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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

Jonatan V. Dias[a], Victor Cutillas[b], Ana Lozano[b], Ionara R. Pizzutti[a], Amadeo R. Fernández-Alba[b,*]

[a] Federal University of Santa Maria (UFSM), Chemistry Department, Center of Research and Analysis of Residues and Contaminants (CEPARC), 97105-900, Santa Maria, RS, Brazil
[b] European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables (EURL-FV), University of Almeria, Agrifood Campus of International Excellence (ceiA3), Almeria, Spain

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ABSTRACT

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

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].

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].

* Corresponding author.
E-mail address: amadeo@ual.es (A.R. Fernández-Alba).

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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