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Simultaneous Quantification of Pesticide Residues in Olive Oil via LC-MS/MS and GC-MS/MS Using a Streamlined, Automatable cSPE Clean-up Workflow.

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1. Introduction

Olive oil, prized for its rich flavor and health benefits, is a staple in culinary traditions worldwide, used in cooking, dressings, and as a finishing touch to dishes. Ensuring its purity and safety is essential for consumers to fully enjoy its nutritional advantages. However, the presence of harmful pesticide residues in olive oil poses a significant concern. The European Union has established maximum residue limits (MRLs) governing the concentration of pesticides in different commodities. This study presents a validated method, utilizing LCMS-8045RX and GCMS-TQ8050 NX, to detect pesticides in olive oil, employing solid-phase extraction (SPE) (Biotage ISOLUTE®) techniques for efficient and accurate sample analysis.

Representative structures of pesticides are shown in Fig. 1. This validation study is conducted with the collaboration between Biotage, UK and Shimadzu Middle East and Africa, UAE.

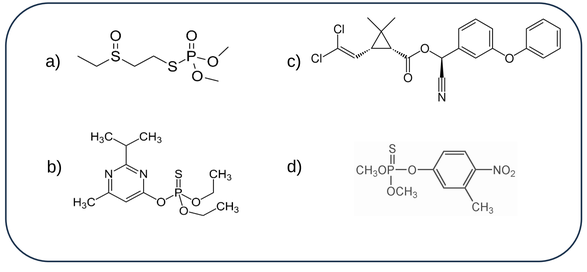

Fig. 1 Representative structures of pesticides a) Oxydemeton-methyl, b) Diazinon, c) Cypermethrin & d) Fenitrothion.

2. Methods and Materials

The LC Multiresidue Pesticide Standards Kit (31971) and GC Multiresidue Pesticide Standards Kit (32562) are procured from Restek Corporation.

Three olive oil samples of different brands are procured from the local market for this study. The same samples are used to prepare matrix-matched calibration standards and spiked samples. The calibration standards are analyzed in the range of 1 to 100 µg/L for LC-MS/MS and 5 to 200 µg/L for GC-MS/MS. Calibration curves are generated by using the external standard calibration method and weighted regression of 1/C or $1/C^2$. All samples are spiked at a concentration level of 10 µg/kg. Each spiked sample is prepared in duplicate to ensure consistency. This approach is taken to evaluate the efficiency of the extraction procedure. The results will help confirm the method's reliability and reproducibility.

The LCMS-8045RX with LC-40 series and GCMS-TQ8050 NX Fig. 2, manufactured by Shimadzu Corporation Japan, are used to quantify residual pesticides in the olive oil sample.

Fig. 2 Shimadzu LCMS-8045RX and Shimadzu GCMS-TQ8050 NX.

Shimadzu's LC-MS/MS Method Package for Residual Pesticides Ver. 3 and Smart Pesticides Database Ver. 2 for GC-MS/MS facilitated rapid instrumental method optimization, enhancing throughput for a majority of compounds. The method includes 1 target and 2 reference MRM transitions. Data processing is efficiently managed using Shimadzu's LabSolutions Insight software, simplifying the evaluation of validation parameters.

Solid-phase extraction (SPE) is used for the extraction of pesticides from oil samples[1]. Approximately 5 g of the oil sample is taken in a 50 mL centrifuge tube, and 10 mL of acetonitrile is added. The mixture is vortexed for 2 minutes and then centrifuged at 5000 rpm for 5 minutes. 1 mL of the supernatant is then collected and passed through QuEChERS 'dispersive SPE' sorbent blends: Biotage ISOLUTE® cSPE (Q0050-0035-BG ISOLUTE® AOAC Waxed 350 mg/3 mL). The eluent is collected in 1.5 mL HPLC vial and transferred to LC-MS/MS and GC-MS/MS systems for quantification. Detailed acquisition methods are outlined in Tables 1 and 2, respectively.

Manual SPE for multiple samples is labor-intensive and time-consuming. To address this, the automated Extrahera™ Classic unit Fig. 3 from Biotage, Sweden, is used. It automates key steps such as sample loading and elution, improving consistency and reducing errors. This enhance efficiency in complex sample preparation and supports higher throughput.

Fig. 3 Biotage® Extrahera™ Classic

Table 1: LC-MS method parameters

Column	Shim-pack Velox Biphenyl, 2.1 x 100 mm; 2.7 µm
Mobile phase	A: Water + 2 mM ammonium formate, 0.01 % formic acid
Mobile phase	B: Methanol + 2 mM ammonium formate, 0.01 % formic acid
Flow rate	0.4 mL/min
Gradient	3 %B (0 min) → 10 %B (1 min) → 55 %B (3 min) → 100 %B (10.5 → 12 min) – 3 %B (12.0 →15 min)
Injection volume	4 µL (Co-injection with water)
Column temperature	35 °C
Ionization mode	ESI (positive, negative)
Gas flow rates	Nebulizing gas:- 3 L/min; Heating gas:- 10 L/min; Drying:- 10 L/min
MS temperature	Interface:- 350 °C; Desolvation:- 250 °C; Heat block:- 300 °C

Table 2: GC-MS method parameters

Column	Rtx-5MS (30 m x 0.25 mm ID x 0.25 µm)
Injection mode	Splitless
Carrier gas	Helium
Injection volume	1.0 µL
Injector temperature	250 °C
Oven Temperature	90 °C (1 min), 40 °/min to 200 °C (0 min), 15 °C/min to 310 °C (8 min)
MS Temperature	Ion source:- 230 °C; Interface:- 290 °C

3. Results

Matrix matched calibration standards are employed for the linearity study. The correlation coefficients observed for all matrix matched calibration standards are ≥ 0.99, and the accuracy falls within 80-120% range, meeting all SANTE guideline criteria. Fig. 3, shows the representative matrix matched calibration curves and chromatograms of LC-MS/MS amenable analytes.

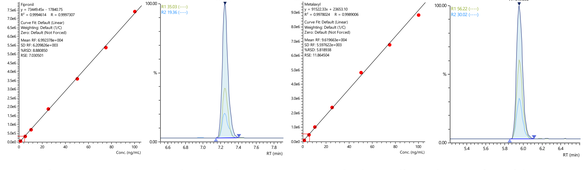

Fig. 3 Representative chromatograms of Fipronil and Metalaxyl in a 5 µg/kg matrix match standard.

The studies confirmed the method's accuracy and precision using fortified samples spiked at concentrations of 10 µg/kg for both LC-MS/MS and GC-MS/MS analyses. Most compounds showed recoveries between the range of 70-120% (refer to Fig. 4). The Biotage® Extrahera™ Classic significantly enhances the efficiency and reliability of solid-phase extraction workflows. Its user-friendly interface and automated operation reduces manual handling, making it exceptionally easy to use even for less experienced operators. This level of automation minimizes user-to-user variability and supports the generation of robust, high-quality data.

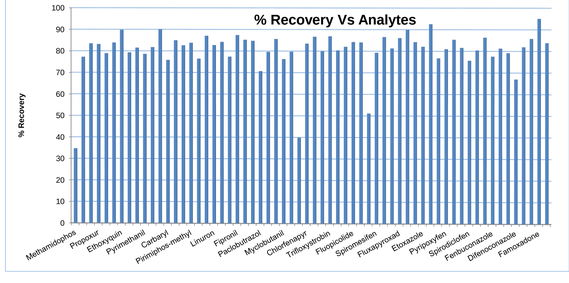

Fig. 4 Plot of % Recovery Vs. Analytes acquired by GC-MS/MS.

4. Conclusion

- A highly sensitive LC-MS/MS and GC-MS/MS method is developed for more than 200 pesticides using Shimadzu LCMS-8045RX and Shimadzu GCMS-8050 NX following SANTE 11312/2021 guidelines.
- The Biotage® Extrahera™ offers a simplified and efficient solid-phase extraction workflow for oil samples, ensuring high recovery and consistent results. Its automation enhances reproducibility while significantly reducing manual effort in sample preparation.

Reference

1) **Development and validation of a new multiresidue method for the determination of multiclass pesticide residues using GC-QqQ-MS/MS in edible oils.** Elena Hakme1, Samanta Uclés1, Piedad Parrilla-Vázquez1, Miguel Gamón2, Víctor Cutillas1, Amadeo R. Fernández-Alba1 1Agrifood Campus of International Excellence (ceiA3), European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables. University of Almería (Spain), 2Pesticide Residue Laboratory (Agro-Food Analysis Service) of the Generalitat Valenciana, Burjassot, Valencia, Spain