PESTICIDE RESIDUE RESEARCH GROUP

European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables
UNIVERSITY OF ALMERÍA, ALMERÍA, SPAIN

UNIVERSITY OF ALMERIA

Development and validation of a new multiresidue method for the determination of multiclass pesticide residues using GC-QqQ-MS/MS in edible oils

Elena Hakme[1], Samanta Uclés[1], Piedad Parrilla-Vázquez[1], Miguel Gamón[2], Víctor Cutillas[1], Amadeo R. Fernández-Alba[1]

[1]Agrifood Campus of International Excellence (ceiA3), European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables. University of Almería (Spain)
[2]Pesticide Residue Laboratory (Agro-Food Analysis Service) of the Generalitat Valenciana,, Burjassot, Valencia, Spain

Introduction

Determination of multiresidue pesticides in oil matrices is a challenging analytical task because of the complexity of the matrix due the high lipid product content. Sample preparation and clean up is the crucial step in the analysis of pesticides in oil since even small amount of lipids can cause damage to analytical instrumentation and harm liner, column and detector in case of GC. On the other hand, lipid from high lipid content products may adversely affect target analytes recoveries and matrix effects, may change peak shape and decreases in the separation efficiency. The traditional approaches for pesticides residues in oil are either time consuming, solvent consuming or resulting in bad recoveries and repeatability and blank interferences.

In the present work, a new approach to evaluate pesticide residues in edible oils by GC-QqQ-MS/MS was developed. The method consists in an improvement of the clean up step that removes in a high percentage the naturally occurred co-extracted compounds (i.e. fatty acids). A modified QuEChERS protocol with a fast freezing out step followed by the application of d-SPE by using a new sorbent named Enhanced Matrix Removal-lipid (EMR™). The efficiency of the new proposed method was evaluated in olive oil by comparison with the d-SPE procedures (PSA/$MgSO_4$ and Supel™ Z-Sep) and to a SPE procedure based on Z-Sep cartridges. The best results of repeatability and recovery were obtained with the EMR sorbent. Recoveries with EMR ranged typically from 40 to 120% with relative standard deviation lower than 5% for all studied pesticides. Limits of quantification of 10 µg kg^{-1} for most of the compounds were obtained. The extraction with EMR sorbent was later studied on different kinds of olive, sunflower and soybean oils. This method was also applied on real samples.

Extraction methods studied in olive oil

d-SPE (EMR)

- 15 g of sample + 15mL of AcN
- Shake 4 min automatically
- Add 6g $MgSO_4$ + 1.5g NaCl + 1.5 g NaCit·$2H_2O$ + 0.75 g NaCit·1.5 H_2O
- Shake 4 min automatically
- Centrifuge for 5 min at 3500 rpm
- Take 8 mL from the upper layer and freeze it out for about 10 min
- Transfer the 5 mL freezing layer into a d-SPE tube (EMR-Lipid) with 5 mL of water
- Vortex 30 s.
- Centrifuge for 5 min at 3500 rpm
- Transfer 5 mL upper layer into Polish tubes
- Centrifuge for 5 min at 3500 rpm
- Analysis

d-SPE (PSA)

- 10g of sample + 10 mL of AcN
- Shake 4 min automatically
- Add 4g $MgSO_4$ + 1 g NaCl + 1 g NaCit·$2H_2O$ + 0.5 g NaCit·1.5 H_2O
- Shake 4 min automatically
- Centrifuge for 5 min at 3500 rpm
- Take 5 mL from the upper layer and freeze it out for about 7 min
- Transfer the 2 mL freezing layer to a d-SPE tube (300 mg $MgSO_4$ and 50 mg PSA)
- Vortex 30 s.
- Centrifuge for 5 min at 3500 rpm
- Analysis

d-SPE (Z-Sep)

- 10g of sample + 10g of AcN
- Shake 4 min automatically
- Add 4 g $MgSO_4$ + 1 g NaCl + 1 g NaCit·$2H_2O$ + 0.5g NaAc·1.5 H_2O
- Shake 4 min automatically
- Centrifuge for 5 min at 3500 rpm
- Take 5 mL from the upper layer and freeze it out for about 7 min
- Transfer the 2 mL freezing layer to a d-SPE tube (300 mg $MgSO_4$ and 50 mg Z-Sep)
- Vortex 30 s.
- Centrifuge for 5 min at 3500 rpm
- Analysis

SPE (Z-Sep)

- 10g of sample + 10g of AcN
- Shake 4 min automatically
- Add 4 g $MgSO_4$ + 1 g NaCl + 1 g NaCit·$2H_2O$ + 0.5g NaCit·1.5 H_2O
- Shake 4 min automatically
- Centrifuge for 5 min at 3500 rpm
- Take 5 mL from the upper layer and freeze it out for about 7 min
- Conditioning the SPE cartridge with 1 mL of AcN and transfer 1 mL sample through the cartridge
- Analysis

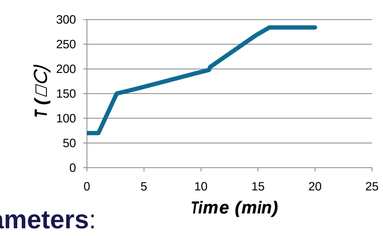
Enhanced Matrix Removal-Lipid

GC-MS/MS specifications

System: 7890 GC System coupled to a 7000 GC/MS Triple Quad (Agilent)

GC parameters:

Injection volume: 2 µL
Splitless mode
Column: HP5MSUI (15 m x 250 µm x 0.25 µm)
Carrier gas: Helium
Constant pressure: 16.19 psi
Total run time: 20 min + 3 min backflush at 280°C
Oven temperature program:

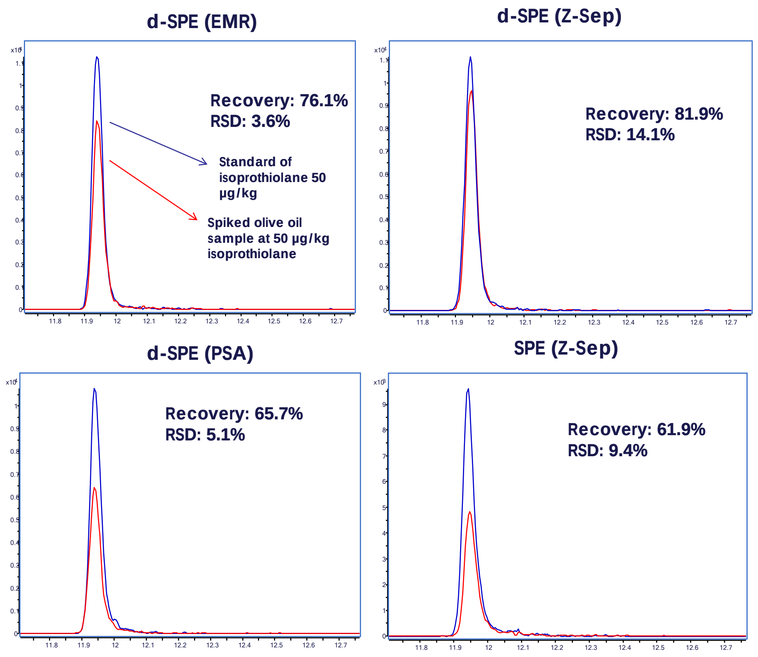


MS parameters:

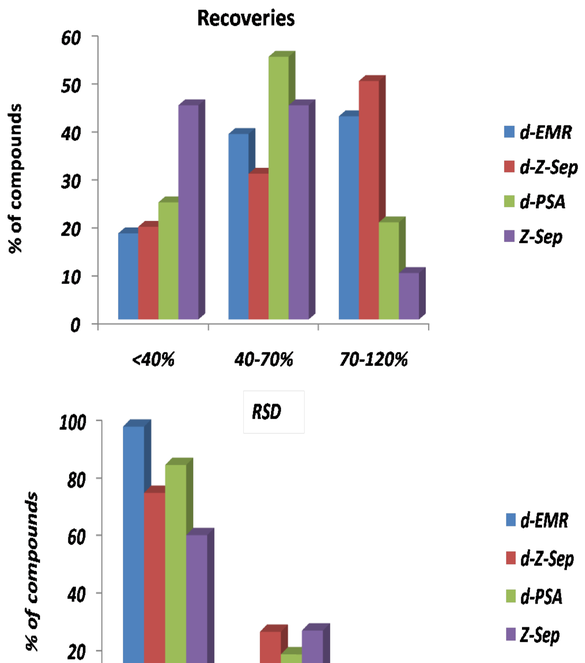
EI mode
MRM acquisition mode
39 time segments
Collision gas: Nitrogen
Collision gas flow: 1.5 mL/min
Quenching gas: Helium
Quenching gas flow: 2.25 mL/min

Recoveries and RSDs obtained with the 4 different extraction and clean-up procedures

For each extraction procedure, a blank of olive oil and 5 replicates of spiked olive oil with a Mix of 213 pesticides at 50 µg kg^{-1} level were extracted. QueChERS protocol followed by clean-up step with d-SPE (Z-Sep) gave the best recoveries with a mean of 67.4% but clean-up step with EMR gave the lowest RSD (4%).



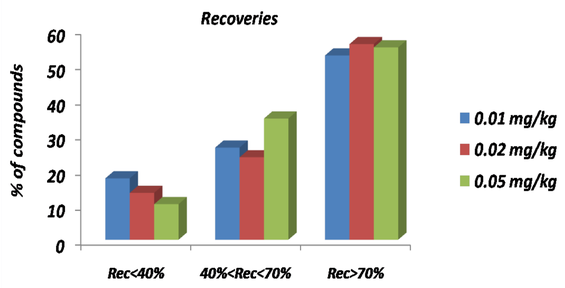
Isoprothiolane with different extraction methods

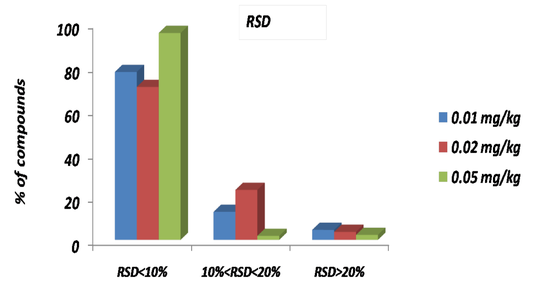


Method validation using EMR procedure

Recoveries

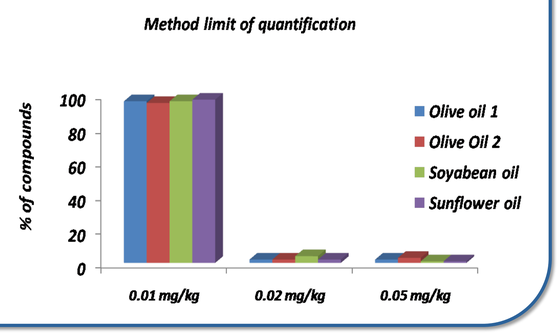
Recovery and reproducibility where evaluated by spiking pesticides standard in olive oil samples at the levels of 10, 20 and 50 µg kg^{-1}. Recoveries were between 70 and 120% for 55% of the compounds.



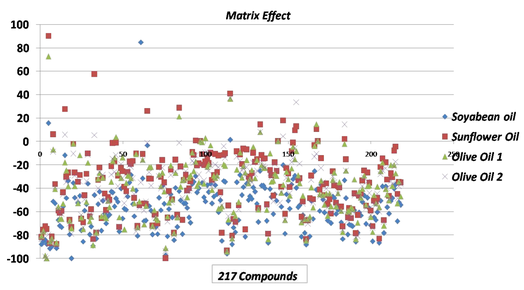


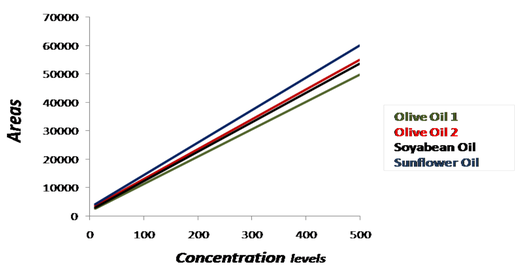
Linearity

Linearity of the method were evaluated by assessing the signal responses of target analytes from matrix-matched calibration solutions by spiking blank extracts at five concentration levels (10, 20, 50, 100 and 500 µg.kg^{-1}). In all cases the residuals were below 20% and coefficients of correlation (R^2) were higher than 0.99.



Matrix effect

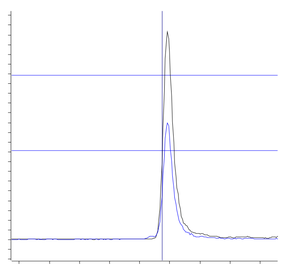




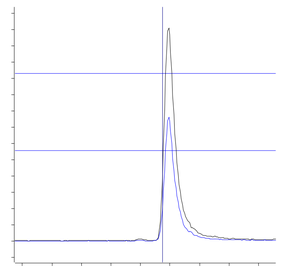
Similar matrix effect was noticed for different kind of oils with a RSD lower than 20% for 80% of the compounds, being soya bean oil slightly different from the rest .

Real oil samples analysis

A total of 17 different oil samples were analyzed and quantified using procedural standard calibration. The pesticides present were chlorpyrifos, chlorpyrifos methyl, deltamethrin, endosulfan sulphate, phosmet, tetraconazole and fluopyram. All the detected pesticides in oil samples were below the EU-MRLs. It was noticed that the detected pesticides were only in olive oil samples with no detection of any in soybean or sunflower ones.



Phosmet std
100 µg/kg



Phosmet identified in olive oil sample at a concentration of 156 µg/kg

Conclusions

This new method based on QuEChERS protocol followed by clean-up step using EMR sorbent have been validated and demonstrated to be efficient for the analysis of pesticides residues in oils. It shows good repeatability (RSD lower than 5%) and recoveries between 40 and 120%. The low recoveries results can be compensated by using procedural standard calibration for the quantification of pesticides residues in real samples. 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. Real samples analyzed contained pesticides below EU-MRLs.

THE AUTHORS ACKNOWLEDGE FUNDING SUPPORT FROM THE EUROPEAN COMMISSION, DG SANCO, OF THE ANNUAL FINANCING DECISION (2011/889/EU)