FP 201

Multiresidue analysis of pesticides in Milk by GC-MS/MS using QuEChERS' extraction method

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1. Overview

Milk is an important food in the diet, especially for infants and children. The presence of any contamination in milk is a common food safety concern. Hence great efforts have been taken throughout the dairy industry to ensure the safety of milk. One of the main classes of contaminants in milk is pesticides, which can come from animals ingesting contaminated feed or water. The maximum residue limits for pesticides in milk are often much lower than for general fruits and vegetables[1]. Therefore, the analysis of pesticides in milk requires a sample preparation method for better matrix removal and analytical instrument methods for increased sensitivity. The aim of this study is to develop a simple and efficient workflow for determining a wide range of pesticides that are broadly controlled in milk worldwide.

2. Introduction

Shimadzu Application Development Center (ADC) has developed a highly sensitive method to simultaneously quantify pesticides in milk matrix using Shimadzu GCMS-TQ8040 NX. Residual analysis in milk has always been complicated due to complex matrix that results in ion suppression, ion enhancement, instrument contamination and co-elution. Optimal cleanup of samples is required to remove the proteins, sugars and solids 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 167 pesticides in Milk using GC-MS/MS. A multi-residue extraction was performed with the modified QuEChERS[2] method for simultaneous determination of 167 pesticides of different chemistries and physicochemical properties.

3. Materials and methods

The reference standards for 167 pesticides under study were procured from Restek with below catalogue numbers:

GC multi-residue pesticides kit – 32562

Milk sample procured from local market, was used to prepare matrix-matched calibration standards and fortified samples. Described data is subset of an extensive validation data generated for 167 pesticides in milk. This method is validated for criteria as mentioned in SANTE Guidelines[3].

GCMS-TQ8040 NX (Figure 1), manufactured by Shimadzu Corporation Japan, was used to quantify residual pesticides in milk 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 compounds, 1 target and 2 reference MRM transitions were included in the method. Shimadzu's data processing software 'LabSolutions Insight' was used for data processing, which helped in evaluating validation parameters with ease. This greatly reduced the development and optimization time of instrumental parameters. Pretreatment method was optimized based on modified QuEChERS. The workflow for pretreatment was fine tuned to give higher and more consistent recoveries.

3-2. Sample extraction

This study uses extraction procedure for GC-MS/MS in which modified QuEChERS method was adopted. Initially, the sample was deproteinized with acetonitrile and was extracted using AR grade anhydrous magnesium sulphate ($MgSO_4$) salt.

After phase separation, upper acetonitrile layer was subjected to clean up using C18 and $MgSO_4$ followed by solvent exchange in mobile phase solution. This clean up was followed by solvent exchange in ethyl acetate. The final reconstitution volume was adjusted to avoid dilution of the sample. All samples were analyzed as per conditions shown in table 1.

3-3. Analytical Conditions

Figure 1. Shimadzu GCMS-TQ8040 NX

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

System Configuration	
Instrument	: GCMS-TQ8040 NX
Auto-injector	: AOC-20i + s
Column	: SH-Rxi-5Sil MS (30 m × 0.25 mm I.D., df = 0.25 µm)
Liner	: Restek Topaz Liner, Splitless (with wool)
GC	
Injector temp.	: 280 °C
Column oven temp	: 60 °C (1 min), 40 °C/min to 170 °C (0 min), 10 °C/min to 310 °C (7.25 min)
Run time	: 25 min
Injection mode	: Splitless (High pressure at 250kPa)
Injection volume	: 2 µL
Carrier gas	: He
Linear Velocity	: 36.5 cm/sec (Constant mode)
MS	
Interface temp.	: 300 °C
Ion source temp.	: 230 °C
Ionization mode	: EI
Solvent cut time	: 3.5 min
Loop Time	: 0.3 sec

4. Results

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

4-1. Linearity

5-Multilevel calibration standards were prepared in solvent and injected in GC-MS/MS system. Calibration curve ranged from 2.5-40 µg/kg and was found to be linear with the corresponding coefficient of determination (r^2) more than 0.99.

To evaluate matrix match linearity, five matrix blank samples were prepared by using Milk as per the extraction protocol. Reconstituted matrix was used to prepare calibration standards ranging from 2.5-40 µg/kg. Matrix matched standard linearity met the acceptance criteria and obtained within 80-120 % accuracy.

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 of more than 20% could be observed. 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 5 and 10 µg/kg (six spiked samples at each level) against matrix match calibration linearity samples plotted between 2.5-40 µg/kg. Average recovery values for 129 pesticides were found to be within 70-120 % and within 40-70 % for remaining 38 pesticides. The trend plot of mean recoveries at LOQ level is shown in figure 2. Recovery values outside the range 70-120 % were found to be acceptable due to consistent, precise and reproducible results with RSD <20 %.

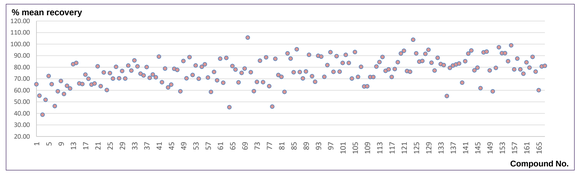

Figure 2. Trend plot of % mean recovery vs Compounds at LOQ level

Precision : Repeatability (RSD_r)

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

Precision : Within laboratory reproducibility (RSD_R)

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

Trend plot of repeatability (RSD_r) and within-laboratory reproducibility (RSD_R) for pre-spiked samples at LOQ level is presented in figure 3.

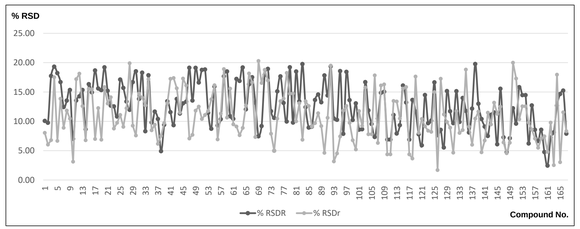

Figure 3. Trend plot of % RSD vs Compounds at LOQ level

The method successfully achieved 5 µg/kg LOQ for 118 compounds. Remaining 49 compounds showed LOQ of 10 µg/kg. Representative chromatograms of few compounds at their LOQ levels are shown in figure 4.

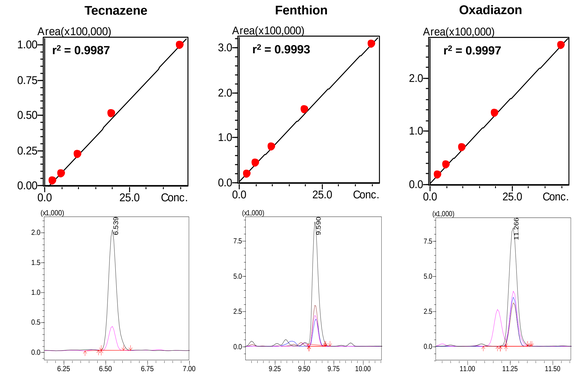

Figure 4. Representative chromatograms at LOQ level

5. Conclusion

- A simple, sensitive and rapid method has been developed and validated as per SANTE guidelines for determination of 167 pesticides in milk matrix. Quantification of pesticides in milk is challenging due to complexity of matrix. A modified QuEChERS' extraction technique was used for sample preparation.
- The method developed on Shimadzu GC-MS/MS proved to be highly sensitive and reproducible as most of the compounds showed good RSD_r and RSD_R (as per SANTE guidelines) at trace levels.
- This highlights the reliability of the method and enables its use in testing laboratories for multi-residue analysis of milk 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