ThP 258

Method Validation for Determining 513 Pesticide Residues in Cucumber Using LCMS-8060NX and 308 Residues by GCMS-TQ8040NX.

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1. Introduction

- Cucumbers are popular raw for their crispness and flavor, often used in various dishes. When consumed raw, cucumbers retain their natural crunch and vibrant flavor, making them a refreshing addition to a variety of dishes. However, to fully enjoy the benefits of raw cucumbers, it's essential to ensure they are free from harmful pesticide residues, emphasizing the importance of pesticide residue analysis to guarantee their safety. The European Union has established maximum residue limits (MRLs) governing the concentration of pesticides in different commodities. This study presents a validated approach for detecting 692 different pesticides in cucumbers using LCMS-8060NX and GCMS-TQ8040NX. To extract the pesticides from the sample matrix, the QuEChERS[1] extraction technique followed by the d-SPE clean-up technique was used. Representative structures of pesticides are shown in Fig. 1. This validation study was conducted with the help of the Saudi Food and Drug Authority (SFDA) Riyadh, Kingdom of Saudi Arabia, and AnalyticaOne, an authorized distributor for Shimadzu in Qatar.

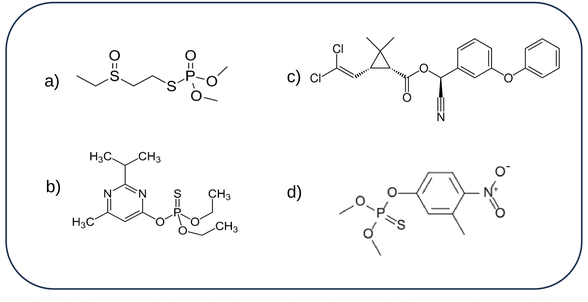

Fig. 1 Representative structures of pesticides a) Oxydemeton-methyl, b) Diazinon, c) Cypermethrin & d) Fenitrothion.

2. Methods

- The LC Multiresidue Pesticide Standards Kit (31971) and GC Multiresidue Pesticide Standards Kit (32562) were procured from Restek Corporation. Additionally, some individual reference standards were procured from AccuStandard and Dr. Ehrenstorfer GmbH.
- Cucumber samples procured from the local market were used to prepare matrix-matched calibration standards and spiked samples. The calibration standards were analyzed in the range of 1 to 75 µg/L for LC-MS/MS and 1 to 100 µg/L for GC-MS/MS.
- Calibration curves were generated by using the internal standard calibration method and weighted regression of $1/C^2$. The LCMS analysis employed Chlorpyrifos-D10 and Triphenyl phosphate as internal standards, while the GCMS analysis utilized PCB-18. Six sets of spiked samples were created, with concentrations set at 10, 20, and 50 µg/kg for LC-MS/MS, and 10 and 20 µg/kg for GC-MS/MS. Internal standards were incorporated into both the samples and matrix-matched standards.
- Shimadzu LCMS-8060NX with LC-40 series and GCMS-TQ8040 NX Fig. 2 , manufactured by Shimadzu Corporation Japan, were used to quantify residual pesticides in the cucumber sample.

Fig. 2 Shimadzu LC-MS 8060NX and Shimadzu GCMS-TQ8040 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 included 1 target and 2 reference MRM transitions. Data processing was efficiently managed using Shimadzu's LabSolutions Insight software, simplifying the evaluation of validation parameters.
- The modified QuEChERS[1] method involved the use of acetonitrile along with anhydrous magnesium sulfate, sodium chloride, sodium citrate dihydrate, and sodium hydrogencitrate sesquihydrate to extract pesticides. Subsequently, a clean-up step was conducted employing PSA and anhydrous magnesium sulfate. Following this clean-up, the supernatant was filtered through a 0.2 µm nylon syringe filter. This ensured the removal of any remaining impurities or particulate matter before analysis.
- Moreover, the filtrate was directly transferred into a 1.5 mL HPLC vial for GCMS analysis. For LCMS analysis the same filtrate was diluted 5 times with LCMS-grade water and used for acquisition. The acquisition method for both LC-MS/MS and GC-MS/MS analysis is a crucial aspect of this study. Detailed acquisition methods specific to LC-MS/MS and GC-MS/MS are outlined in Tables 1 and 2, respectively.
- Early eluting polar compounds in LC-MS/MS had broad peaks. To enhance peak shape, we used SIL-40's "Co-Injection" feature to effectively enhancing the peak shape of early-eluting polar compounds.

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	Nebulizing gas:- 3 L/min; Heating gas:- 10 mL/min; Drying:- 10 L/min
MS temperature	Interface:- 350 °C; Desolvation:- 250 °C; Heat block:- 300 °C

Table 2: GC-MS method parameters

Column	SH-Rxi™-5Sil MS (30 m L x 0.25 mm I.D. x 0.25 µm)
Injection mode	Splitless
Carrier gas	Helium
Injection volume	1.0 µL
Injector temperature	250 °C
Oven Temperature	105 °C (3 min.), 10 °C/min to 130 °C (0 min), 4 °C/min to 200 °C (0 min) to 8 °C/min to 290 °C
MS Temperature	Ion source:- 230 °C; Interface:- 290 °C

3. Results

- Matrix-matched calibration standards were employed for the linearity study. The calibration curve spanned from 1 to 75 µg/L for LC-MS/MS and from 1 to 100 µg/L for GC-MS/MS. All calibration standards met the criteria of falling within the 80 to 120% accuracy range according to SANTE guidelines. In Fig. 3, the linearity graphs and chromatograms of each representative compound from the Restek mixture are presented.
- The extraction process for cucumber, given its high-water content, initially omitted additional water. However, lower recovery rates of certain GC amenable molecules prompted the inclusion of 10 mL extra water alongside acetonitrile, leading to marked improvements. Validation studies confirmed the method's accuracy and precision through fortified samples spiked at concentrations of 10, 20, and 50 µg/kg for LC-MS/MS, and 10 & 20 µg/kg for GC-MS/MS, with most compounds showing recoveries within the 70-120% range. Reproducibility, as per SANTE guidelines, was demonstrated with a 20% RSD at LOQ levels, affirming both accuracy and precision. Precision and repeatability studies further supported accuracy, with %RSD values below 20%.

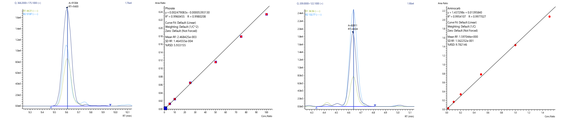

Fig. 3 Representative chromatograms of Phorate and Aminocarb in a 10 µg/kg spiked sample.

- A reproducibility experiment on six fortified samples matched the repeatability study's spiked concentrations. %RSD for their recovery at LOQ levels was below 20%. Mean recoveries for total compounds were within 70-120% on LC-MS/MS and GC-MS/MS. Nonetheless, some compounds exhibited recoveries below 70%. According to SANTE guidelines, all compound recoveries were reproducible, with RSD values at LOQ levels below the threshold. Refer to Fig. 4.

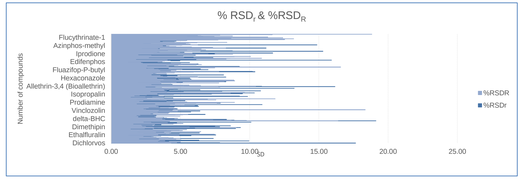

Fig. 4 Comparison of %RSD for Repeatability (RSD_r) and Reproducibility (RSD_R) during GC-MS/MS validation.

4. Conclusion

- A highly sensitive LC-MS/MS and GC-MS/MS method is developed for more than 600 pesticides using Shimadzu LCMS-8060NX and GCMS-8040 NX following SANTE 11312/2021 guidelines.
- The modified QuEChERS extraction technique facilitated precise and efficient sample preparation by confronting the inherent challenges posed by high water content.

Reference

1) EN 15662: Determination of pesticide residues using GC-MS and/or LC-MS (/MS) following acetonitrile extraction/partitioning and cleanup by dispersive SPE – QuEChERS method 2007 Brussels, Belgium, European Committee for Standardization.