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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^{TM}); (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