

Validated method for analysis of 190 pesticides in black tea using LCMS-8045

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1. Overview

Tea is one of the most refreshing and aromatic beverage consumed globally for its potential health promoting properties⁽¹⁾. To improve both the quality and quantity of tea production, broad spectrum of pesticides is frequently applied. Due to potential health hazards of such pesticides, resulting from chronic dietary exposure, EU has established Maximum Residue Limits (MRLs) for many pesticides⁽²⁾.

2. Introduction

Shimadzu Application Development Center (ADC) has developed a highly sensitive method to simultaneously quantify registered and approved pesticides in tea matrix using Shimadzu LCMS-8045. Residual analysis in black tea has always been complicated due to complex matrix that results in ion suppression, instrument contamination and co-elution. Optimal cleanup of samples is required to remove the pigments without affecting pesticides during the extraction. At trace level, quantification of pesticides is highly challenging if the sample preparation, processing, cleanup and extraction are not chosen appropriately. This study implements a simple and high throughput processing method for estimation of 190 pesticides in black tea. A multiple residue QuEChERS method has been employed for simultaneous determination of 190 pesticides of different chemistries and physicochemical properties.

3. Materials and methods

Described data is subset of an extensive validation data generated for 190 pesticides in black tea. This method is validated for criteria as mentioned in SANTE Guidelines⁽³⁾. There by confirming general applicability and transferability of this method on LCMS-8045.

3-1. Method development

Instrumental method is developed based on chromatographic and mass spectrometric parameters as described in Method Package for Residual Pesticide Ver.3 from Shimadzu Corporation⁽⁴⁾. This greatly reduces the development and optimization time of instrumental parameters. Pretreatment method was optimized based on QuEChERS. The workflow for pretreatment was fine tuned to give higher and more consistent recoveries. Early eluting polar pesticides are essentially known to have peak shape problem resulting in higher variability of analytical parameters. This was managed systematically by water co-injection using auto-sampler pretreatment function.

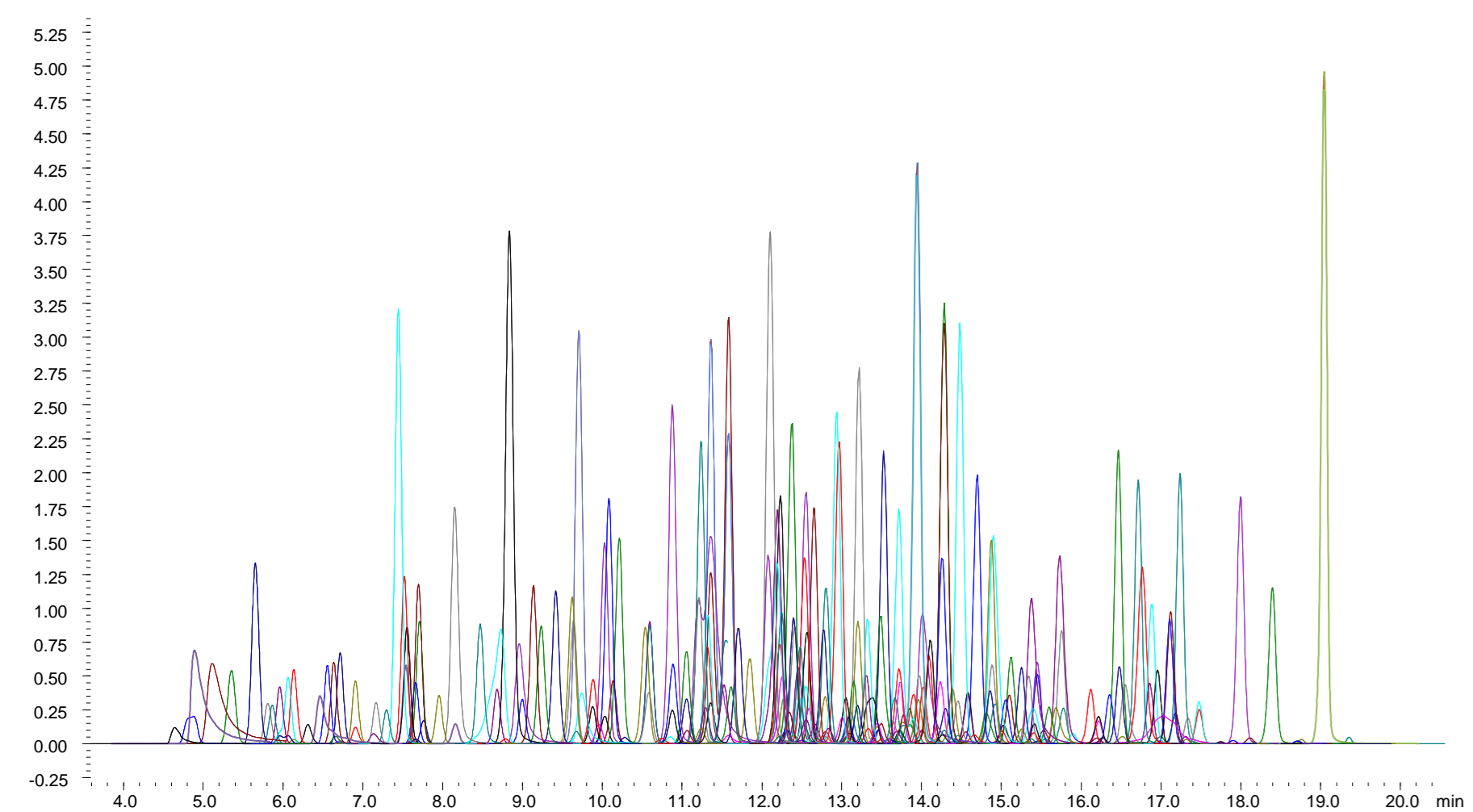


Figure 1. Representative chromatogram for analysis of 190 pesticides in black tea, analysed at 10 ppb

3-2 Sample extraction

Five milliliter water was added to 0.5 g of black tea samples and vortexed for a minute to mix the sample. 5 mL of acetonitrile containing acetic acid was added and processed by using QuEChERS technique.

Cleanup was done with dispersive solid phase extraction by using Primary secondary amine, C18 powder and graphitized carbon black with optimum combination. After cleanup, supernatant was evaporated in N₂ stream and reconstituted in formic acid in water: methanol (1:1, v/v). Samples were filtered through 0.22 micron syringe filter in auto sampler vial and injected into LCMS 8045.

3-3. LC-MS/MS analysis



Figure 2. Nexera X2 with LCMS-8045

LCMS-8045 triple quadrupole mass spectrometer by Shimadzu (Figure 2), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UF sensitivity), ultra fast scanning speed of 30,000 u/sec (UF scanning) and polarity switching speed of 5 msec (UF switching). This system ensures highest quality of data, with very high degree of reliability.

Table 1. Instrument parameters

UHPLC condition (Nexera X2)		MS parameters (LCMS-8045)	
Column	Shim-pack XR ODS II column 150 mm×3 mm, 2.7 μm.	MS interface	Electro Spray Ionization (ESI)
Mobile phase	A: 2mM Ammonium formate + 0.002% formic acid in water B: 2mM Ammonium formate + 0.002% formic acid in methanol	Nitrogen gas flow	Nebulizing gas- 3 L/min; Drying gas- 10 L/min
Flow rate	0.4 mL/min	Zero air flow	Heating gas- 10 L/min
Gradient	0 to 1 min 10% B, 1 – 4.5 min B 55%, 4.5 – 15.75 min B 100%. 15.75 – 18 min B 100%, 18.2 min B 5%, 18.2 – 21 min B 5%.	MS temp	Desolvation line- 250 °C; Heating block- 400 °C; Interface- 300 °C
Column temp	40 °C		

4. Results

4-1. Linearity

5-Multilevel calibration standards were prepared in solvent and injected in LC-MS/MS system. Calibration curve ranged from 1-50 μg/kg and was found to be linear with the corresponding coefficient of determination, (r²) more than 0.99.

To evaluate matrix match linearity, five matrix blank samples were prepared by using organic tea matrix as per the extraction protocol. Reconstituted matrix was used to prepare calibration standards ranging from 1-50 μg/kg. Matrix match standard linearity met the acceptance criteria and obtained a coefficient of determination (r²) ≥0.99.

4-2. 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 determined by calculating % CV of the peak area and retention time of the pesticides is 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 reporting level. Response in reagent/matrix blank was well within 30% of the reporting limit and met the acceptance criteria.

4-3. Matrix effect

Matrix effect was assessed by comparing the slopes of matrix match linearity with the slope of aqueous standard linearity samples. Matrix effect was found to be predominant; more than 20% in 124 pesticides and with-in 20% for 66 pesticides. Therefore, quantification of the unknown analytes should be performed against the matrix match standards for further experimentation.

4-4. Recovery

Recovery was evaluated by analyzing pre-spiked samples at 10, 25, 40, 100 and 200 μg/kg (six spiked samples at each level) against matrix match calibration linearity samples plotted between 1-50 μg/kg. Average recovery values for 158 pesticides were found to be within 70-120% and within 40-70% for remaining 32 pesticides. Recovery values outside the range 70-120% were found to be acceptable due to consistent, precise and reproducible results with RSD <20%.

As mentioned in SANTE guidelines, the relative ion ratios of the samples should be within ±30% of reference ion ratios obtained from calibration standards measured in the same sequence and under same conditions. The range of values of ion ratios for spiked samples at 10, 25, 40, 100 and 200 μg/kg and matrix match standard were measured and found to be acceptable.

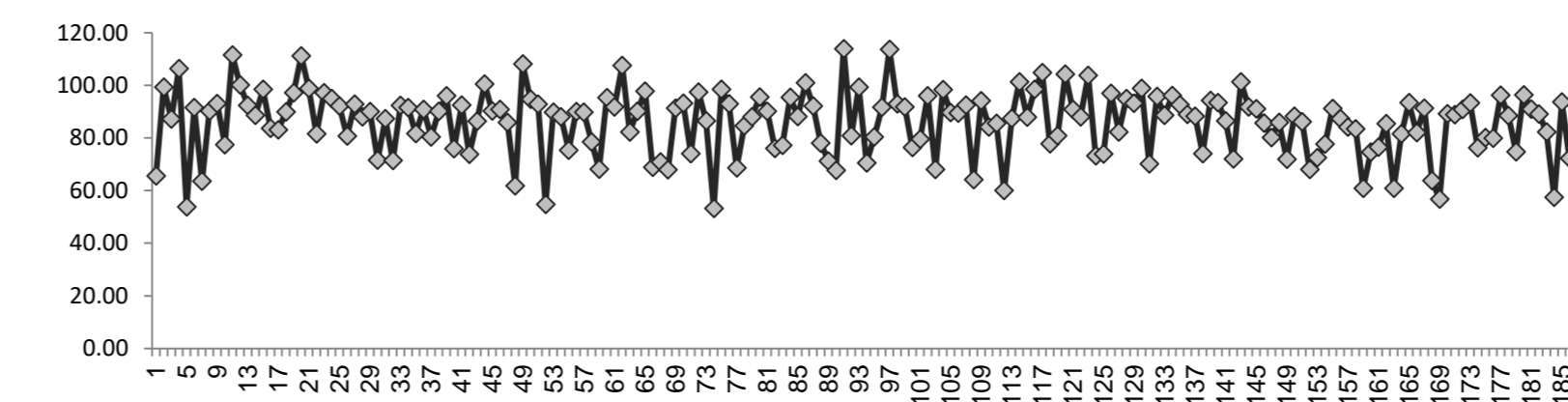


Figure 3. Trend plot of mean recovery at 10 μg/kg level

4-5. Precision : Repeatability (RSDr)

Pre-spiked tea samples, at level of 10, 25, 40, 100 and 200 μg/kg (six spiked samples at each level) were injected and checked against matrix match calibration linearity samples. Back calculated concentrations of these samples showed excellent reproducibility.

4-6. Precision : Within laboratory reproducibility (RSDwr)

Six aliquots of pre-spiked samples at 10, 25, 40, 100 and 200 μg/kg were prepared in matrix, processed as per the extraction protocol and injected against matrix match calibration linearity samples to evaluate RSDwr of the respective pesticides. % CV of each sample at 10, 25, 40, 100 and 200 μg/kg injected for recovery, repeatability and reproducibility experiments, met the acceptance criteria and found to be within 20%. Repeatability precision (RSDr) and within-laboratory reproducibility (RSDwr) for pre-spiked samples is presented in figure 4

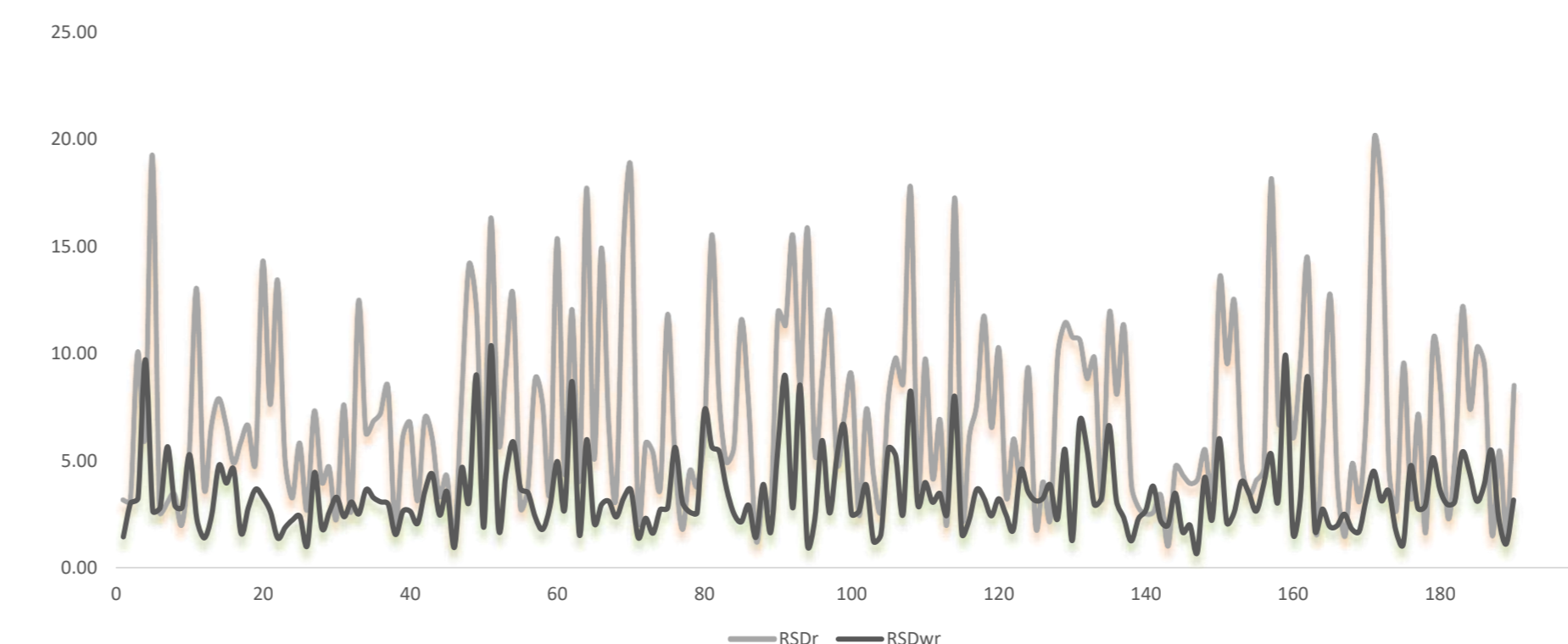


Figure 4. Trend plot of % RSD at LOQ level

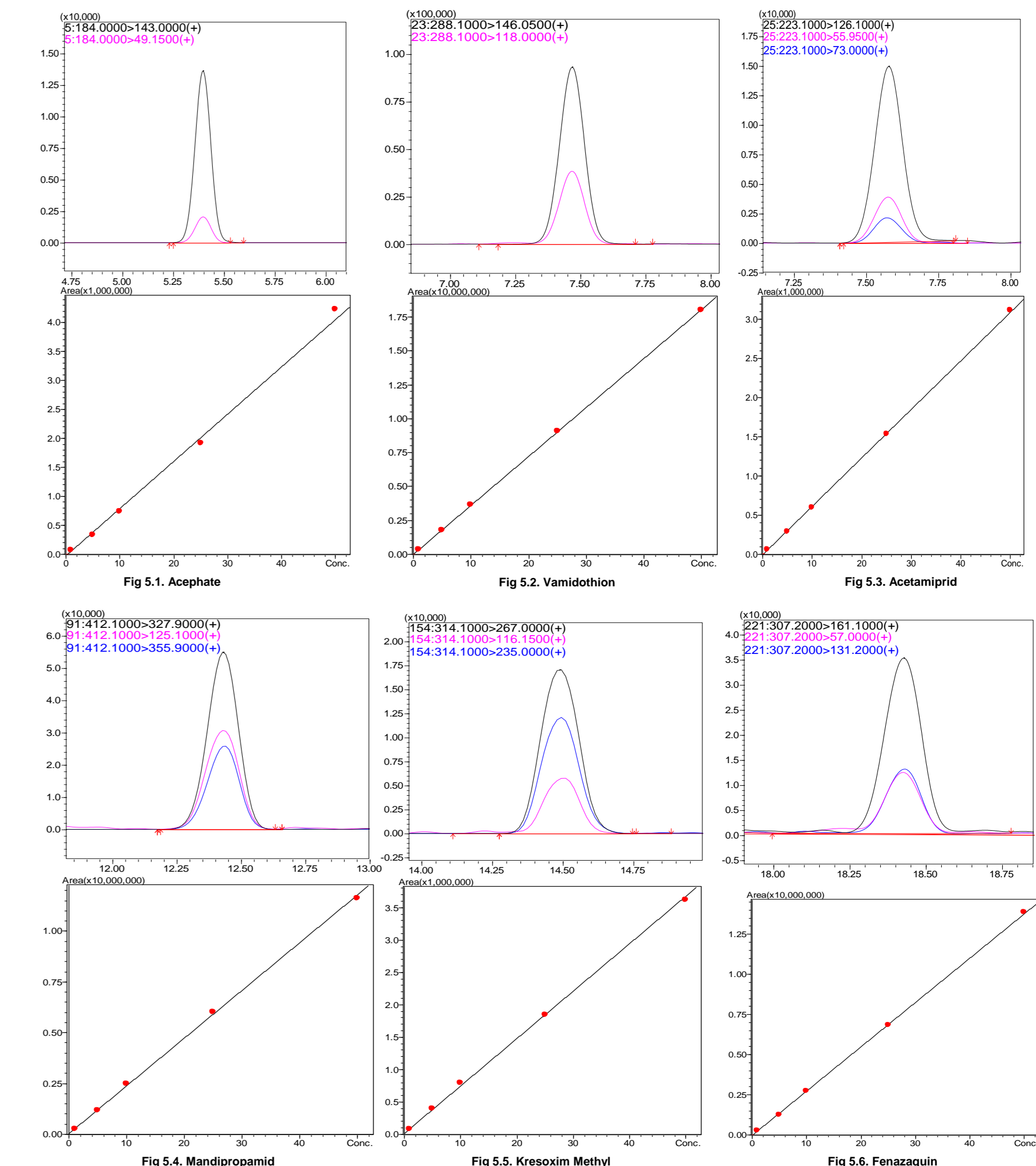


Figure 5. Representative chromatograms at LOQ level

5. Conclusion

A simple, sensitive and rapid method has been developed and validated as per SANTE guidelines for determination of 190 pesticides in tea matrix. Extraction QuEChERS technique was used as a sample preparation method. Shimadzu LCMS 8045 method proved to be a reliable tool to quantify pesticides due to its superior sensitivity and good repeatability. All the pesticides exhibited very good RSDr and RSDwr (as per SANTE guidelines) allowing method to be used for calculating residual pesticides in actual sample after applying recovery factors.

6. References

- Effects of Tea Consumption on Nutrition and Health, C.S. Yang, J.M. Landau, J. Nutr. 130 (2000) 2409–2412
- European Commission. Plants. EU Pesticides database. [Online]. <http://ec.europa.eu/food/plant/pesticides/eu-pesticidesdatabase> (accessed October 17, 2021).
- Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/11813/2017, 21 – 22 November 2017 rev.0
- LC/MS/MS Method Package for Residual Pesticides (English); Part No.: 225-32405-92.

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