Application Note

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Accurate Identification and Quantification of Pesticide Residues in Ghee

Analysis by QuEChERS with GC/MS/MS using the Agilent 8890 GC and Agilent 7010 GC/TQ

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Abstract

This application note outlines a technique for analyzing pesticides in ghee (clarified butter). Analysis of pesticide residues in ghee is challenging because of its fat content. In this application note, we have developed a QuEChERS-based extraction and cleanup method followed by GC/MS/MS analysis for the determination of over 150 pesticides in ghee. Ghee samples were weighed and acidified using 0.1% acetic acid in water. They were then extracted using acetonitrile and subjected to dispersive cleanup before being injected onto the GC/MS/MS. Instrument limit of quantification (LOQ) was determined in the range of 0.25 to 2.5 ng/g. The correlation coefficient (R^2) for calibration ranging over 0.25 to 25 ng/g was demonstrated to be above 0.98. Recovery studies were conducted at 2.5, 5, and 10 ng/g level of fortification. Average recoveries were in the range of 70 to 130%. This application note showcases the effectiveness of this sample preparation and analysis approach in determining pesticides from ghee samples, ensuring accuracy in detection.

Introduction

India is one of the largest producers of milk, contributing about 23% of global milk production.[1] The dairy processing sector includes a wide range of products, such as milk, butter, cheese, ghee, yogurt, flavored milk, lactose-free dairy products, and value-added products like ice creams and yogurts. Amongst these, ghee production is significant because of its popularity, low cost of production, and versatile use. According to the Food Safety and Standards Authority of India (FSSAI), ghee is mentioned as a "product obtained exclusively from milk, cream or butter by a process that almost completely removes water and non-fat solids; it has a specially developed flavor and physical structure".[2] Milk-producing animals can be exposed to pesticides through their diet, such as feed and fodder containing pesticide residues, or through the direct application of pesticides for controlling ectoparasites on their bodies, in animal sheds, and in milk processing areas. Due to their persistent and lipophilic nature, pesticides tend to accumulate in the fat-rich tissues of animals. The FSSAI has specified maximum residue levels (MRLs) for 55 pesticides, and a tolerance limit of 0.01 mg/kg for the remaining pesticides for which an MRL has not been fixed.

Ghee consists of 98.9% fat, irrespective of the animal source. Among the short- and medium-chain saturated fatty acids, all samples contain butyric (C4:0), caproic (C6:0), caprylic (C8:0), and capric acid (C10:0).[3] Since its introduction, the QuEChERS method has been widely used for the analysis of pesticides in several matrices. Some modifications to the original QuEChERS method have been introduced to ensure efficient extraction of pH-dependent compounds, to minimize degradation of susceptible compounds (such as base- and acid-labile pesticides), and to expand the spectrum of matrices covered. Ghee matrix has a very high lipid load, making it a difficult commodity for analysis pesticide residues. The primary challenge lies in obtaining an extract that contains the target analytes while minimizing the amount of fat. Co-extracted lipids in the extracts can be removed, to a high degree, by a freezing-out step or a C18 cleanup.

During sample extraction, the sample is often diluted. To obtain a quantification limit of 0.01 mg/kg, the instrument should be able to detect the residues at a lower concentration. This application note addresses these requirements by combining a sample preparation technique covering multiclass pesticides, target-specific multiple reaction monitoring (MRM) data acquisition, and efficient software to simplify quantitative analysis. The Agilent 7010 triple quadrupole GC/MS system enabled trace-level detection and confident quantitation of 150 pesticides in ghee.

Experimental

Equipment

An Agilent 8890 GC coupled to a 7010 GC/TQ system equipped with high efficiency source was used for the analysis. The GC system was equipped with a multimode inlet with air cooling and midcolumn backflush based on an Agilent purged Ultimate union controlled by a pneumatic switching device. The method parameters are displayed in Table 1.

Sample preparation

The sample preparation involved the extraction of 2 g sample with acidified water and acetonitrile, followed by dispersive cleanup with a combination of 150 mg C18, 900 mg $MgSO_4$. The sample preparation workflow is displayed in Figure 1.

The samples were quantified against matrix-matched standards.

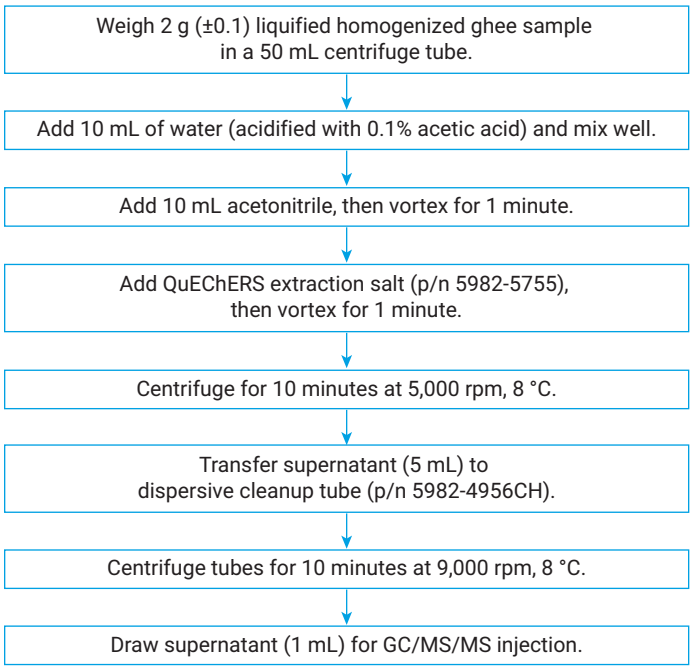

Figure 1. Sample preparation for pesticide analysis in ghee.

Table 1. Instrument method parameters.

Parameter	Value
Agilent 8890 GC Parameters	
Injection Volume	2 µL
Liner	Agilent Ultra Inert inlet liner, splitless, dimpled, 2 mm id (p/n 5190-2297)
Carrier Gas	Helium
Injection Mode	Splitless
Injector Program	70 °C (hold time 0.2 min) 280 °C (ramp at 900 °C/min)
Oven Program	60 °C (Hold for 1 min) 170 °C (Ramp at 40 °C/min, hold for 0 min) 310 °C (Ramp at 10 °C/min, hold for 3 min) Total run time: 20.75 min Backflush at 325 °C for 7 min
Columns 1 and 2	Agilent J&W HP-5ms column, ultra inert (15 m × 250 µm × 0.25 µm)
Column Flow 1 and 2	1 and 1.2 mL/min
Agilent 7010 GC/TQ	
Source Temperature	280 °C
Quadrupole Temperature	Q1 = Q2 = 150 °C
Collision Cell Gas Flows	N_2: 1.5 mL/min He: 4 mL/min
MRM Parameters	As per Agilent P&EP MRM database, version 4.0

Results and discussion

Sample extraction and cleanup

Sample preparation involved the dilution of the sample by up to five times. The fortification of 10 µg/kg pesticide residue resulted in a final extract of 2 µg/kg. The matrix (ghee) was rich in both saturated and unsaturated fats.

The dispersive cleanup tubes (part number 5982-4956CH) containing 150 mg C18, 900 mg $MgSO_4$ effectively reduced the matrix components eluting from 9 to 14 minutes (Figure 2). Extracted ion chromatograms of the tested pesticides (including organochlorine, organophosphorus, and synthetic pyrethroids) are shown in Figure 3.

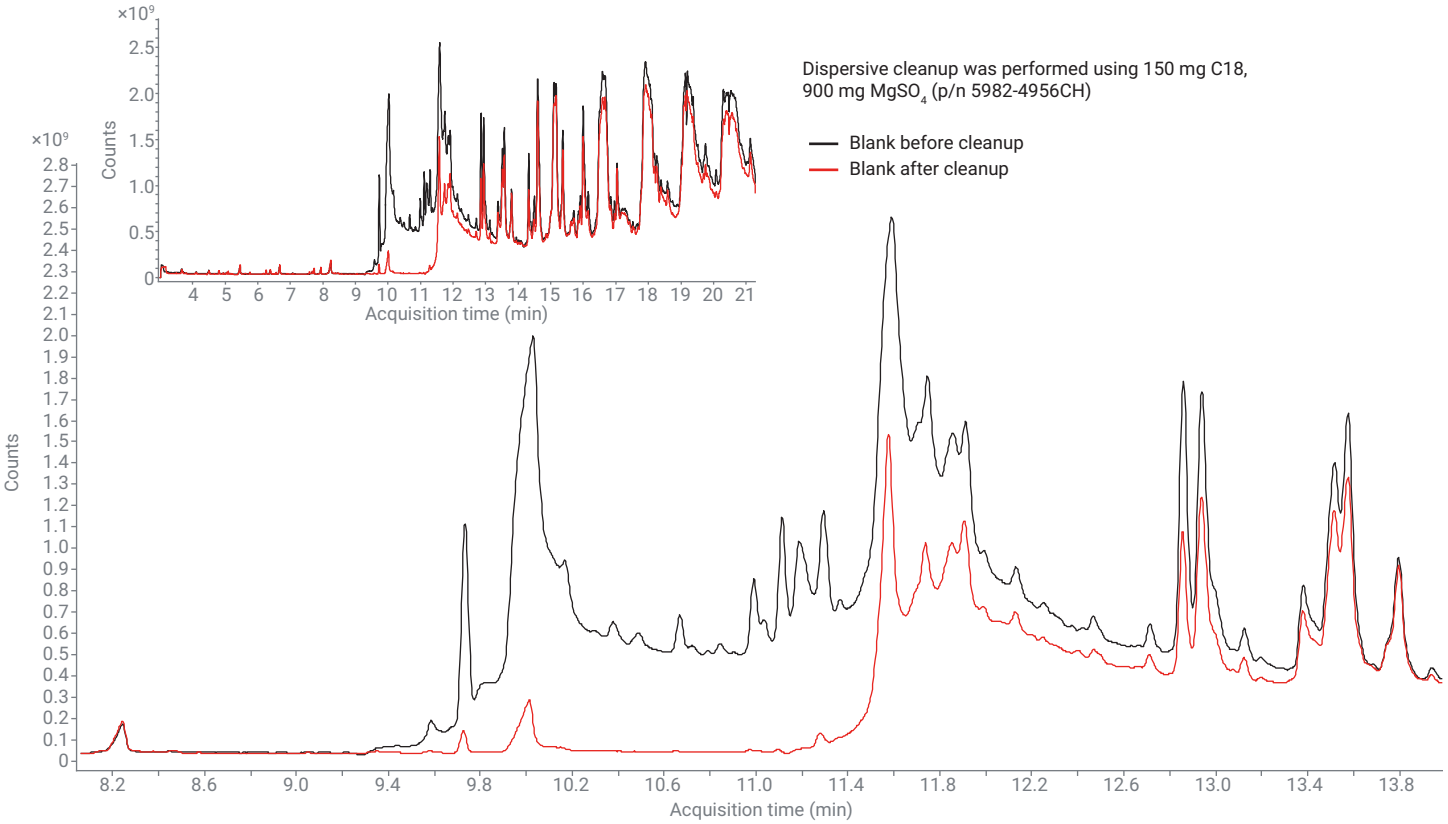

Figure 2. Reduction in matrix components with dispersive cleanup (p/n 5982-4956CH containing 150 mg C18, 900 mg $MgSO_4$).

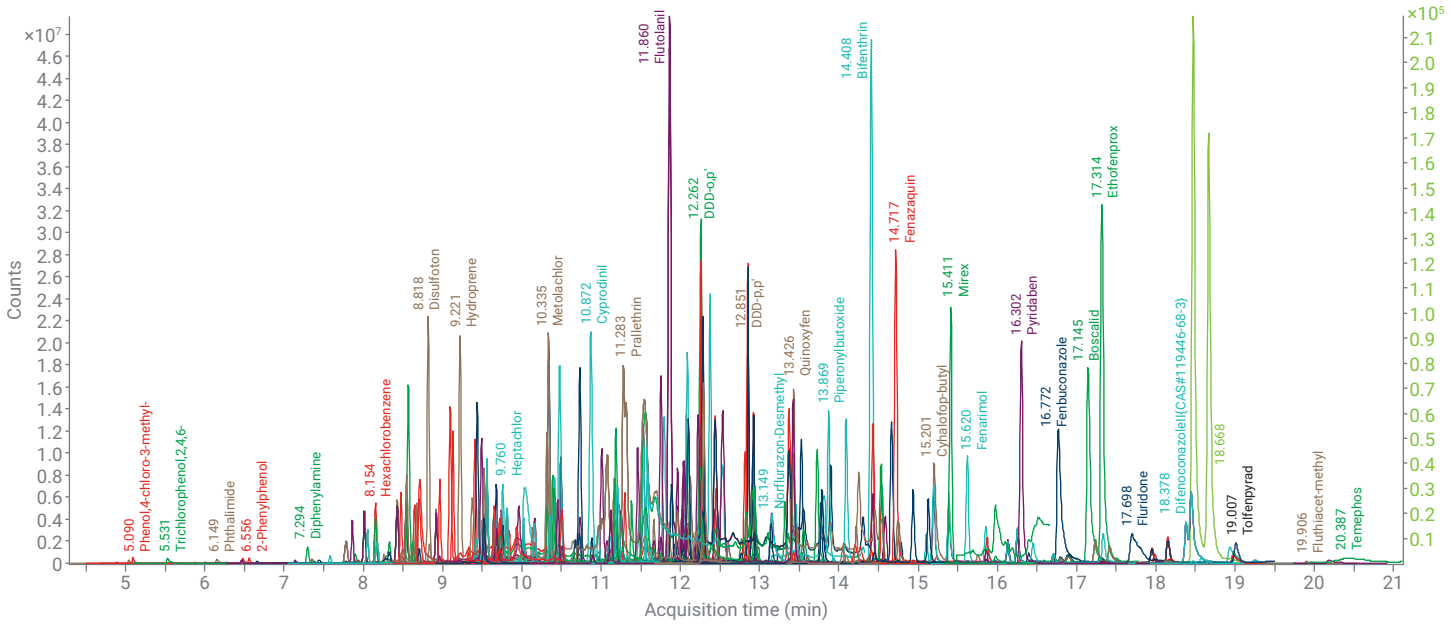

Figure 3. MRM chromatograms of 150 pesticides in ghee matrix.

Sensitivity and linearity

Linear matrix-matched calibration curves for all targets were plotted from the LOQ up to the highest matrix-matched calibration level of 50 µg/kg. Linear regression was applied, ignoring the origin and using a 1/x weighting. All 150 targets met the calibration curve linearity criteria of $R^2 > 0.98$. LOQ ranged from 2.5 to 5 µg/kg target list of over 150 compounds.

Method precision and accuracy

Precision and accuracy were determined using six injections of matrix-matched calibration levels. Precision of all targets were within 20% while accuracy was within 70 to 130%.

Recovery and repeatability

Recovery was calculated using target response in matrix-spiked QCs, and measured response using matrix-matched calibration curve equations. Intrabatch recovery repeatability was measured as %RSD of recovery values calculated using technical preparations of QC levels (n = 4). Based on the MRL values of a target, an appropriate level of QC sample was used to evaluate method recovery and intrabatch repeatability.

The target recovery and repeatability results are summarized in Figure 4. Chromatograms of representative pesticides in recovery sample at 10 ppb level of fortification are displayed in Figure 5.

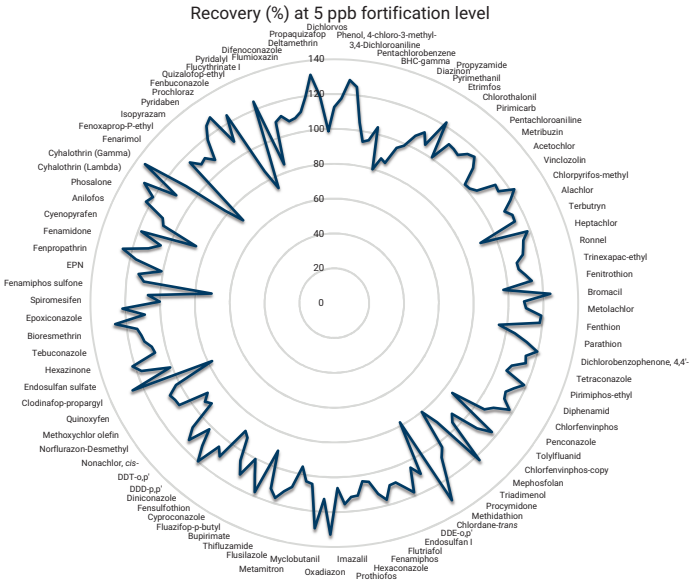

Figure 4. Average recovery at 5 ppb fortification level.

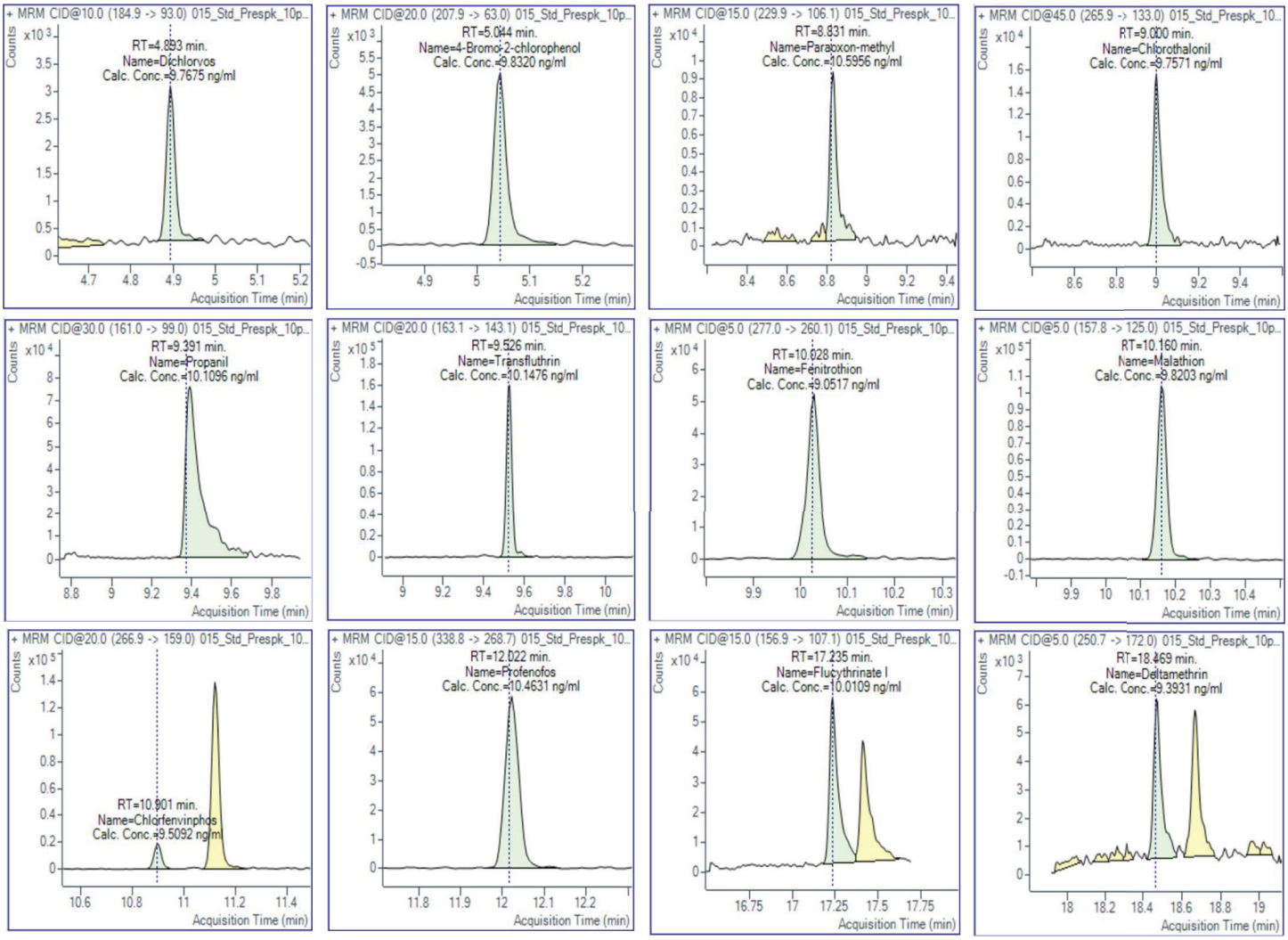

Figure 5. Chromatograms of representative pesticides in recovery sample at 10 ppb level of fortification.

Conclusion

The limits of quantitation (LOQs) for the target list of over 150 compounds ranged from 2.5 to 5 mg/kg for the ghee matrix. To minimize interferences from the ghee matrix, numerous multiple reaction monitoring (MRM) transitions were employed. This approach helped to accurately identify and quantify the target compounds despite the complex sample matrix. The samples were diluted five-fold to ensure that the analytes were within the required detection range of the instrument. This dilution also helped in reducing the matrix effects, leading to more reliable results. The average analyte recoveries were between 70 and 130%, indicating that the method has good accuracy and precision. This range of recoveries demonstrates the method's robustness and suitability for routine analysis of these compounds.

References

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