Talanta 112 (2013) 1–10

Contents lists available at SciVerse ScienceDirect

Talanta

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

Development and validation of an easy multiresidue method for the determination of multiclass pesticide residues using GC–MS/MS and LC–MS/MS in olive oil and olives

C. Anagnostopoulos *, G.E. Miliadis

Laboratory of Pesticide Residues, Department of Pesticides Control and Phytopharmacy, Benaki Phytopathological Institute, 8 St. Delta str., GR-145 61 Kifissia (Athens), Greece

ARTICLE INFO

Article history:
Received 22 November 2012
Received in revised form
14 March 2013
Accepted 20 March 2013
Available online 27 March 2013

Keywords:
GC–MS/MS
LC–MS/MS
Olive
Olive oil
Validation
Dispersive solid phase extraction

ABSTRACT

Olives and olive oil are two of the most important commodities produced in the Mediterranean region. Due to their significant economical importance, the usage of pesticides in their production is systematic, by using a wide range of plant protection products with a variety of modes of action. As a consequence, monitoring of their residue levels in this products is a necessity. In the present study a gas and liquid chromatography-tandem mass spectrometry multiresidue method, with a short sample preparation step, based on acetonitrile extraction is developed and validated according to the European Union guidelines (SANCO Doc. No. 12495/2011) in olives and olive oil, with a large scope that includes pesticides of different chemical classes. Good sensitivity and selectivity of the method were obtained with limits of quantification at 10 μg/kg. All pesticides had recoveries in the range of 70–120%, with relative standard deviation values less than 20–25%, at both validation levels. Excellent linearity was achieved with $r \geq 0{,}99$ for both matrices. The method is easy, with low consumption of reagents, is characterized by reliability, sensitivity and therefore is suitable for the monitoring the levels of multiclass pesticides residues in olives and olive oil. The method was applied to 262 samples of the Greek market, of which 7% were found positive for the present of pesticides. In some of the samples 2–8 different analytes were detected.

1. Introduction

The most important cultivation in the Mediterranean region since ancient times to today is the olive tree. The European Union is the biggest consumer of olive oil by 1.3 million tons annually. According to the Greek Ministry of Rural Development and Food, in 2009 over 150 million trees were cultivated in Greece to produce table olives and olive oil. The importance of olive tree cultivation in Europe and worldwide makes the intensive use of agrochemicals essential. In Greece alone, about 180 formulations are approved for use in the cultivation of olive trees, containing active substances belonging to various chemical groups.

Monitoring of pesticide residues is crucial, as it assures that applications are made according to the proposed good agricultural practices and the product is safe for the consumer. Due to the importance of olive production, the number and the variety, as to their chemical class, of the active substances used in plant protection products is continuously growing and as a result the determination of their residues in the final product is becoming more and more challenging.

The main ingredients of olives and olive oil that play important role in the determination of residues of pesticides, in the terms of coextracted matrix interferences, are lipids [1] and pigments. Most analytical methods focus on the removal of lipids, since besides separation and sensitivity problems, the continuous analysis of many samples, during routine work, can cause problems in the chromatographs (e.g. block the analytical column or liner contamination). The most common approaches for cleanup are solid phase extraction (SPE) [2–4], dispersive solid phase extraction [5–7] and gel permeation chromatography (GPC) [8,9]. Other approaches like on-line reversed phase LC-GC [10], are also used in a smaller scale. The above extraction procedures are mostly combined with GC or LC and mass spectrometry (MS). The use of tandem mass spectrometry (MS/MS) in combination with GC or LC is gaining ground as it provides better sensitivity and confirmation reliability.

In the current study the use of a tandem mass spectrometry (MS/MS) technique combined with GC and LC was investigated for the determination of pesticide residues in olives and olive oil. The sample preparation procedure, based on acetonitrile, was developed as to be easy, economic and with sufficient clean up for both fatty component and pigments. The method was assessed as for the sub-sampling homogeneity, the matrix effect and was validated according to EU guidelines [1] for a variety of analytes, with

* Corresponding author. Tel.: +30 210 8180359.
E-mail address: c.anagnostopoulos@bpi.gr (C. Anagnostopoulos).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.03.051

different physicochemical properties, as to assure the flexibility of its scope.

2. Experimental

2.1. Reagents and standards

Pesticide reference standards (purity > 98%) of all analytes were purchased from ChemService and Ehrenstorfer GmbH. All solvents, namely acetonitrile, methanol and water were of HPLC grade. Primary secondary amine sorbent (PSA, 40 μm, Bondesil) was purchased from Varian Inc., USA. Magnesium sulfate dried was purchased from Acros Organics and graphitized carbon black (GCB) was purchased from Sigma-Aldrich Chemie GmbH.

2.2. Apparatus

GC–MS/MS analysis was performed by a Varian 3800 gas chromatograph connected to a triple quadrupole mass spectrometer (Varian model 1200 L). Samples were injected with a CP 8400 autosampler, using a 10 μL syringe, into a 1079 programmed temperature injector operated with the large volume injection technique (PTV-LVI). A Factor Four Capillary Column VF-5 ms 30 × 0.25 mm I.D. × 0.25 μm film thickness, with a guard column (fused silica untreated capillary column 5 × 0.53 mm I.D. cyanophenyl-methyl deactivated) from Varian Inc. was used for the chromatographic separation of the compounds. The mass spectrometer was operated at the electron impact mode (EI). For instrument control, data acquisition and processing, Varian MS Workstation software version 6.8 was used.

The LC–MS/MS analysis was performed by an Agilent Series 1200 liquid chromatograph with a degasser (G1379B), autosampler (Hip/ALS G1367A) with thermostat (FC/ALS Therm G1330B), binary pump (G1312A), a thermostated column department (TCC G1316A) equipped with a reverse phase Zorbax Eclipse XDB C_{18} 3.5 μm particle size, 150 mm × 2.1 mm analytical column (Varian, Palo Alto, CA, USA). and a triple quadrupole mass spectrometer (Agilent Triple Quad 6410) equipped with an electrospray ionization interface operating at positive mode (ESI+).

2.3. Selection of pesticides

A wide variety and number of pesticides are used in olive tree cultivation. For the validation of the method, a representative group of analytes was selected as to ensure that acceptable performance is achieved for all other analytes of the same chemical classes. The selected pesticides were insecticides, fungicides and herbicides of 32 different chemical groups, including: *Acylalanines*, *Anilinopyrimidines*, *Aromatic hydrocarbons*, *Aryloxyphenoxypropionic acids*, *Aryloxyphenoxypropionic esters*, *Benzamides*, *Benzenedicarboxylic acids/esters*, *Benzilates*, *Benzimidazoles*, *Benzofurans*, *Carbamates*, *Chloroacetamides*, *Cinnamic acids*, *Dicarboximides*, *Dinitroanilines*, *Imidazoles*, *Morpholines*, *Neonicotinoids*, *Organochlorines*, *Organophosphorous*, *Oxadiazoles*, *Phenylureas*, *Phosphorothiolates*, *Pyrethroids*, *Pyridinecarboxamides*, *Pyrimidinamines*, *Pyrimidinols*, *Strobilurins*, *Tetrazines*, *Triazines*, *Triazoles* and *Ureas*. Important pesticide metabolites e.g. aldicard sulfone and sulfoxide, metabolites of the carbamate fungicide aldicab, were also selected, since they are of high toxicity and are therefore included in the residue definition of the parent compound.

2.4. Preparation of standard solutions

The stock solutions of the individual pesticide standards were prepared by accurately weighing 10–50 mg of each analyte in volumetric flasks (certified 'A' class) and dissolving in 10 mL acetone, acetonitrile or methanol depending on the analytes solubility. The stock standard solutions were stored at −20 °C. A single composite working standard solution was prepared by combining aliquots of each stock solution and diluting in acetonitrile to obtain a final concentration of 1 mg/L. The working standard solution was also stored at −20 °C and before each use was left to reach room temperature. From this working standard solution, 3 series of calibration standards were prepared within the range of 5–100 μg/L by serial dilution in acetonitrile, olive extract and oil extract respectively.

2.5. Extraction procedure and analysis

Five grams of homogenized sample were weighted in a 50 mL polypropylene centrifuge tube and extracted with 10 mL acetonitrile for 1 min, shaking vigorously by hand. The sample was then centrifuged at 4000 rpm for 5 min and stored in the freezer (−20 °C) for at least 12 hours. Freezing is a critical part of the extraction procedure as it helps to partly remove some additional co-extractives with limited solubility in acetonitrile while the major part of fat and waxes solidify and precipitate. No additional cleanup of fatty components is conducted or required. An aliquot of 6 mL of the still cold acetonitrile phase was transferred into a 15 mL centrifuge tube containing 150 mg of PSA, 12.5 mg GCB and 900 mg of $MgSO_4$, the tube was shaken vigorously for 1 min and centrifuged for 5 min at 4000 rpm. The final extract was transferred into a screw cup storage vial, taking care to avoid sorbent particles of being carried over, and stored in the freezer until analysis. Before injecting in the chromatographic system, the final solution was filtered through a 0.45 μm disposable PTFE syringe filter,. Following this extraction procedure the concentration C in mg/kg of the analytes in the sample correspond to $2 \times C$ in μg/mL of the analytes in the final extract.

2.6. Method Validation

The validation of the method was preformed according to the newest EU guidelines [1]. Analytical parameters evaluated were mean recovery (as a measure of trueness), repeatability (as a measure of precision) linearity, homogeneity during sub-sampling, and sensitivity.

2.7. Confirmation criteria

For initial identification of the analytes the retention time (R.T.) criterion was used. The R.T. of the analyte was matched based on a calibration standard at a tolerance of ± 0.5% for GC and ± 2.5% for LC. The final confirmation of a target compound, initially identified by RT, was done according to the criteria laid down in document SANCO 12495/2011 [1]. The permitted tolerances for the relative ion intensities (% of base peak) in MS/MS techniques according to this document are as follows:

- For relative intensity more than 50% the tolerance is ± 20%.
- For relative intensity between 20–50% the tolerance is ± 25%.
- For relative intensity between 10–20% the tolerance is ± 30%.
- For relative intensity less than 10% the tolerance is ± 50%.

3. Results and discussion

3.1. Optimization of MS/MS parameters

The ionization and fragmentation of the pesticides and metabolites was studied prior to the validation of the method. A large volume injection was used in GC in order to increase sensitivity.