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Multi-Residue Pesticide Analysis in Honey using GC-MS/MS

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1. Overview

The use of honey (Figure 1) has grown and has been adopted into consumption habits due to its high nutritional value, palatable flavor, and medicinal properties. Several reports indicate the presence of pesticide residues from different classes, mainly neonicotinoid insecticides, organophosphates and pyrethroids in honey.

Therefore, to protect human health, the European Union has set maximum residue limits (MRLs) for the presence of insecticides in honey[1]. Thus, increasing the importance of having analytical method for determination of residual pesticides present in honey.

Figure 1. Honey

2. Introduction

This study reports a validated method for the determination of 214 pesticides in honey using Shimadzu GCMS-TQ8040 NX. The multi-residue extraction was performed using modified QuEChERS' extraction method[2].

3. Materials and Methods

The reference standards for this study were procured from Restek with below catalogue number:
GC multi-residue pesticides kit – 32562
Honey sample procured from local market, was used to prepare matrix-matched calibration standards and spiked samples. This method is validated for criteria as mentioned in SANTE Guidelines[3].
GCMS-TQ8040 NX (Figure 2), manufactured by Shimadzu Corporation Japan, was used to quantify residual pesticides in honey sample.

3-1. Method development

Instrumental method was developed based on chromatographic and mass spectrometric parameters. Smart Pesticides Database Ver.2 for GC-MS/MS enabled quick instrumental method optimization for higher throughput. For most of the pesticides, 1 target and 2 reference MRM transitions were included in the method. Shimadzu's 'LabSolutions Insight' software was used for data processing, which helped in evaluating validation parameters with ease. This greatly reduced the development and optimization time of method parameters. Pretreatment method was optimized based on modified QuEChERS to give better and consistent recoveries.

3-2. Sample extraction

For honey sample extraction, sodium sulphate (Na_2SO_4) salt was used in optimized proportion to maximize recoveries of pesticides. Ethyl acetate was used as extraction solvent. After extraction, the aliquot of ethyl acetate was used for further clean up.

After extraction, clean up was performed using optimum combination of PSA (Primary secondary amine) and anhydrous $MgSO_4$ to minimize matrix interference. The extract was filtered through 0.22 µm nylon filter. Final reconstitution volume was adjusted such that recovery samples' concentration is diluted by two times. All samples were analyzed as per conditions shown in table 1.

3. Materials and Methods

3-3. Analytical Conditions

Table 1 Instrument configuration and Analytical Conditions: GC-MS/MS

System Configuration	
Instrument	: GCMS-TQ8040 NX
Auto-injector	: AOC-20i + s
Column	: SH-I-Rxi-5Sil MS (30 m × 0.25 mm I.D., df = 0.25 µm)
Liner	: Restek Topaz Liner, Splitless (with wool)
GC	
Injector temp.	: 250 °C
Column oven temp	: 80 °C (2 min), 20 °C/min to 180 °C (0 min), 10 °C/min to 310 °C (7.25 min)
Run time	: 34 min
Injection mode	: Splitless (High pressure at 250kPa)
Injection volume	: 1 µL
Carrier gas	: He
Linear Velocity	: 40.4 cm/sec (Constant mode)
MS	
Interface temp.	: 280 °C
Ion source temp.	: 230 °C
Ionization mode	: EI
Solvent cut time	: 5.0 min
Loop Time	: 0.3 sec

Figure 2. Shimadzu GCMS-TQ8040 NX

4. Results

Validation parameters like specificity, linearity, recovery and precision were studied as per SANTE guidelines.

4-1. System precision and specificity

Stability of method was tested by checking system precision. This was evaluated by injecting 10 µg/kg concentration of each pesticide in six replicates. System precision was determined by calculating the %CV of peak area and retention time of all the pesticides. The %CV was less than 10% for peak area and less than 1% for retention time. Specificity of the method was determined by comparing the response of blank (reagent and matrix) against matrix matched standard at reporting level. Response in reagent/ matrix blank was well within 30% of the reporting limit and met the acceptance criteria.

4-2. Matrix effect

Matrix effect was assessed by comparing the slopes of matrix matched linearity with the slope of aqueous standard linearity samples. For most of the pesticides, matrix effect of more than 20% could be observed. Therefore, quantification of the unknown analytes should be performed against the matrix matched standards for further experimentation.

4-3. Linearity

For linearity study and quantifying spiked samples, matrix matched calibration standards were used. Multilevel calibration curve included 1, 2.5, 5, 10, 25 and 50 µg/L concentration levels. All calibration standards were found within 80 to 120% accuracy as per SANTE guidelines.

4. Results

4-4. Recovery

Recovery was evaluated by analyzing spiked samples at 5, 10, 20 and 25 µg/kg (six spiked samples at each level) against matrix matched linearity plotted between 1 to 50 µg/L. Out of 214 pesticides, mean recoveries for 207 were found to be within 70-120%. For remaining 7 pesticides, recoveries were within 40 to 70%. The bar chart of mean recoveries at LOQ level is shown in figure 3. As per SANTE guidelines, recovery values outside the range 70-120% were found to be acceptable due to reproducible results with less than 20% RSD.

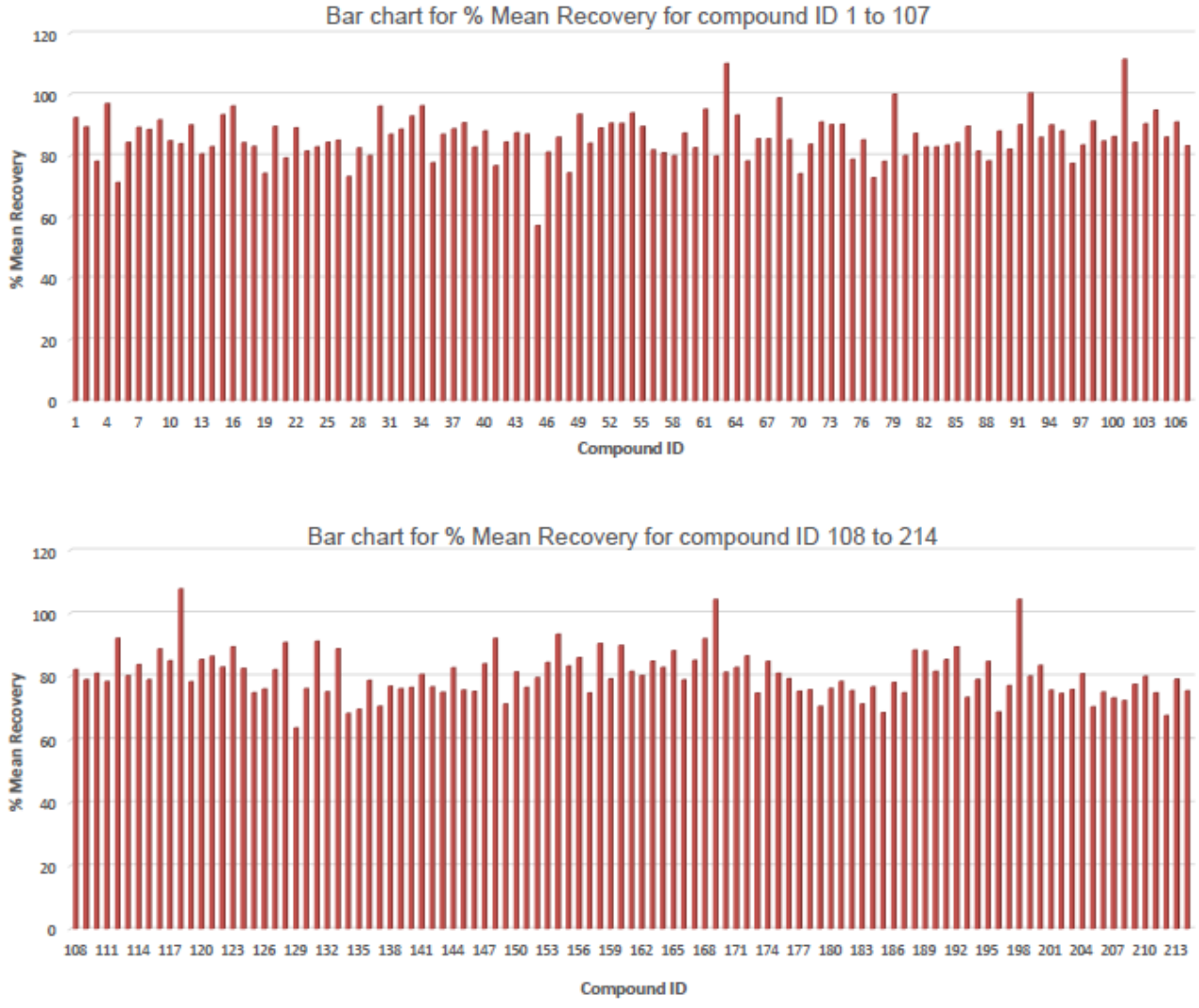

Figure 3. Bar chart of % Mean Recovery vs Compound ID at LOQ level

Precision : Repeatability (RSDr)

Repeatability experiment was performed by injecting six replicates of spiked samples at 5, 10, 20 and 25 µg/kg concentration levels. The %RSD for repeatability of six injections at their respective LOQ levels was found to be less than 20%.

Precision : Within laboratory reproducibility (RSDR)

Reproducibility experiment for recoveries was performed on six different spiked samples at 5, 10, 20 and 25 µg/kg concentration levels. The %RSD for recovery of six spiked samples at their respective LOQ levels was found to be less than 20%.

Trend plot of repeatability (RSDr) and within-laboratory reproducibility (RSDR) for spiked samples at LOQ level is presented in figure 4.

The method successfully achieved 5, 10, 20 and 25 µg/kg LOQ for 158, 50, 1 and 5 pesticides, respectively.

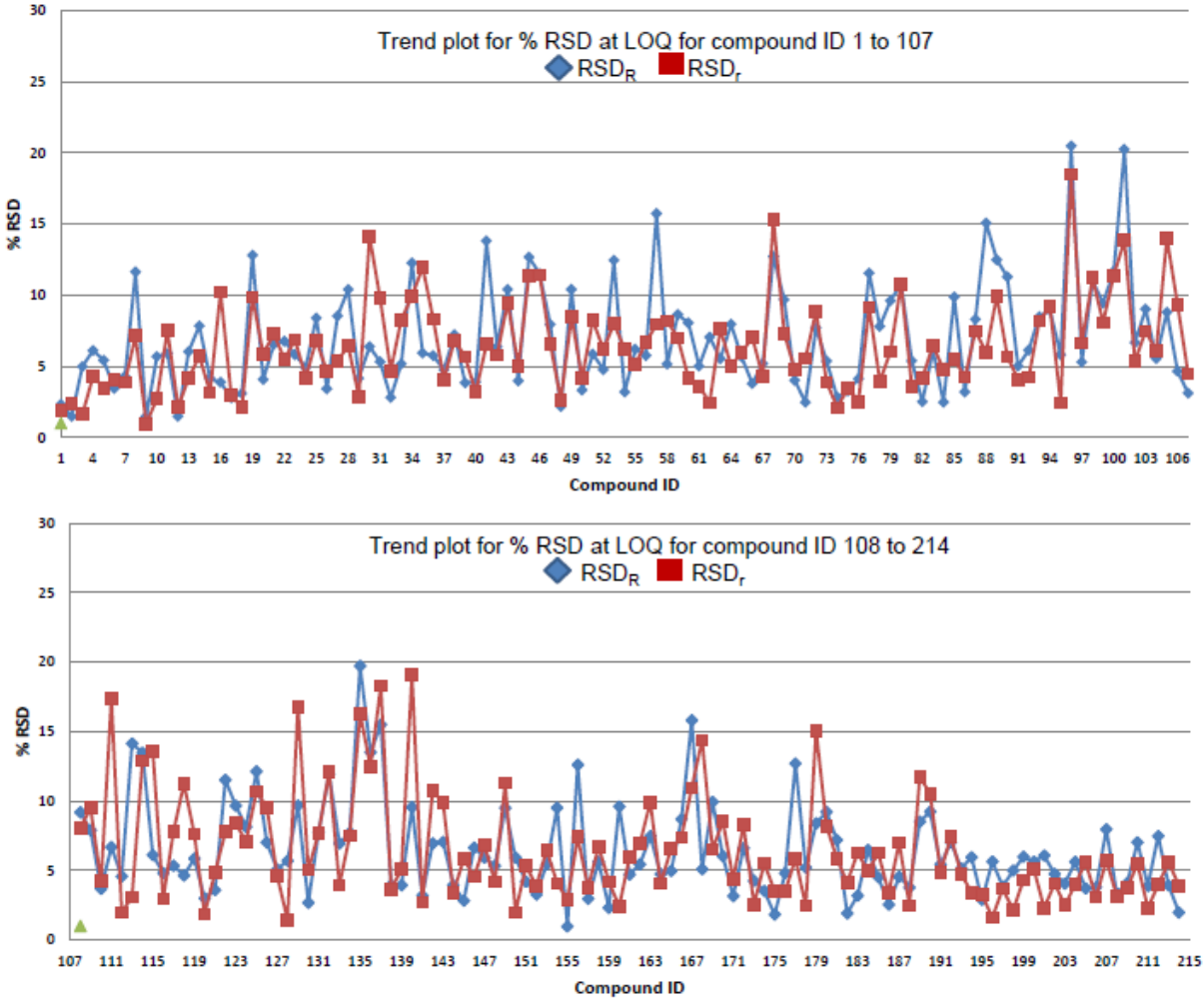

Figure 4. Trend plot of % RSD vs Compound ID at LOQ level

5. Conclusion

- A simple, sensitive and rapid method has been developed and validated as per SANTE guidelines for determination of 214 pesticides in honey sample. Quantification of pesticides in honey is challenging due to presence of high sugar content. A modified QuEChERS' extraction technique was used for sample preparation.
- The method developed on Shimadzu GC-MS/MS proved to be highly sensitive and consistent as all the pesticides showed repeatability and reproducibility < 20% (as per SANTE guidelines) at trace levels.
- The combination of sensitive instrument and reliable method enables its use in testing laboratories for multi-residue analysis of honey samples.

6. References

1. GB 2763-2019 National Food Safety Standard—Maximum Residue Limits for Pesticides in Food : https://www.codeofchina.com/standard/GB2763-2019.html.
2. M. Anastassiades, S. J. Lehotay, D. Štajnbaher, F. J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, J. AOAC Int., 86: 412–431, 2003
3. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/12682/2019

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