Document N° SANTE/11945/2015 [30] describes the LOQ as the minimum concentration which meets the criteria of a mean recovery within the 70–120% range and an RSD $\leq$ 20%. Due the spiking levels evaluated by the authors, the LOQs have values of 10 or 20 $\mu g\,kg^{-1}$. In olive oil, 162 pesticides had limits of quantitation equal to 10 $\mu g\,kg^{-1}$; whilst 50 pesticides had recoveries outside the 70–120% range. In sunflower oil, 184 pesticides had LOQs at 10 $\mu g\,kg^{-1}$ and 3 pesticides had LOQs at 20 $\mu g\,kg^{-1}$. The remaining 26 pesticides had recoveries that were low or were not detected. In soybean oil, 136 pesticides had LOQs of 10 $\mu g\,kg^{-1}$ and 30 had 20 $\mu g\,kg^{-1}$. The remaining 47 pesticides were not detected or their recoveries were below 70%. Detailed limits of quantitation values are shown in Table S3 (in the Supplementary data).

However, the use of Procedural Standard Calibration and the good repeatability of the proposed method ensure the applicability of the proposed method for analytes with mean recoveries outside the above mentioned 70–120% range. Thus, in cases where a LOQ value of 20 $\mu g\,kg^{-1}$ were obtained, it may be practicable to include these compounds as a LOQ of 10 $\mu g\,kg^{-1}$.

### 3.2.3. Linearity

Linear calibration was checked in the range from 10 $\mu g\,kg^{-1}$ up to 500 $\mu g\,kg^{-1}$. Oil samples were spiked at different analyte concentrations in quintuplicate then, extractions were performed and weighted linear regressions (1/x) were calculated for each pesticide. Residuals were calculated and showed a deviation by less than ±20% from the calibration curve for each calibration level. Good linearity was achieved in all cases with correlation coefficients better than 0.990 (Table S3). The lowest calibrated level always had a qualifying transition with S/N ≥6. In all studied matrices, detector saturation was not a problem due to the very effective cleaning procedure proposed.

### 3.2.4. Inter- and intra-day precision

Repeatability (intra-day precision) and reproducibility (inter-day precision) were evaluated through recovery studies using spiked blank oil samples at two concentration levels (10 and 20 $\mu g\,kg^{-1}$). Intra-day precision was assessed by five determinations at each spiking level in the same day. Inter-day precision was assessed by one determination at each spiking level for five days. RSD values were generally below 10% (5% on average) and never exceeded 15% for the intra-day precision, except for aldrin, biphenyl and carbosulfan in soybean oil. RSD values were also generally below 10% (6% on average) and never exceed 19% for the inter-day precision, except for biphenyl and carbosulfan in soybean oil (Table S3).

### 3.2.5. Matrix effects

As consequence of coeluting sample components, the analyte signal may be enhanced or suppressed compared to the signal of the same analyte when injected in solvent. In most multiresidue procedures employing different clean-up techniques and MS detection methods, matrix effect has been described for the determination of pesticide residues in edible oils [3,14,15,18,19]. These methods suffered medium (suppression or enhancement of 20–50%) [3,18,19] or strong (suppression or enhancement >50%) [14,15] matrix effects for 27–60% of compounds. This drawback, however, may be overcome by applying Procedural Standard Calibration since this procedure of quantification corrects for matrix effects.

The assessment of ME was carried out by comparing the slopes of analyte calibration plots in the oils studied, including two organic extra virgin olive oils samples from different trademarks (Type 1 and Type 2), one organic sunflower oil and one organic soybean oil. Thus, the variability of the response when calibrating olive oils from different sources was checked since it is likely that for example signal value may be affected by differences in the variety of olives.

To calculate, the matrix effect value equation was used considering Type 1 olive oil as reference oil:

$$ME(\%) = \left(\frac{\text{Slope of calibration curve in studied matrix}}{\text{Slope of calibration curve in Type 1 olive oil}} - 1\right) \times 100$$

In sunflower and Type 2 olive oils, calculated ME was negligible indicating similar behavior of analytes in these matrices with respect to Type 1 olive oil. However, differences in the analytical signals were observed for soybean oil. Medium signal suppression was found for most compounds. Since mean recoveries and RSD values of pesticides in soybean oil were quite similar compared to the other studied oils, the proposed explanation for this was supported by the endogenous matrix compounds of soybean oil that could suppress the signals. This signal suppression for soybean oil can be compensated by using Procedural Standard Calibration.

## 3.3. Analysis of real oil samples

The validated method was applied for the analysis of 17 oil samples collected locally from Almería markets. The samples (seven extra virgin olive oil, two virgin olive oil, two refined olive oil, three sunflower oil and three soybean oil samples) were analyzed the same as the modified validated method for the analysis of 213 pesticides. The results of the detected pesticides are shown in Table 2.

Chlorpyrifos, chlorpyrifos methyl, deltamethrin, endosulfan sulfate, phosmet, tetraconazole and fluopyram pesticides were detected with values higher than the LOQ. Chlorpyrifos was detected in three extra virgin oil sample (Type 1, 3 and 4) with a concentration of 26.6, 23.0 and 32.4 $\mu g\,kg^{-1}$, respectively. Chlorpyrifos methyl was detected in one extra virgin olive oil sample (Type 5) with a concentration of 21.2 $\mu g\,kg^{-1}$. Deltametrin was detected in one refined olive oil sample (Type 2) with a concentration of 17.7 $\mu g\,kg^{-1}$. Endosulfan sulfate was detected in one extra virgin olive oil samples (Type 4) with a concentration of 10.2 $\mu g\,kg^{-1}$. Phosmet was found in two extra virgin olive oil (Type 3 and 7) with a concentration of 156.0 and 26.7 $\mu g\,kg^{-1}$, respectively. Tetraconazole was found in one extra virgin olive oil sample (Type 3) with a concentration of 31.0 $\mu g\,kg^{-1}$. Fluopyram was determined in one extra virgin olive oil sample (Type 3) with a concentration of 21.0 $\mu g\,kg^{-1}$. All the detected pesticides in the oil samples were with values lower than the MRLs ones (chlorpyrifos 50 $\mu g\,kg^{-1}$,

**Table 2**
Mean concentrations of pesticides found in the edible vegetable oils.

| Sample | Pesticides detected ($\mu g\,kg^{-1}$) |
|---|---|
| Olive oil | |
| Extra virgin1 | Chlorpyrifos (26.6) |
| Extra virgin2 | – |
| Extra virgin3 | Chlorpyrifos (23.0), Phosmet (156.0), Tetraconazole (31.0), Fluopyram (21.0), Tebuconazole (2.2[a]), Lambda cyhalothrin (1.5[a]), Chlorpropham (4.3[a]) |
| Extra virgin4 | Chlorpropham (6.3[a]), Chlorpyrifos (32.4), Chlorpyrifos methyl (3.4[a]), Cypermethrin (6[a]), Endosulfan sulfate (10.2) |
| Extra virgin5 | Chlorpyrifos (5.0[a]), Chlorpyrifos methyl (21.2), Endosulfan sulfate (2.8[a]) |
| Extra virgin6 | – |
| Extra virgin7 | Chlorpyrifos (7.4[a]), Lambda cyhalothrin (3.9[a]), Phosmet (26.7) |
| Virgin1 | – |
| Virgin2 | – |
| Refined1 | Cypermethrin (4.0[a]) |
| Refined2 | Cypermethrin (1.5[a]), Deltamethrin (17.7) |
| Sunflower oil | |
| Sunflower1 | – |
| Sunflower2 | Pyridaben (4.7[a]) |
| Sunflower3 | Pyridaben (4.7[a]) |
| Soybean oil | |
| Soybean1 | Fenthion (1.3[a]) |
| Soybean2 (Soya + Walnut) | – |
| Soybean3 (Soya + Omega3) | – |

[a] This value is lower than the validated LOQ.

chlorpyrifos methyl 50 $\mu g\,kg^{-1}$, deltamethrin 1000 $\mu g\,kg^{-1}$, endosulfan sulfate 50 $\mu g\,kg^{-1}$, phosmet 3000 $\mu g\,kg^{-1}$, tetraconazole 20 $\mu g\,kg^{-1}$ and fluopyram 10 $\mu g\,kg^{-1}$). These MRLs values were established for the olives used for oil production, where, the exact MRLs values for oil processing should be 5 times of magnitude of these values assigned above except for deltamethrin (1.5 factor).

It was noticed that the detected pesticides were only in olive oil samples with no detection of any in soybean or sunflower ones. It was noticed also that the higher concentrations of chlorpyrifos and chlorpyrifos methyl were found in two of extra virgin olive oil samples. On the other hand, phosmet was found with higher value in extra virgin olive oil (Type 3) than value found in extra virgin olive oil one (Type 7). Regarding the different analyzed matrices, clearly results show a major occurrence of pesticide residues in extra virgin olive oil comparing with the "few" residues found in virgin and refined oil samples. The proposed explanation for this was supported by the pesticide degradation during the manufacturing process (only cold pressure for extra virgin olive oil and hot pressure for refined oil).

## 4. Conclusions

The combination of freeze-out with dry ice ($CO_2$ at −76 °C) and d-SPE with EMR-Lipid for the clean-up of edible oil samples led to a development of a simple, efficient, selective, robust and sensitive method for the determination of 213 pesticides by GC–MS/MS. In general, better recoveries were achieved using QuEChERS with d-SPE (EMR-Lipid) method for the target pesticides. Using this extraction protocol, 83% of the analytes were recovered in the range 70–120%, while using the d-SPE (PSA), d-SPE (Z-Sep) and SPE (Z-Sep) extraction procedures, the percentage of analytes was 32%, 49% and 9%, respectively (see Table S2 in Material Supplementary for detailed data). An advantage is the low RSD achieved (between 1 and 15%) that allows the application of Procedural Standard approach to compensate the low recoveries obtained in some cases. As a result of selective extraction and effective removal of coextractives, negligible matrix effect was observed in extra virgin olive and sunflower oils. Soybean oil is the more complex matrix; medium matrix effect resulting in suppression of the response was found in this matrix.

The analysis of real oil samples showed that values of pesticides chlorpyrifos, chlorpyrifos methyl, deltamethrin, endosulfan sulfate, phosmet, tetraconazole and fluopyram were detected with values higher than the LOQ but still below the MRLs guidelines values established by EU.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.08.008.

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# Determination of pesticides in edible oils by liquid chromatography-tandem mass spectrometry employing new generation materials for dispersive solid phase extraction clean-up

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ABSTRACT

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The goal of this work was to evaluate the efficiency of several sorbents on removal fats from edible oils (olive, soya and sunflower) during the clean-up step for posterior determination of 165 pesticides by UHPLC-QqQ-MS/MS system. The extraction procedure employed in this work was the citrate version of QuEChERS method followed by a step of freezing out with dry ice and clean-up evaluation using i) PSA with magnesium sulfate (d-SPE); ii) magnesium sulfate and Z-sep sorbent (d-SPE); iii) Z-sep (column SPE) and iv) Agilent Bond Elut QuEChERS Enhanced Matrix Removal-Lipid (EMR-Lipid). After evaluation of the recovery results at 10, 20 and 50 $\mu g\,kg^{-1}$, the EMR-Lipid showed important advantages comparing to the other sorbents evaluated, such as better recovery rates and RSD%. The method was validated at the three concentrations described above. Analytical curves linearity was evaluated by spiking blank oil samples at 10, 20, 50, 100 and 500 $\mu g\,kg^{-1}$. The method demonstrated good recoveries values between the acceptable range of 70–120% and RSD% < 20 for most of evaluated pesticides. In order to evaluate the performance of the method, this same procedure was employed to other oils such as soya and sunflower with very good results.



## 1. Introduction

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Olive oil is the principal source of lipids in the Mediterranean diet, and its consumption in the world is increasing due to related potential health benefits, such as a lower incidence of cardiovascular diseases, neurological disorders, breast and colon cancers, as well as its hypolipidemic and antioxidant properties [1]. According to the data published in November 2015 by International Olive Oil Council, Spain is the main producer of olive oil in Europe with about 840 thousand tons during 2014/2015 production. Related to consumption in Europe, Italy is the main consumer with circa 520 thousand tons in 2014/2015 and in second place Spain with approximately 490 thousand tons [2].

Pesticides are chemical substances applied to crops at various stages of cultivation and post-harvest storage of crops. The use of pesticides is intended to prevent the destruction of food crops by controlling agricultural pests or unwanted plants and to improve plant quality. The widespread use of pesticides for improving agricultural productivity has raised public concern about the possible presence of residues in crops and its byproducts. In agricultural practice for olive groves, the use of insecticides and herbicides provides an unquestionable benefit for crop protection. However, these pesticides can persist up to the harvest and processing stage, making the contamination of olives, and consequently of olive oil, possible [3,4].

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The large number of pesticides to be monitored associated with low concentration of the maximum residue limits (MRL) established and non-registered residues in food require sensitive and selective methods for their identification and quantification. However, olive oil contains high level of lipid substances which can cause problems during pesticide residue analysis because they are soluble in many organic solvents used for extraction. The lipids must be removed from the extracts prior to analysis or the chromatographic and detection system can be damaged [5].

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In the last few years many studies were published aiming the development of sensitive and accurate methods for pesticide residues determination inhigh fat content matrices. The most common sorbent employed in these works during clean-up step was PSA [6–10] which was also evaluated in combination with other sorbents such as C18 [6,7] and GCB [8,9], due to the well know power of PSA in removing lipid content. Some methods employing Oasis Hydrophilic-Lipophilic Balance (HLB) were also reported [11]. Most recently, a new sorbent based on zirconium dioxide has been employed instead of PSA due to its higher ability on removing fat content from olive oil [12,13].

These clean-up methods were applied, in most of the cases, in combination with QuEChERS methodologies and its variations [6,7,10,13,14]. Mini-Luke was also evaluated in combination with UPLC–MS/MS in order to determine residues of 169 pesticides in soya grain [15].

Taking all these points into account and considering the importance of olive oil in Europe, the goal of this study was to develop and validate an analytical method for pesticides residue determination in olive oil by UHPLC-QqQ-MS/MS employing new sorbents for clean-up step. Four different methods using different sorbents were employed: i) PSA with magnesium sulfate (d-SPE); ii) Z-Sep sorbent with magnesium sulfate (d-SPE); iii) Z-Sep (cartridge SPE) and iv) Agilent Bond Elut QuEChERS Enhanced Matrix Removal-Lipid (EMR-Lipid). Furthermore, a step of low temperature precipitation (freezing-out) was evaluated before SPE clean-up. The method was fully validated in olive oil and applied for sunflower oil and soya oil in order to compare the results and check the possibility of employing only one kind of oil to quantify all of them. The method was applied in oil real samples of olive, sunflower and soya collected in local supermarkets of Almería city, in the southeastern of Spain.

## 2. Experimental

### 2.1. Chemicals and reagents

Acetonitrile, HPLC grade (99.9%), formic acid, analytical grade (>96%) and magnesium sulfate (98%) were purchased from Sigma Aldrich (Steinheim, Germany). Water, Optima®, HPLC grade was supplied by Fisher Scientific (New Jersey, USA). Sodium chloride was obtained from J. T. Baker (Deventer, Netherlands). Sodium citrate tribasic dihydrate (≥99%) and disodium hydrogencitrate sesquihydrate (99%) were obtained from Fluka (Steinheim, Germany). PSA and Z-Sep were purchased from Supelco (Bellefonte, USA). Bond Elut Enhanced Matrix Removal d-SPE and Bond Elut Final Polish from Agilent Technologies (Santa Clara, USA). Pesticides standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany), from Riedel-de-Haën (Seelze, Germany) and from Sigma Aldrich (Steinheim, Germany).

### 2.2. Pesticides standards solutions

Individual pesticide standard stock solutions were prepared in acetonitrile and stored in amber screw-capped glass vials at −20 °C. A standard mixture solution of the pesticides was prepared in acetonitrile at 10 mg $L^{-1}$. This solution was used as spike solution for recovery experiments and also to prepare the analytical curves solution for linearity studies.

### 2.3. Final extraction procedure

The final extraction procedure employed was the citrate version of QuEChERS method [16] using the EMR-Lipid from Agilent Technologies. An amount of 15 g of olive oil was weighed in a 50 mL PTFE centrifuge tube and 15 mL of acetonitrile was added plus 15 μL of procedure internal standard solution at 10 mg $L^{-1}$ in acetonitrile containing triphenyl phosphate (TPP), dichlorvos-d6, malathion-d10 and carbendazin-d3. The tubes were shaken in an automatic axial extractor (AGYTAX®, Cirta Lab. S.L., Spain) during 4 min. Thereafter, 6 g of magnesium sulfate, 1.5 g of sodiumchloride, 1.5 g of sodium citrate tribasic dihydrate and 0.75 g of disodium hydrogencitrate sesquihydrate were added and the samples were again shaken during 4 min in the automatic axial extractor. The extracts were centrifuged at 3500 rpm for 5 min and 8 mL were transferred to a 15 mL PTFE centrifuge tube. The tubes containing the extract were allowed to stand in dry ice during approximately 6 min in order to precipitate the fat content. The upper acetonitrile extract (5 mL) was collected and transferred to an EMR-Lipid d-SPE 15 mL tube already containing the adsorbent for clean-up step (1 g) and 5 mL of water. The mixture were homogenized in vortex during 1 min, centrifuged (3500 rpm, 5 min) and 5 mL of extract was transferred to an EMR-Lipid polish tube containing 2 g of a mixture of sodium chloride and magnesium sulfate (1:4, w/w). The mixture was homogenized during 1 min in vortex and centrifuged. Hereafter, 2 mL of extract were transferred to a vial and acidified with 20 μL of formic acid (5% in acetonitrile). Before UHPLC–MS/MS analysis, the extracts (100 μL) were diluted 5-fold with water HPLC grade and 10 μL of injection internal standard solution at 2.5 mg $L^{-1}$ containing dimethoate-d6 was added to the vials.

### 2.4. Instrumentation

An Agilent UHPLC 1290 Series (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent Technologies 6490 TripleQuad LC/MS was used for this study. Data acquisition and processing were developed by using Agilent MassHunter QQQ Acquisition and Quantitative Analysis B.07.00 software using Dynamic MRM software features with a retention time window of 0.8 min. The injection volume was 5 μL, and the chromatographic separation was carried out with a Zorbax Eclipse Plus C8 column (Agilent), 1.8 μm × 2.1 mm × 100 mm, maintained at 35 °C. The mobile phases employed was a solution of formic acid 0.1% in milliQ water (mobile phase A) and 0.1% formic acid and 5% water in acetonitrile (mobile phase B) at a constant flow rate of 0.3 mL $min^{-1}$, with the following gradient: 20% of B for 2 min, a linear gradient up to 100% of B in 13 min and finally an isocratic mode at 100% of B for 2 min. Afterwards, an equilibration step coming back to 20% of B (2.5 min) was performed. The system was provided with a Jet-Stream electrospray ion source, employing nitrogen as nebulizer gas. This ion source was configured as follows: 120 °C for drying gas temperature, 13 L $min^{-1}$ for drying gas flow, 45 psi for pressure of the nebulizer, 375 °C for sheath gas temperature and 10 L $min^{-1}$ for the sheath gas flow. The MS used nitrogen as collision gas (99.999% purity), 380 V for the fragmentor and 3000 V for the capillary voltage both in positive and negative mode.

For the optimization of the MS parameters, all pesticides at 100 μg $L^{-1}$ (acetonitrile:water, 1:1, v/v) were injected directly in the MS system in full scan mode with a mass range of 50–800 *m/z*. From this injection the precursor ion was selected and one more injection in product ion mode was needed to choose two fragment ions and the optimum collision energy (CE) for each transition. Retention times, transitions and CEs for each compound are collected in Table 1. The most intense transition was selected as the quantifier transition (SRM1), while the second most intense was chosen as the qualifier transition (SRM2).

### 2.5. Validation of the analytical procedure

Validation study was performed in order to evaluate accuracy (recovery), precision, linearity, limit of quantification, matrix effects and repeatability in accordance with the Document No. SANTE/11945/2015 [17]. Recovery and precision were determined

**Table 1**
Acquisition conditions for mass spectrometer: retention time, precursor ion, quantifier and qualifier transition and polarity of acquisition.

| Compound | Retention time (min) | Precursor Ion | 1st transition | | 2nd transition | | Polarity |
|---|---|---|---|---|---|---|---|
| | | | Product ion | CE* | Product ion | CE* | |
| 2,4-D | 7.8 | 218.96/220.96 | 160.96 | 15 | 162.95 | 15 | Negative |
| Acephate | 1.1 | 184.00 | 143.00 | 5 | 125.00 | 15 | Positive |
| Acetamiprid | 4.4 | 223.00 | 126.00 | 20 | 56.00 | 15 | Positive |
| Aldicarb | 5.7 | 213.00 | 116.00 | 10 | 89.00 | 15 | Positive |
| Aldicarb-sulfone | 1.7 | 223.00 | 148.00 | 5 | 86.00 | 10 | Positive |
| Aldicarb-sulfoxide | 1.2 | 207.00 | 132.00 | 5 | 89.00 | 10 | Positive |
| Azinphos-methyl | 9.1 | 318.00 | 261.00 | 0 | 132.10 | 8 | Positive |
| Azoxystrobin | 9.6 | 404.00 | 372.00 | 10 | 344.00 | 20 | Positive |
| Bifenazate | 10.2 | 301.10 | 198.20 | 10 | 169.90 | 20 | Positive |
| Bitertanol | 10.2 | 338.20 | 269.20 | 5 | 99.10 | 10 | Positive |
| Boscalid | 9.7 | 343.00 | 307.10 | 16 | 272.10 | 32 | Positive |
| Bromuconazole | 9.5 | 378.00 | 159.00 | 20 | 70.00 | 20 | Positive |
| Bupirimate | 8.6 | 317.00 | 272.00 | 20 | 166.00 | 20 | Positive |
| Buprofezin | 10.1 | 306.00 | 201.00 | 10 | 116.00 | 15 | Positive |
| Carbaryl | 7.4 | 202.00 | 145.00 | 10 | 127.00 | 20 | Positive |
| Carbendazim | 1.2 | 192.00 | 160.00 | 15 | 132.00 | 20 | Positive |
| Carbendazim-d3 (LS) | 1.2 | 195.10 | 159.80 | 20 | 131.90 | 20 | Positive |
| Chlorantraniliprol | 8.6 | 483.90 | 452.90 | 16 | 285.90 | 8 | Positive |
| Chlorfenvinphos | 10.8 | 358.90 | 155.00 | 8 | 99.20 | 28 | Positive |
| Chlorpyrifos-methyl | 11.7 | 321.90 | 289.90 | 14 | 125.00 | 16 | Positive |
| Chlorpyriphos | 12.9 | 352/349.93 | 200.00 | 20 | 198.00 | 20 | Positive |
| Clofentezin | 11.4 | 303.00 | 138.00 | 12 | 102.00 | 40 | Positive |
| Clomazone | 8.6 | 240.10 | 127.80 | 10 | 124.90 | 20 | Positive |
| Coumaphos | 11.4 | 363.00 | 307.00 | 20 | 227.00 | 28 | Positive |
| Cyazofamid | 11.1 | 325.00 | 261.20 | 10 | 108.10 | 15 | Positive |
| Cymoxanil | 5.0 | 199.10 | 128.00 | 4 | 110.90 | 12 | Positive |
| Cyproconazole | 9.1 | 292.10 | 125.00 | 32 | 70.00 | 16 | Positive |
| Cyprodinil | 8.0 | 226.20 | 92.90 | 40 | 76.90 | 40 | Positive |
| Cyromazine | 0.9 | 167.00 | 125.00 | 15 | 59.90 | 20 | Positive |
| Demeton-S-methylsulfone | 2.3 | 263.02 | 169.00 | 12 | 109.00 | 24 | Positive |
| Demeton-S-methylsulfoxide | 1.4 | 247.00 | 169.00 | 8 | 109.00 | 24 | Positive |
| Diazinon | 11.4 | 305.00 | 169.00 | 15 | 153.00 | 20 | Positive |
| Diclorvos | 6.5 | 220.80 | 108.80 | 15 | 78.90 | 30 | Positive |
| Diclorvos-d6 (LS) | 7.8 | 226.90 | 132.90 | 20 | 115.00 | 20 | Positive |
| Dicrotophos | 2.0 | 238.09 | 112.10 | 8 | 72.10 | 28 | Positive |
| Diethofencarb | 9.2 | 268.00 | 226.00 | 5 | 180.00 | 15 | Positive |
| Difenoconazole | 10.9 | 406.00 | 337.00 | 15 | 251.00 | 20 | Positive |
| Diflubenzuron | 10.1 | 311.00 | 158.00 | 8 | 141.00 | 32 | Positive |
| Dimethoate | 4.2 | 230.00 | 199.00 | 5 | 171.00 | 10 | Positive |
| Dimethoate-d6 (LS) | 4.2 | 236.00 | 205.00 | 4 | 131.00 | 16 | Positive |
| Dimethomorph | 8.7 | 388.00 | 301.00 | 20 | 165.00 | 20 | Positive |
| Diniconazole | 10.5 | 326.10 | 159.00 | 28 | 70.00 | 28 | Positive |
| Dodine | 8.6 | 228.20 | 60.10 | 20 | 57.20 | 20 | Positive |
| Emamectin B1a | 9.6 | 886.50 | 158.10 | 40 | 81.80 | 50 | Positive |
| EPN | 12.0 | 324.05 | 296.01 | 10 | 156.99 | 20 | Positive |
| Epoxiconazole | 9.6 | 330.10 | 121.00 | 16 | 101.20 | 52 | Positive |
| Ethion | 13.3 | 385.10 | 199.00 | 5 | 171.00 | 10 | Positive |
| Ethirimol | 2.5 | 210.16 | 140.10 | 20 | 43.10 | 52 | Positive |
| Ethoprophos | 9.8 | 243.10 | 130.90 | 15 | 97.00 | 30 | Positive |
| Fenamidone | 9.7 | 312.00 | 92.20 | 28 | 65.10 | 56 | Positive |
| Fenamiphos | 9.5 | 304.10 | 234.00 | 12 | 217.10 | 20 | Positive |
| Fenamiphos-sulfone | 6.9 | 336.10 | 266.00 | 16 | 188.00 | 24 | Positive |
| Fenamiphos-sulfoxide | 5.8 | 320.11 | 292.10 | 8 | 108.10 | 44 | Positive |
| Fenarimol | 9.3 | 331.00 | 268.00 | 20 | 259.00 | 20 | Positive |
| Fenazaquin | 12.5 | 307.30 | 161.30 | 15 | 147.20 | 15 | Positive |
| Fenbuconazole | 10.1 | 337.10 | 125.10 | 40 | 70.00 | 33 | Positive |
| Fenhexamid | 9.7 | 302.00 | 97.00 | 25 | 55.00 | 30 | Positive |
| Fenoxycarb | 10.3 | 302.20 | 116.20 | 5 | 88.20 | 20 | Positive |
| Fenpropimorph | 7.6 | 304.30 | 147.10 | 30 | 130.00 | 25 | Positive |
| Fenpyrazamine | 10.0 | 332.20 | 272.10 | 10 | 230.20 | 20 | Positive |
| Fenpyroximate | 13.1 | 422.21 | 366.20 | 12 | 107.00 | 64 | Positive |
| Fenthion | 11.2 | 279.00 | 247.10 | 8 | 169.10 | 12 | Positive |
| Fenthion-sulfoxide | 7.1 | 295.02 | 280.00 | 16 | 109.00 | 32 | Positive |
| Fipronil | 11.2 | 434.90 | 329.90 | 12 | 249.90 | 28 | Negative |
| Fluazifop | 9.2 | 328.20 | 282.20 | 15 | 254.20 | 20 | Positive |
| Flubendiamide | 11.1 | 680.90 | 273.90 | 15 | 254.00 | 20 | Negative |
| Fludioxonil | 9.3 | 247.00 | 169.00 | 32 | 152.00 | 32 | Negative |
| Flufenacet | 10.6 | 364.10 | 194.10 | 15 | 152.00 | 15 | Positive |
| Flufenoxuron | 12.9 | 489.10 | 158.00 | 20 | 140.90 | 56 | Positive |
| Fluopyram | 10.1 | 397.10 | 208.00 | 20 | 173.10 | 20 | Positive |
| Fluquinconazole | 9.7 | 376.00 | 307.10 | 24 | 108.00 | 56 | Positive |
| Flusilazol | 10.1 | 316.10 | 247.10 | 12 | 165.00 | 24 | Positive |
| Flutriafol | 7.6 | 302.10 | 95.00 | 56 | 70.10 | 16 | Positive |
| Formetanate | 1.0 | 222.13 | 165.10 | 8 | 65.10 | 52 | Positive |

Table 1 (Continued)

| Compound | Retention time (min) | Precursor ion | 1st transition | | 2nd transition | | Polarity |
|---|---|---|---|---|---|---|---|
| | | | Product ion | CE[a] | Product ion | CE[a] | |
| Fosthiazate | 7.6 | 284.00 | 227.80 | 10 | 103.80 | 20 | Positive |
| Haloxyfop | 10.3 | 362.10 | 316.20 | 12 | 288.10 | 24 | Positive |
| Hexaconazole | 10.2 | 314.10 | 159.00 | 30 | 70.10 | 20 | Positive |
| Hexythiazox | 13.0 | 353.10 | 228.20 | 10 | 168.20 | 20 | Positive |
| Imazalil | 6.4 | 297.00 | 255.00 | 15 | 159.00 | 20 | Positive |
| Imidacloprid | 3.8 | 256.00 | 209.00 | 15 | 175.00 | 15 | Positive |
| Indoxacarb | 12.2 | 528.10 | 218.00 | 20 | 203.00 | 45 | Positive |
| Ioxonil | 8.4 | 369.80 | 214.80 | 30 | 126.80 | 30 | Negative |
| Iprovalicarb | 9.5 | 321.20 | 202.90 | 0 | 119.00 | 16 | Positive |
| Isofenfos-methyl | 11.6 | 231.00 | 199.00 | 15 | 121.00 | 15 | Positive |
| Isoprocarb | 8.2 | 194.10 | 152.00 | 5 | 95.10 | 15 | Positive |
| Isoxaflutole | 9.7 | 360.00 | 250.90 | 15 | 219.70 | 50 | Positive |
| Kresoxim-methyl | 11.0 | 314.10 | 267.00 | 0 | 222.10 | 10 | Positive |
| Linuron | 9.1 | 249.02 | 160.10 | 20 | 133.00 | 36 | Positive |
| Lufenuron | 12.6 | 508.90 | 339.00 | 10 | 325.90 | 10 | Negative |
| Malathion | 10.4 | 331.00 | 127.00 | 10 | 99.00 | 20 | Positive |
| Malathion-d10 (I.S) | 10.3 | 341.11 | 132.00 | 12 | 100.00 | 24 | Positive |
| Mandipropamid | 9.8 | 412.13 | 356.10 | 4 | 328.10 | 8 | Positive |
| MCPA | 7.9 | 199.00 | 154.60 | 5 | 140.70 | 10 | Negative |
| Mepanypirim | 9.7 | 224.10 | 206.80 | 10 | 190.60 | 20 | Positive |
| Meptyldinocap | 13.8 | 295.10 | 193.00 | 42 | 163.00 | 50 | Negative |
| Metalaxyl | 7.8 | 280.30 | 220.00 | 5 | 192.40 | 10 | Positive |
| Metconazole | 10.3 | 320.10 | 125.00 | 48 | 70.10 | 24 | Positive |
| Methamidophos | 1.1 | 142.10 | 125.00 | 10 | 94.10 | 10 | Positive |
| Methidathion | 9.1 | 302.90 | 145.00 | 0 | 85.10 | 15 | Positive |
| Methiocarb | 9.0 | 226.10 | 121.10 | 12 | 169.00 | 5 | Positive |
| Methiocarb-sulfoxide | 2.8 | 242.00 | 185.00 | 10 | 170.00 | 20 | Positive |
| Methomyl | 2.0 | 163.10 | 106.00 | 4 | 88.00 | 0 | Positive |
| Methoxyfenozide | 10.2 | 369.30 | 149.00 | 15 | 133.00 | 20 | Positive |
| Metobromuron | 8.1 | 259.00 | 170.00 | 15 | 148.00 | 10 | Positive |
| Monocrotophos | 1.7 | 224.20 | 193.10 | 5 | 127.00 | 10 | Positive |
| Myclobutanil | 9.6 | 289.20 | 125.10 | 20 | 70.20 | 15 | Positive |
| Nitempyram | 1.5 | 271.00 | 225.00 | 10 | 99.00 | 10 | Positive |
| Omethoate | 1.2 | 214.10 | 183.00 | 5 | 125.00 | 20 | Positive |
| Oxadixyl | 6.2 | 279.10 | 219.20 | 5 | 132.30 | 32 | Positive |
| Oxamyl | 1.7 | 237.00 | 90.00 | 5 | 72.00 | 10 | Positive |
| Paclobutrazol | 8.9 | 294.10 | 125.20 | 36 | 70.10 | 16 | Positive |
| Paraoxon-methyl | 6.3 | 247.80 | 201.90 | 15 | 108.70 | 30 | Positive |
| Penconazole | 10.2 | 284.00 | 159.00 | 20 | 70.00 | 15 | Positive |
| Pencycuron | 11.7 | 329.10 | 125.10 | 24 | 89.10 | 60 | Positive |
| Pendimethalin | 13.0 | 282.10 | 212.10 | 4 | 194.10 | 16 | Positive |
| Phenthoate | 11.4 | 321.00 | 247.10 | 4 | 79.10 | 44 | Positive |
| Phosalone | 11.8 | 368.00 | 182.00 | 8 | 110.90 | 44 | Positive |
| Phosmet | 9.4 | 317.99 | 160.00 | 8 | 133.00 | 36 | Positive |
| Phoxim | 11.9 | 299.00 | 129.10 | 4 | 77.10 | 24 | Positive |
| Pirimicarb | 2.5 | 239.20 | 182.10 | 15 | 72.20 | 20 | Positive |
| Pirimicarb-desmethyl | 1.4 | 225.10 | 168.10 | 8 | 72.10 | 20 | Positive |
| Pirimiphos-methyl | 11.2 | 306.20 | 164.20 | 20 | 108.20 | 20 | Positive |
| Prochloraz | 8.8 | 376.00 | 308.00 | 10 | 266.00 | 15 | Positive |
| Profenofos | 12.0 | 374.90 | 347.00 | 5 | 304.90 | 15 | Positive |
| Propamocarb | 1.1 | 189.20 | 144.10 | 10 | 102.10 | 15 | Positive |
| Propaquizafop | 12.2 | 444.10 | 371.00 | 15 | 99.90 | 20 | Positive |
| Propargite | 13.5 | 368.10 | 231.20 | 0 | 175.20 | 8 | Positive |
| Propiconazole | 10.4 | 342.10 | 159.00 | 32 | 69.10 | 16 | Positive |
| Propoxur | 7.0 | 210.11 | 168.10 | 5 | 111.10 | 10 | Positive |
| Propyzamide | 9.8 | 256.00 | 190.00 | 10 | 173.00 | 20 | Positive |
| Proquinazid | 13.2 | 373.00 | 331.00 | 20 | 289.10 | 20 | Positive |
| Prothioconazole | 10.4 | 341.90 | 306.10 | 15 | 99.80 | 20 | Negative |
| Prothiofos | 14.2 | 345.00 | 241.00 | 20 | 161.00 | 40 | Positive |
| Pymetrozine | 0.9 | 218.11 | 105.00 | 20 | 51.00 | 60 | Positive |
| Pyraclostrobin | 11.4 | 388.11 | 193.80 | 8 | 163.10 | 20 | Positive |
| Pyrethrin | 13.6 | 329.21 | 161.00 | 5 | 143.00 | 20 | Positive |
| Pyridaben | 13.8 | 365.20 | 309.20 | 10 | 147.30 | 20 | Positive |
| Pyridate | 14.6 | 379.10 | 351.10 | 5 | 206.80 | 10 | Positive |
| Pyrimethanil | 6.3 | 200.00 | 183.00 | 20 | 107.00 | 20 | Positive |
| Pyriproxyfen | 12.6 | 322.00 | 185.00 | 20 | 96.00 | 10 | Positive |
| Quinoclamine | 6.1 | 208.00 | 105.10 | 25 | 77.00 | 40 | Positive |
| Quinoxyfen | 11.7 | 308.10 | 271.90 | 25 | 196.90 | 35 | Positive |
| Quizalofop-ethyl | 12.1 | 373.09 | 271.20 | 24 | 255.10 | 36 | Positive |
| Rotenone | 10.4 | 395.00 | 213.10 | 20 | 192.10 | 20 | Positive |
| Spinosyn A | 8.7 | 732.50 | 142.00 | 20 | 98.00 | 20 | Positive |
| Spinosyn D | 9.1 | 746.50 | 142.00 | 20 | 98.00 | 20 | Positive |
| Spirodiclofen | 14.2 | 411.10 | 313.00 | 5 | 71.20 | 15 | Positive |
| Spiromesifen | 14.1 | 371.00 | 273.00 | 5 | 255.00 | 20 | Positive |

Table 1 (Continued)

| Compound | Retention time (min) | Precursor Ion | 1st transition | | 2nd transition | | Polarity |
|---|---|---|---|---|---|---|---|
| | | | Product ion | CE[a] | Product ion | CE[a] | |
| Spirotetramat | 9.3 | 374.20 | 330.30 | 15 | 270.10 | 20 | Positive |
| Spiroxamine | 7.6 | 298.00 | 144.00 | 20 | 100.00 | 20 | Positive |
| Tebuconazole | 9.9 | 308.00 | 125.00 | 20 | 70.00 | 20 | Positive |
| Tebufenozide | 10.9 | 353.20 | 296.90 | 5 | 133.10 | 15 | Positive |
| Tebufenpyrad | 12.1 | 334.20 | 145.10 | 20 | 117.00 | 47 | Positive |
| Teflubenzuron | 11.7 | 379.00 | 359.00 | 0 | 339.00 | 4 | Negative |
| Terbuthylazine | 9.0 | 230.00 | 174.00 | 15 | 146.00 | 20 | Positive |
| Tetraconazole | 10.0 | 372.00 | 159.00 | 36 | 70.00 | 20 | Positive |
| Thiabendazol | 1.3 | 202.00 | 175.00 | 30 | 131.00 | 40 | Positive |
| Thiacloprid | 5.5 | 253.00 | 186.00 | 10 | 126.00 | 20 | Positive |
| Thiamethoxam | 2.4 | 292.00 | 211.00 | 10 | 181.00 | 20 | Positive |
| Thiobencarb | 11.5 | 258.00 | 124.70 | 15 | 99.90 | 10 | Positive |
| Thiodicarb | 6.9 | 355.06 | 108.10 | 8 | 88.10 | 8 | Positive |
| Tolclofos-methyl | 11.7 | 300.90 | 269.00 | 10 | 125.00 | 15 | Positive |
| Triadimenol | 9.0 | 296.00 | 227.00 | 5 | 70.00 | 10 | Positive |
| Triazophos | 10.4 | 314.10 | 286.20 | 10 | 162.20 | 20 | Positive |
| Trichlorfon | 3.0 | 256.90 | 221.00 | 4 | 109.00 | 12 | Positive |
| Trifloxystrobin | 12.2 | 409.20 | 206.20 | 10 | 186.20 | 20 | Positive |
| Triflumuron | 11.1 | 359.00 | 156.00 | 8 | 139.00 | 32 | Positive |
| Triticonazole | 9.0 | 318.10 | 125.20 | 41 | 70.20 | 33 | Positive |
| Zoxamide | 11.3 | 336.00 | 187.00 | 16 | 159.00 | 44 | Positive |

[a] CE: collision energy (V).

as average peak areas of five replicates of spiked blank olive oil samples at 10, 20 and 50 μg kg$^{-1}$. The mixture solution containing all the pesticides was added to the blank olive oil sample and shaken during 30 min in order to obtain a homogeneous sample due to insolubility of the acetonitrile in the oil. The linearity was evaluated by assessing signal responses of the target analytes from procedural standard calibration by spiking blank samples of olive oil at five concentration levels from 10 to 500 μg kg$^{-1}$. Matrix effect was calculated by comparison of the slopes obtained from analytical curves prepared in acetonitrile and in blank olive oil using the following equation:

$$\text{Matrix effect}(\%) = \left[\left(\frac{\text{slope analytical curve in matrix}}{\text{slope analytical curve in matrix aceonitrile}}\right) - 1\right] \times 100$$

The linearity was accessed also for other two oils, sunflower and soya, and compared with the results obtained from olive oil. This procedure was done to verify the behavior of analytes in different types of oil in order to evaluate if it is possible to quantify residues of different oils with the same calibration curve.

The limit of quantification (LOQ) for each pesticide was settled as the lowest fortified concentration in blank olive oil samples that could be quantified with acceptable accuracy and precision as preconized by Document No. SANTE/11945/2015 [17].

## 3. Results and discussion

### 3.1. Clean-up evaluation

In this study a procedure of low temperature precipitation (freezing-out) was evaluated before clean-up employing various sorbents and combination of sorbents.

Primary-secondary amine (PSA) is a well know clean-up sorbent employed when removal of fat content is necessary. The chemical structure of PSA provides high retention of free fatty acids and other polar matrix compounds [18]. Z-sep is a mixture of two sorbents, C18 and silica, coated with zirconium dioxide. Distinct classes of active sites make the lipid removal efficient when this sorbents is used [19]. Agilent Bond Elut QuEChERS Enhanced Matrix Removal Lipid (EMR-Lipid) is the new generation of sample preparation products for dispersive solid phase extraction (d-SPE) employed for highly selective matrix removal without impacting analyte recovery, especially for high-fat samples [20].

Blank samples of olive oil were spiked with the mixture of analytes at 50 μg kg$^{-1}$, extracted, allowed to freezing out procedure and submitted to clean-up step. For clean-up using PSA, 2 mL of the extract was purified using 300 mg of magnesium sulfate and 50 mg of PSA; for clean-up employing Z-sep (d-SPE), 2 mL of extract were mixed with 500 mg of magnesium sulfate and 50 mg of Z-sep sorbent; for clean-up using Z-sep (cartridge SPE), 1 mL of extract was passed through a cartridge containing 40 mg of Z-sep solid and the collected fraction in acetonitrile was analyzed; for clean-up employing EMR-Lipid, the purification was done as described in Section 2.3. Fig. 1 shows the recoveries and mean relative standard deviation obtained for all clean-up methods evaluated at 50 μg kg$^{-1}$. In terms of recovery Z-sep using cartridges SPE showed the worst results with almost 40% of the pesticides no recovered properly followed by Z-sep using dispersive clean-up. When PSA and EMR were employed the best results were obtained, but PSA showed a mean RSD of 15%. For the EMR method none of the pesticides was completely lost and more compounds showed recovery percentage between 70 and 120% and mean RSD of 7%.

To evaluate the effectiveness of clean-up procedure the blank sample of olive oil was extracted employing EMR-Lipid clean-up and analyzed in a LC-QToF system (6530 Accurate Mass QTOF-MS, Agilent Technologies, Santa Clara, USA) in full scan mode. To obtain the number of co-extractives present in the matrix the data were processed employing MassHunter software. The results can be accessed by visual comparison of full scan chromatograms in Fig. 2, where is possible to see the differences between the full scan chromatograms for each clean-up procedure. Fig. 3 shows the matrix components present in olive oil extract related to its retention times when evaluated the different clean-up procedures employed. As can be seen in Fig. 3, the EMR is the clean-up procedure where more co-extractives are present, but with the best recoveries results as well as acceptable levels for routine analysis. Taking these results into account, the EMR method was chosen as clean-up procedure for pesticide determination in olive oil.

### 3.2. Validation

#### 3.2.1. Accuracy (recovery), precision and repeatability

In order to assess accuracy and precision, blank samples of olive oil were spiked with the pesticide mixture at 10, 20 and 50 μg kg$^{-1}$ with five replicates of each concentration. Table 2 shows the

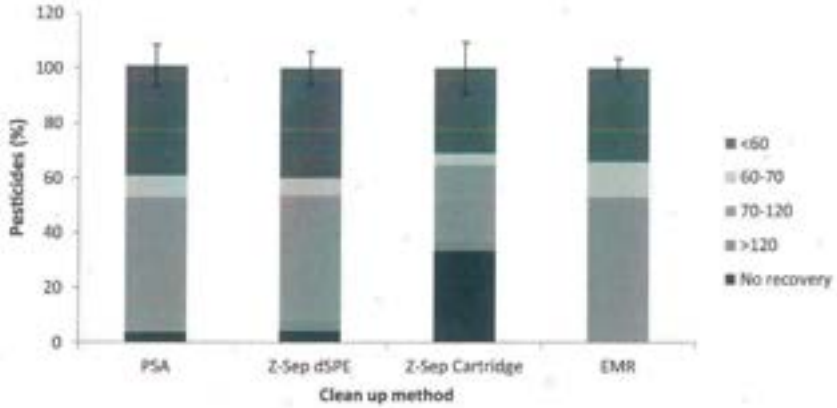


**Fig. 1.** Recovery (%) and RSD (%) for the pesticides determined by LC–QqQ–MS/MS employing four different clean-up methods.

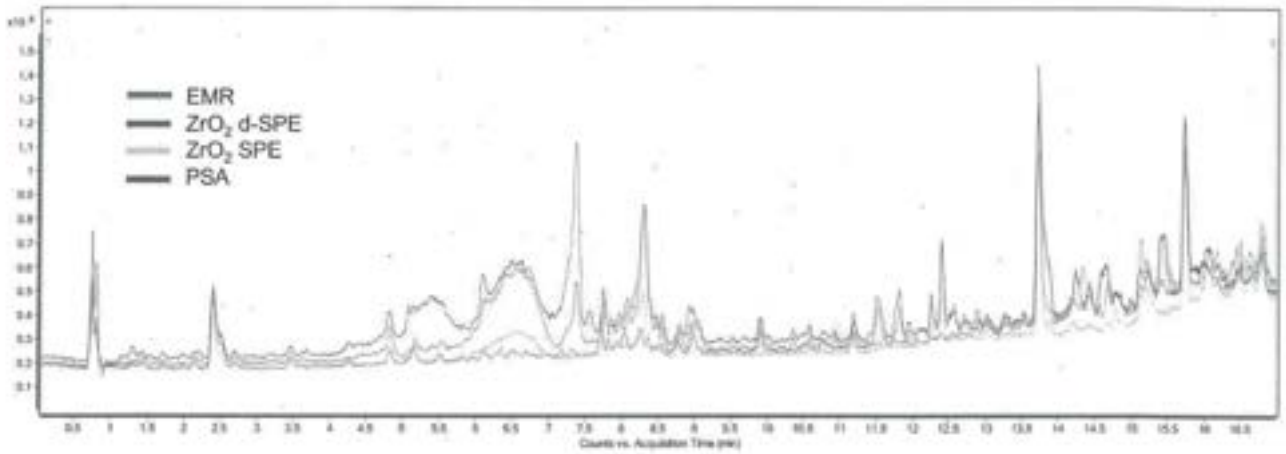


**Fig. 2.** LC-QToF full scan chromatograms from olive oil extracts employing different clean-up procedures.

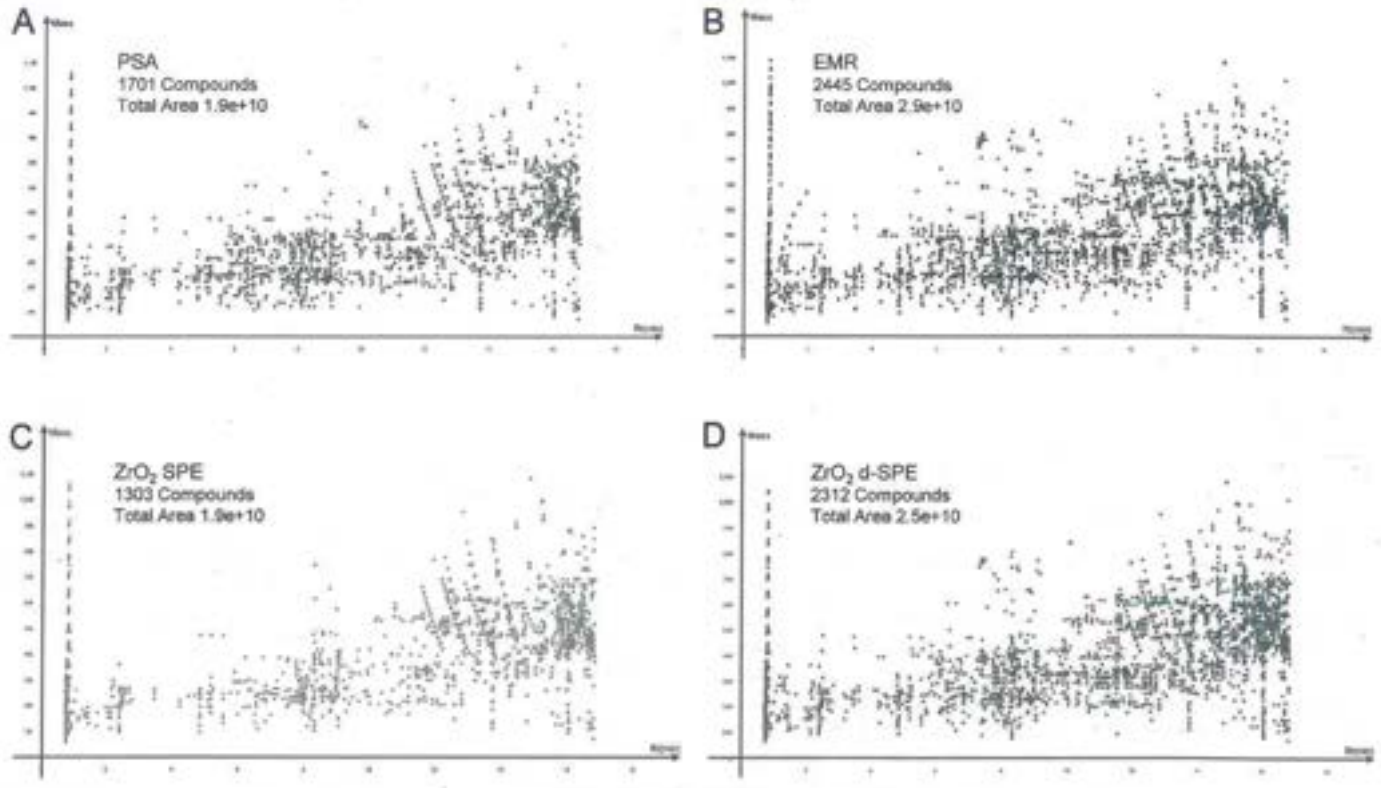


**Fig. 3.** Matrix components of olive oil when extracted employing PSA (A), EMR-Lipid (B), $ZrO_2$ cartridges SPE (C) and $ZrO_2$ dispersive SPE. Each point corresponds to one matrix component.

**Table 2**
Recoveries, relative standard deviation at 10, 20 and 50 μg kg$^{-1}$ (n = 5) and LOQ for the pesticides spiked in blank olive oil sample.

| Compound | Spike level (μg kg$^{-1}$) | | | | | | LOQ (μg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | 10 | | 20 | | 50 | | |
| | Rec (%) | RSD (%) | Rec (%) | RSD (%) | Rec (%) | RSD (%) | |
| 2,4-D | 19 | 11 | 18 | 18 | 14 | 15 | 10 |
| Acephate | 48 | 47 | 46 | 7 | 69 | 6 | 20 |
| Acetamiprid | 116 | 2 | 94 | 8 | 91 | 2 | 10 |
| Aldicarb | 97 | 2 | 102 | 4 | 102 | 6 | 10 |
| Aldicarb sulfone | 144 | 7 | 100 | 13 | 90 | 5 | 10 |
| Aldicarb sulfoxide | 91 | 6 | 73 | 9 | 74 | 8 | 10 |
| Azinphos-methyl | 95 | 12 | 87 | 12 | 79 | 5 | 10 |
| Azoxystrobin | 100 | 8 | 103 | 8 | 110 | 3 | 10 |
| Bifenazate | 40 | 23 | 53 | 9 | 63 | 11 | 20 |
| Bitertanol | 59 | 11 | 61 | 7 | 61 | 8 | 10 |
| Boscalid | 89 | 4 | 74 | 5 | 85 | 3 | 10 |
| Bromuconazole | 60 | 10 | 60 | 7 | 57 | 7 | 10 |
| Bupirimate | 76 | 8 | 73 | 6 | 73 | 5 | 10 |
| Buprofezin | 51 | 6 | 50 | 9 | 48 | 4 | 10 |
| Carbaryl | 97 | 11 | 88 | 6 | 80 | 4 | 10 |
| Carbendazim | 37 | 11 | 38 | 6 | 41 | 6 | 10 |
| Chlorantraniliprol | 95 | 8 | 85 | 6 | 84 | 3 | 10 |
| Chlorfenvinphos | 92 | 6 | 82 | 10 | 83 | 7 | 10 |
| Chlorpyrifos-methyl | 76 | 13 | 58 | 11 | 57 | 4 | 10 |
| Chlorpyriphos | 57 | 13 | 48 | 6 | 51 | 2 | 10 |
| Clofentezin | 61 | 10 | 54 | 5 | 57 | 4 | 10 |
| Clomazone | 82 | 5 | 83 | 7 | 71 | 4 | 10 |
| Coumaphos | 87 | 8 | 87 | 8 | 80 | 7 | 10 |
| Cyazofamid | 108 | 10 | 94 | 5 | 119 | 3 | 10 |
| Cymoxanil | 101 | 3 | 104 | 6 | 101 | 3 | 10 |
| Cyproconazole | 56 | 4 | 53 | 7 | 58 | 6 | 10 |
| Cyprodinil | 34 | 7 | 37 | 5 | 37 | 4 | 10 |
| Cyromazine | 0 | 17 | 1 | 56 | 1 | 33 | 50 |
| Demeton-S-methylsulfone | 105 | 6 | 100 | 6 | 113 | 3 | 10 |
| Demeton-S-methylsulfoxide | 17 | 19 | 16 | 14 | 24 | 15 | 10 |
| Diazinon | 68 | 7 | 65 | 7 | 67 | 5 | 10 |
| Diclorvos | 84 | 7 | 104 | 6 | 93 | 5 | 10 |
| Dicrotophos | 70 | 10 | 75 | 5 | 80 | 7 | 10 |
| Diethofencarb | 92 | 8 | 89 | 5 | 87 | 4 | 10 |
| Difenoconazole | 70 | 7 | 69 | 5 | 66 | 5 | 10 |
| Diflubenzuron | 96 | 11 | 72 | 15 | 82 | 6 | 10 |
| Dimethoate | 115 | 5 | 105 | 8 | 95 | 3 | 10 |
| Dimethomorph | 107 | 6 | 88 | 7 | 86 | 6 | 10 |
| Diniconazole | 43 | 9 | 49 | 7 | 49 | 7 | 10 |
| Dodine | 2 | 17 | 2 | 74 | 1 | 54 | 50 |
| Emamectin B1a | 2 | 16 | 3 | 46 | 7 | 31 | 10 |
| EPN | 56 | 20 | 70 | 11 | 62 | 14 | 10 |
| Epoxiconazole | 89 | 5 | 85 | 8 | 78 | 8 | 10 |
| Ethion | 67 | 21 | 73 | 4 | 69 | 4 | 10 |
| Ethirimol | 7 | 11 | 10 | 38 | 17 | 24 | 50 |
| Ethoprophos | 74 | 3 | 72 | 9 | 68 | 4 | 10 |
| Fenamidone | 86 | 6 | 88 | 5 | 91 | 4 | 10 |
| Fenamiphos | 87 | 8 | 87 | 6 | 85 | 6 | 10 |
| Fenamiphos – sulfone | 93 | 3 | 89 | 7 | 95 | 5 | 10 |
| Fenamiphos – sulfoxide | 15 | 13 | 13 | 12 | 18 | 17 | 10 |
| Fenarimol | 58 | 9 | 51 | 6 | 51 | 6 | 10 |
| Fenazaquin | 20 | 23 | 22 | 7 | 22 | 3 | 10 |
| Fenbuconazole | 90 | 8 | 78 | 12 | 85 | 8 | 10 |
| Fenhexamid | 65 | 5 | 63 | 12 | 56 | 7 | 10 |
| Fenoxycarb | 88 | 6 | 76 | 8 | 79 | 3 | 10 |
| Fenpropimorph | 51 | 13 | 50 | 19 | 72 | 8 | 10 |
| Fenpyrazamine | 102 | 7 | 96 | 6 | 92 | 4 | 10 |
| Fenpyroximate | 48 | 27 | 50 | 6 | 49 | 4 | 10 |
| Fenthion | 65 | 15 | 75 | 11 | 70 | 10 | 10 |
| Fenthion sulfoxide | 117 | 4 | 101 | 5 | 93 | 4 | 10 |
| Fipronil | 102 | 5 | 103 | 8 | 100 | 2 | 10 |
| Fluazifop | 80 | 10 | 72 | 10 | 77 | 3 | 10 |
| Flubendiamide | 102 | 4 | 100 | 3 | 101 | 5 | 10 |
| Fludioxonil | 90 | 3 | 104 | 8 | 90 | 8 | 10 |
| Flufenacet | 97 | 8 | 91 | 5 | 92 | 2 | 10 |
| Flufenoxuron | 83 | 8 | 84 | 9 | 79 | 7 | 10 |
| Fluopyram | 107 | 6 | 100 | 9 | 93 | 5 | 10 |
| Fluquinconazole | 85 | 13 | 74 | 9 | 76 | 9 | 10 |
| Flusilazol | 84 | 8 | 71 | 7 | 76 | 4 | 10 |
| Flutriafol | 63 | 7 | 66 | 4 | 72 | 7 | 10 |
| Formetanate | 18 | 10 | 19 | 23 | 40 | 13 | 10 |
| Fosthiazate | 101 | 6 | 94 | 9 | 93 | 5 | 10 |

Table 2 (Continued)

| Compound | Spike level (μg kg$^{-1}$) | | | | | | LOQ (μg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | 10 | | 20 | | 50 | | |
| | Rec (%) | RSD (%) | Rec (%) | RSD (%) | Rec (%) | RSD (%) | |
| Haloxyfop | 83 | 8 | 76 | 9 | 87 | 11 | 10 |
| Hexaconazole | 36 | 16 | 41 | 5 | 45 | 9 | 10 |
| Hexythiazox | 35 | 18 | 38 | 7 | 36 | 3 | 10 |
| Imazalil | 23 | 12 | 27 | 25 | 46 | 15 | 10 |
| Imidacloprid | 116 | 5 | 94 | 7 | 96 | 3 | 10 |
| Indoxacarb | 100 | 6 | 100 | 8 | 97 | 9 | 10 |
| Ioxonil | 68 | 4 | 63 | 6 | 60 | 4 | 10 |
| Iprovalicarb | 92 | 8 | 85 | 7 | 88 | 7 | 10 |
| Isofenfos methyl | 93 | 7 | 86 | 5 | 79 | 3 | 10 |
| Isoprocarb | 101 | 4 | 86 | 3 | 75 | 2 | 10 |
| Isoxaflutole | 118 | 4 | 117 | 8 | 104 | 6 | 10 |
| Kresoxim methyl | 100 | 4 | 92 | 9 | 85 | 5 | 10 |
| Linuron | 78 | 5 | 82 | 8 | 68 | 7 | 10 |
| Lufenuron | 72 | 26 | 86 | 9 | 84 | 3 | 10 |
| Malathion | 108 | 5 | 94 | 7 | 101 | 4 | 10 |
| Mandipropamid | 125 | 10 | 115 | 6 | 104 | 3 | 10 |
| MCPA | 25 | 14 | 0 | 30 | 21 | 18 | 50 |
| Mepanypirim | 65 | 15 | 62 | 7 | 58 | 3 | 10 |
| Meptyldinocap | 16 | 42 | 18 | 15 | 38 | 9 | 20 |
| Metalaxyl | 91 | 8 | 86 | 8 | 95 | 5 | 10 |
| Metconazole | 52 | 9 | 49 | 5 | 51 | 6 | 10 |
| Methamidophos | 57 | 8 | 56 | 9 | 48 | 4 | 10 |
| Methidathion | 100 | 6 | 93 | 5 | 72 | 7 | 10 |
| Methiocarb | 82 | 4 | 79 | 7 | 72 | 7 | 10 |
| Methiocarb sulfoxide | 94 | 4 | 88 | 7 | 90 | 4 | 10 |
| Methomyl | 106 | 5 | 98 | 7 | 100 | 3 | 10 |
| Methoxyfenozide | 109 | 6 | 92 | 12 | 95 | 5 | 10 |
| Metobromuron | 83 | 1 | 78 | 5 | 71 | 2 | 10 |
| Monocrotophos | 120 | 6 | 96 | 6 | 77 | 5 | 10 |
| Myclobutanil | 85 | 7 | 82 | 7 | 82 | 3 | 10 |
| Nitempyram | 29 | 5 | 24 | 13 | 34 | 7 | 10 |
| Omethoate | 64 | 14 | 55 | 7 | 63 | 5 | 10 |
| Oxadixyl | 106 | 3 | 100 | 5 | 108 | 2 | 10 |
| Oxamyl | 134 | 6 | 102 | 10 | 89 | 5 | 10 |
| Paclobutrazol | 75 | 6 | 71 | 6 | 73 | 6 | 10 |
| Paraoxon methyl | 112 | 10 | 104 | 9 | 100 | 6 | 10 |
| Penconazole | 50 | 7 | 56 | 10 | 62 | 5 | 10 |
| Penicycuron | 65 | 11 | 61 | 5 | 59 | 4 | 10 |
| Pendimethalin | 42 | 18 | 46 | 7 | 47 | 5 | 10 |
| Phenthoate | 93 | 10 | 80 | 9 | 79 | 7 | 10 |
| Phosalone | 74 | 16 | 70 | 3 | 80 | 11 | 10 |
| Phosmet | 123 | 2 | 106 | 3 | 91 | 5 | 10 |
| Phoxim | 89 | 11 | 83 | 11 | 88 | 6 | 10 |
| Pirimicarb | 80 | 5 | 76 | 6 | 81 | 3 | 10 |
| Pirimicarb, desmethyl- | 50 | 10 | 48 | 5 | 57 | 5 | 10 |
| Pirimiphos-methyl | 67 | 6 | 64 | 7 | 63 | 4 | 10 |
| Prochloraz | 53 | 7 | 54 | 6 | 57 | 4 | 10 |
| Profenofos | 58 | 9 | 54 | 4 | 49 | 6 | 10 |
| Propamocarb | 2 | 20 | 3 | 52 | 6 | 26 | 50 |
| Propaquizafop | 69 | 21 | 71 | 7 | 73 | 3 | 10 |
| Propargite | 64 | 36 | 79 | 7 | 66 | 11 | 20 |
| Propiconazole | 66 | 15 | 66 | 6 | 65 | 4 | 10 |
| Propoxur | 110 | 3 | 94 | 9 | 92 | 3 | 10 |
| Propyzamide | 82 | 5 | 82 | 8 | 72 | 4 | 10 |
| Proquinazid | 17 | 20 | 18 | 6 | 17 | 5 | 10 |
| Prothioconazole | 2 | 65 | 4 | 48 | 4 | 23 | 50 |
| Prothiofos | 31 | 34 | 38 | 35 | 30 | 11 | 50 |
| Pymetrozine | 0 | 0 | 0 | 0 | 3 | 17 | 50 |
| Pyraclostrobin | 87 | 7 | 80 | 9 | 75 | 5 | 10 |
| Pyrethrin | 56 | 27 | 64 | 7 | 56 | 4 | 20 |
| Pyridaben | 33 | 34 | 36 | 6 | 38 | 5 | 20 |
| Pyridate | 31 | 43 | 29 | 25 | 20 | 16 | 50 |
| Pyrimethanil | 53 | 4 | 41 | 8 | 47 | 6 | 10 |
| Pyriproxyfen | 41 | 18 | 44 | 7 | 43 | 3 | 10 |
| Quinoclamine | 147 | 16 | 90 | 22 | 68 | 7 | 10 |
| Quinoxyfen | 23 | 16 | 24 | 6 | 22 | 5 | 10 |
| Quizalofop-ethyl | 65 | 11 | 64 | 7 | 63 | 3 | 10 |
| Rotenone | 84 | 5 | 90 | 9 | 77 | 5 | 10 |
| Spinosyn A | 23 | 12 | 26 | 29 | 44 | 17 | 10 |
| Spinosyn D | 24 | 16 | 26 | 28 | 45 | 17 | 10 |
| Spirodiclofen | 59 | 5 | 60 | 6 | 56 | 6 | 10 |
| Spiromesifen | 75 | 25 | 77 | 10 | 69 | 5 | 10 |
| Spirotetramat | 98 | 7 | 86 | 8 | 90 | 6 | 10 |

Table 2 (Continued)

| Compound | Spike level (μg kg$^{-1}$) | | | | | | LOQ (μg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | 10 | | 20 | | 50 | | |
| | Rec (%) | RSD (%) | Rec (%) | RSD (%) | Rec (%) | RSD (%) | |
| Spiroxamine | 50 | 9 | 46 | 16 | 68 | 10 | 10 |
| Tebuconazole | 51 | 10 | 57 | 6 | 56 | 7 | 10 |
| Tebufenozide | 103 | 5 | 93 | 6 | 92 | 4 | 10 |
| Tebufenpyrad | 46 | 13 | 50 | 4 | 44 | 4 | 10 |
| Teflubenzuron | 60 | 11 | 65 | 7 | 68 | 4 | 10 |
| Terbuthylazine | 60 | 4 | 62 | 4 | 58 | 4 | 10 |
| Tetraconazole | 93 | 5 | 92 | 5 | 94 | 5 | 10 |
| Thiabendazol | 32 | 7 | 33 | 9 | 44 | 9 | 10 |
| Thiacloprid | 109 | 2 | 98 | 3 | 101 | 2 | 10 |
| Thiamethoxam | 112 | 7 | 94 | 8 | 96 | 4 | 10 |
| Thiobencarb | 49 | 6 | 51 | 9 | 44 | 5 | 10 |
| Thiodicarb | 111 | 5 | 103 | 5 | 104 | 4 | 10 |
| Tolclofos-methyl | 63 | 7 | 58 | 17 | 56 | 11 | 10 |
| Triadimenol | 67 | 6 | 69 | 7 | 69 | 7 | 10 |
| Triazophos | 105 | 5 | 87 | 5 | 88 | 7 | 10 |
| Trichlorfon | 102 | 4 | 94 | 5 | 93 | 5 | 10 |
| Trifloxystrobin | 100 | 8 | 95 | 4 | 93 | 5 | 10 |
| Triflumuron | 77 | 7 | 77 | 8 | 75 | 5 | 10 |
| Triticonazole | 48 | 5 | 48 | 7 | 56 | 10 | 10 |
| Zoxamide | 84 | 6 | 78 | 3 | 70 | 3 | 10 |

recovery, RSD% and method LOQ results for the pesticides quantified by matrix matched calibration. As can be seen in the Table 2, the mean recovery for all spike levels was about 70% with mean RSD% of 11, 10 and 7 for the levels 10, 20 and 50 μg kg$^{-1}$, respectively. The majority of compounds that did not fulfill the requirements for recovery demonstrated good RSD values, most of them lower than 10%. Taking these results into account, the linearity studies as well as evaluation of real samples were done applying procedural standard calibration approach (PSC), an alternative type of calibration that can compensate for low extraction recoveries and matrix effects [17].

Repeatability was evaluated by preparing standards solutions in olive oil blank extract at 10, 20 and 50 μg kg$^{-1}$, and injected 5 times. As can be observed in Table S1 (see in the online version at DOI: http://dx.doi.org/10.1016/j.chroma.2016.07.072), all pesticides showed a RSD ≤20% for the three evaluated concentrations and excellent reproducibility making the use of procedural standard calibration feasible.

### 3.2.2. Calibration curves, linearity and matrix effect

Linearity of analytical curves was evaluated via procedural standard calibration (PSC) by spiking blank olive oil portions at 10, 20, 50, 100 and 500 μg kg$^{-1}$. From the 165 evaluated pesticides, 154 (93%) showed correlation coefficient ($r^2$) ≥ 0.99 for analytical curves evaluated using procedural standard calibration approach. However, the other 11 compounds showed $r^2 \geq 0.98$ demonstrating the good linearity of the method in the range from 10 to 500 μg kg$^{-1}$. For analytical curves solutions in acetonitrile, just prothioconazole and pymetrozine showed $r^2 < 0.99$, both had $r^2 = 0.98$.

The same procedure (PSC) was applied for sunflower and soya oil in order to check the behavior of the analytes in different oils. For soya oil only six compounds showed $r^2 < 0.99$ but higher than 0.98. For sunflower oil, six compounds showed $r^2$ between 0.97 and 0.99 and all other compound demonstrated correlation coefficient >0.99. For all analytical curves evaluated in all oils the residuals were <20%.

For matrix effect evaluation, results obtained for analytical curves prepared in matrix extract by PSC for all oils studied were compared. As expected, the matrix effect was very similar among all oils studied as can be seen in Fig. 4 and in Table S1 (see in the online version at DOI: http://dx.doi.org/10.1016/j.chroma.2016.07.072). In the Table S1 (see in the online version at DOI: http://dx.doi.org/10.1016/j.chroma.2016.07.072), the pesticides were grouped according to their chemical group in order to demonstrate the behavior of the different pesticides classes when evaluated in different oils. Taking the results obtained is possible to see the similarities of signals among all different oils and, with this, the quantification of pesticides is possible to be done with the same analytical curve for any oil.

### 3.2.3. Analysis of real samples

In order to apply the developed method in real samples, 15 samples (9 of olive oil, 3 of soya oil and 3 of sunflower oil) were purchased from local markets in Almería city, southeastern of Spain. Two samples of olive oil came from farmers that produce their own oil. From 9 olive oil samples from supermarket (extra virgin oil), four showed no contamination by any evaluated pesticide. In the other five at least one pesticide was found being dimethoate found in five samples. With exception of one sample that was contaminated with chlorpyrifos at 11 μg kg$^{-1}$ all the other five olive oil samples showed contamination below the method limit of quantification (10 μg kg$^{-1}$). From the domestic olive oil samples, one of them showed contamination by phosmet at 194 μg kg$^{-1}$, tetraconazol at 31 μg kg$^{-1}$, chlorpyrifos at 23 μg kg$^{-1}$ and fluopyran at 22 μg kg$^{-1}$, besides tebuconazol where the residue was found below the limit of quantification of 10 μg kg$^{-1}$. The other domestic olive oil was contaminated with chlorpyrifos methyl at 25 μg kg$^{-1}$

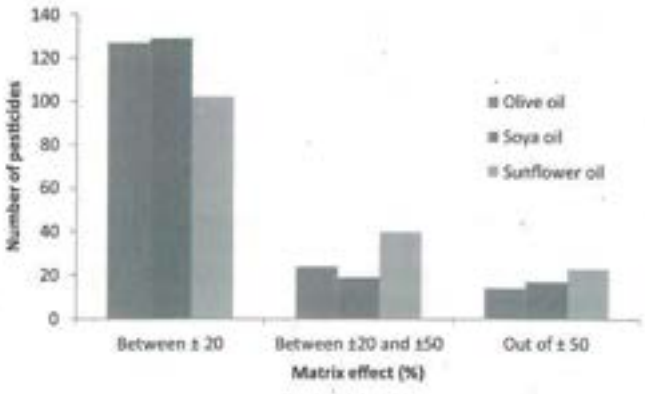


**Fig. 4.** Matrix effect evaluated by comparing slopes obtained from analytical curves prepared in acetonitrile and in matrix extract by procedural standard calibration.

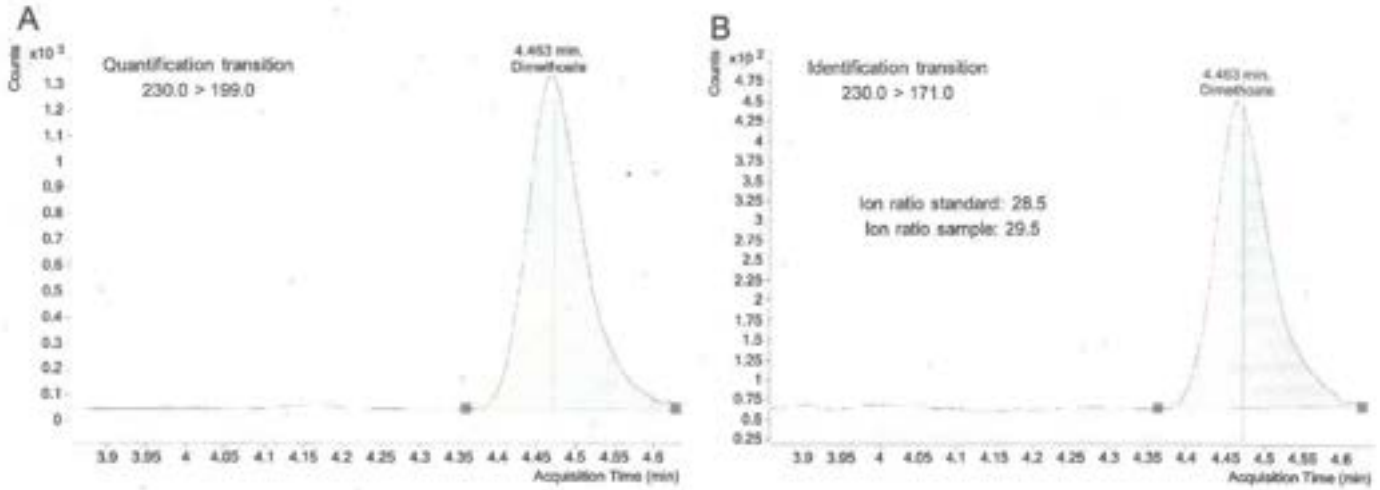


**Fig. 5.** SRM transitions peaks for dimethoate found in samples showing (A) quantification transition and (B) identification transition as well as ion ratio.

besides diazinon, dimethoate and pyriproxyfen below the LOQ. Dimethoate was the most found compound in olive oil samples but below the LOQ. Despite of that, in Fig. 5 is possible to see that despite the signal was below the LOQ the peak shape, quantification and confirmation transitions, retention time and ion ratio matched the criteria for quantification, demonstrating the good performance of the method.

For sunflower oil (three samples) no contamination was registered by any of the evaluated pesticides. On the other hand for soya oil, from three evaluated samples two of them showed contamination by azoxystrobin below the LOQ. For the other sample, no contamination was observed.

## 4. Conclusions

A multiresidue method for the determination of 165 pesticides in edible oils (olive oil, sunflower oil and soya oil) employing low temperature precipitation procedure and clean-up using Agilent Bond Elut QuEChERS Enhanced Matrix Removal-Lipid (EMR-Lipid) was developed and validated allowing quantification levels of 10 $\mu g\,kg^{-1}$ for 91% of studied compounds. The use of EMR-Lipid in combination with freezing-out showed important advantages such as more pesticides with recovery between 70 and 120% range and no pesticide losses when compared with other clean-up procedures evaluated in this study such as PSA and Z-sep sorbent. The EMR-Lipid showed as the only drawback the use of more extract (5 mL) for clean-up procedure when compared to the other approaches. Validation was done using procedural standard calibration approach (PSC), an alternative type of calibration that compensate for recovery losses, showing good results. The method was employed to analyze 15 samples of oils sold in supermarkets as well as two domestic olive oil samples.

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ALL. C

## GENERICI

**1 Una cartella può contenere?**

- File o cartelle
- Solo i file
- File e menù
- File e CD

**2 Cos'è la RAM ?**

- Un software
- Un file
- Una cartella
- Una memoria

## WORD PROCESSOR

**3 Quali comandi devo usare per spostare del testo?**

- Sposta e Copia
- Copia e Incolla
- Copia e Sposta
- Taglia e Incolla

**4 Formattare un testo significa ...**

- Copiare il formato del testo
- Cambiare forma al testo
- Cancellare il testo
- Copiare il testo

**5 Per NON salvare le modifiche fatte ad un documento?**

- Dalla barra dei menu, clicco su File - Esci
- Chiudo il documento e dalla finestra che appare clicco su ANNULLA
- Dalla barra dei menu, clicco su File - Salva
- Chiudo il documento e dalla finestra che appare clicco su NO

**6 Se applico l'elenco puntato, cosa succede ?**

- Viene inserita una tabella con punti e simboli
- All'inizio di ogni paragrafo viene inserito un punto o un simbolo
- Viene inserito un elenco di punti e simboli
- All'inizio di ogni riga viene inserito un punto o un simbolo

## FOGLIO DI CALCOLO

**7 Come viene identificata una cella in Excel?**

- Mediante la barra di stato
- Mediante la barra della formula
- Mediante la selezione della cella
- Mediante una riga e una colonna

**8 Se in una cella digito: =5+3*2 e premo invio, il risultato è ?**

- 11
- 0
- 16
- errore

**9 Quale tipo di dati puoi inserire in singola cella?**

- Solo numeri
- Solo numeri e formule
- Solo testo e numeri
- Testo, numeri e formule

**10 Se in una cella digito ,35 (virgola tre cinque) e premo invio, cosa verrà visualizzato ?**

- ,35
- #nome
- 0,35
- errore

**Cognome Nome**

**Firma**