

Trace-Level Analysis of Melamine in Milk Products on Agilent 7890A/5975C GC/MSD Using a New Agilent J&W DB-5ms Ultra Inert Column and SampliQ SCX Cartridges

Application Note

Gas Chromatography/Mass Spectrometry/Food Safety

Authors

Min Cai and Yun Zou
Agilent Technologies (Shanghai) Co. Ltd.
412 Ying Lun Road
Waigaoqiao Free Trade Zone
Shanghai 200131
China

Abstract

A GC/MS method is presented for the quantitative determination and confirmation of melamine residues in milk products. The milk sample was cleaned up using Agilent's new SampliQ SCX SPE cartridges before derivatization. The derived extracts were analyzed by GC/MS with EI in synchronous SIM/scan mode on a new Agilent J&W DB-5ms Ultra Inert column. The method has good linearity in the range of 0.025 to 2.000 $\mu\text{g/mL}$ with a correlation coefficient of 0.9986. The average recoveries of melamine, for milk spiked at 0.080, 0.800, and 1.600 mg/kg, were over 80 percent, with RSDs of less than 5 percent. The LOD is 0.0025 mg/kg in the milk matrix, which is suitable for trace-level analysis of melamine in milk products.



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Introduction

Melamine is an organic base chemical most commonly found in the form of white crystals rich in nitrogen. Recently, melamine in adulterated milk product has attracted much attention all over the world. The main analytical methods available for melamine quantification in milk involve GC/MS, HPLC, and LC/MS, such as China GB/T method 22388-2008 [1]. An HPLC method can be used to quantitatively analyze melamine at ppm level. But it is inadequate for qualitative analysis and trace-level analysis. LC/MS is a sensitive technique for quantitative and qualitative analysis. However, due to the high price of equipment, it is not widely used in practical applications. GC/MS is an economic and commonly used technique for most of analytical laboratories.

Milk products are complex matrixes that contain interfering compounds, such as protein and fat. These compounds may interfere with the analysis of the target analytes or contaminate the analytical system if the sample is introduced without selective sample preparation.

Solid-phase extraction (SPE) is commonly used as a sample preparation method for a variety of analytical procedures. Agilent's mixed-mode SampliQ SCX polymeric resin is a sulfonic acid-modified divinyl benzene polymer with both ion exchange and reverse-phase retention properties. The SampliQ SCX resin exhibits excellent retention for both basic and neutral compounds. It ensures fast, reliable extraction of basic compounds like melamine.

GB/T method 22388-2008 requires melamine analysis with an LOQ of 0.05 mg/kg in a GC/MS method. For a complex matrix like milk sample, an inert GC/MS column is crucial for trace-level analysis. The new Agilent J&W Ultra Inert column allows for the best and most consistent column inertness [3], providing better sensitivity and peak shape for active compounds and reliable results for trace-level analysis. Combined SampliQ SCX SPE with the Agilent J&W DB-5ms Ultra Inert column, a sensitive GC/MS method, is put forward on trace-level analysis of melamine in milk products.

Experimental

Standards and Reagents

The standards and reagents used in the experiment are listed in Table 1.

A stock solution 1,000 µg/mL of melamine was prepared in methanol. Working solutions were prepared by diluting the

stock solution with methanol. After being transferred to an autosampler vial, the 1-mL working solution was allowed to evaporate to dryness with nitrogen. 600 µL pyridine and 200 µL BSTFA were then added, vortexed briefly to mix, and incubated at 70 °C for 30 minutes. With this procedure, a series of standard solutions of melamine trimethylsilyl (TMS) derivatives (0.025, 0.050, 0.200, 0.500, 1.000, and 2.000 µg/mL) were prepared for linearity analysis.

Table 1. Standards and Reagents

Melamine	> 99% purity
Methanol	HPLC grade
Pyridine	Certified A.C.S. reagent
1% trichloroacetic acid (TAA) solution	Dissolve 10.0 g of TAA in reagent water. Dilute to 1.0 L with reagent water.
22 g/L lead acetate solution	Dissolve 22.0 g of lead acetate in reagent water. Dilute to 1.0 L with reagent water.
5% ammonium hydroxide solution	Mix 5 mL of ammonium hydroxide and 95 mL of methanol together for use.
Silylating reagent	BSTFA with 1% TMCS: bis(trimethylsilyl) trifluoroacetamide with 1% Trimethylchlorosilane

Instruments

Table 2 lists the GC chromatography and mass spectrometry conditions of the method.

Table 2. Gas Chromatograph and Mass Spectrometer Conditions

GC Conditions	
Instruments	Agilent 7890A/5975C GC/MSD Agilent 7683 Automatic Liquid Sampler (ALS)
Column	Agilent J&W DB-5ms Ultra Inert, 30 m × 0.25 mm × 0.25 µm (P/N 122-5532UI)
Inlet temperature	EPC, split/splitless @ 250 °C
Injection volume	1 µL, split 3:1
Carrier gas	Helium, constant flow mode, 1.3 mL/min
Oven program	75 °C (1 min); 30 °C/min to 300 °C (2 min)
Transfer line	290 °C
MS Conditions	
MS	EI, SIM/scan
Solvent delay	4.2 min
MS temperature	230 °C (source); 150 °C (quad)
Scan mode	Mass range (40 to 450 amu)
SIM mode	Ion (342, 327*, 171, 99)

*Quantitative ion

Sample Preparation

Sample preparation was performed according to the scheme in Figure 1. A SampliQ SCX SPE cartridge (60 mg, 3 mL, P/N 5982-3236) was used to clean up sample extracts. All SPE elution steps, including conditioning, sample loading, washing, and elution were performed without the application of vacuum. Vacuum was only applied to dry the cartridges.

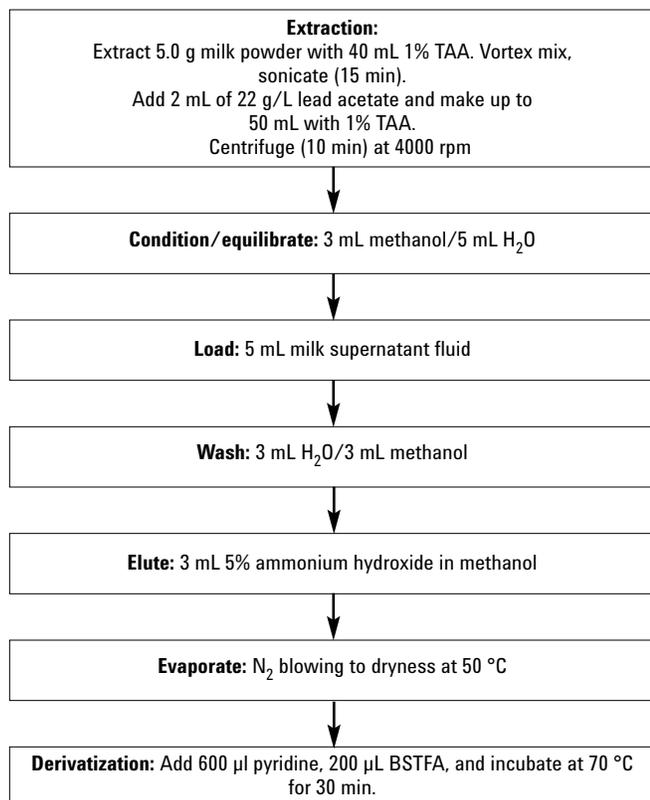


Figure 1. Scheme of sample preparation process.

The derivatization of melamine is shown in Figure 2.

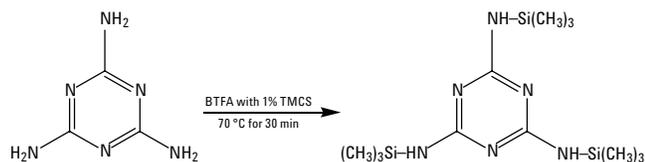


Figure 2. Melamine derivatization reaction.

Results and Discussion

GC/MS Analysis of Melamine

GC/MS was used for the identification (full-scan mode) and quantification (SIM mode) of melamine. It helped simplify the process of confirming positive or negative results.

Figure 3A shows the chromatogram of a sample that was only extracted with methanol without any SPE cleanup [4]. It highlights that there was much sample matrix interference in milk extract for the analytes of interest. Especially after 10 minutes, large amounts of high boilers eluted from the column. Undoubtedly, it increased the analysis time to ensure that the late eluters were eluted. Furthermore, it could contaminate the analytical system and shorten the lifetime of the ion source.

Figure 3B shows the chromatogram of a sample prepared according to the procedure in Figure 1. Figure 3C is the chromatogram of melamine tri-TMS for reference. The SCX SPE effectively removed interferences from the sample matrix. Downtime is reduced due to less detector contamination.

Figure 4 is the corresponding mass spectrum of melamine tri-TMS in Figure 3. After SPE cleanup, it eliminates some interference ions from the matrix and the mass spectra shows better "match" factors over 90. It further demonstrates that it makes the qualitative and quantitative analysis easier and more accurate.

As you can see in Figure 3, sharp, symmetrical peaks were observed for melamine tri-TMS on Agilent J&W DB-5ms Ultra Inert GC columns. Sharper peaks greatly enhance the height of the signal and therefore provide better signal-to-noise ratios and greater sensitivity. The ultra inert column ensures minimal compound adsorption, for more accurate quantification. It proved that Agilent J&W Ultra Inert GC columns allow you to perform trace-level analysis of melamine with the utmost confidence.

Linearity and Recovery

The linearity of the method was studied using the standard working solutions after derivatization. Figure 5 is the calibration curve for melamine tri-TMS. The method has good linearity in the range of 0.025 to 2.000 µg/mL, with a correlation coefficient of 0.9986.

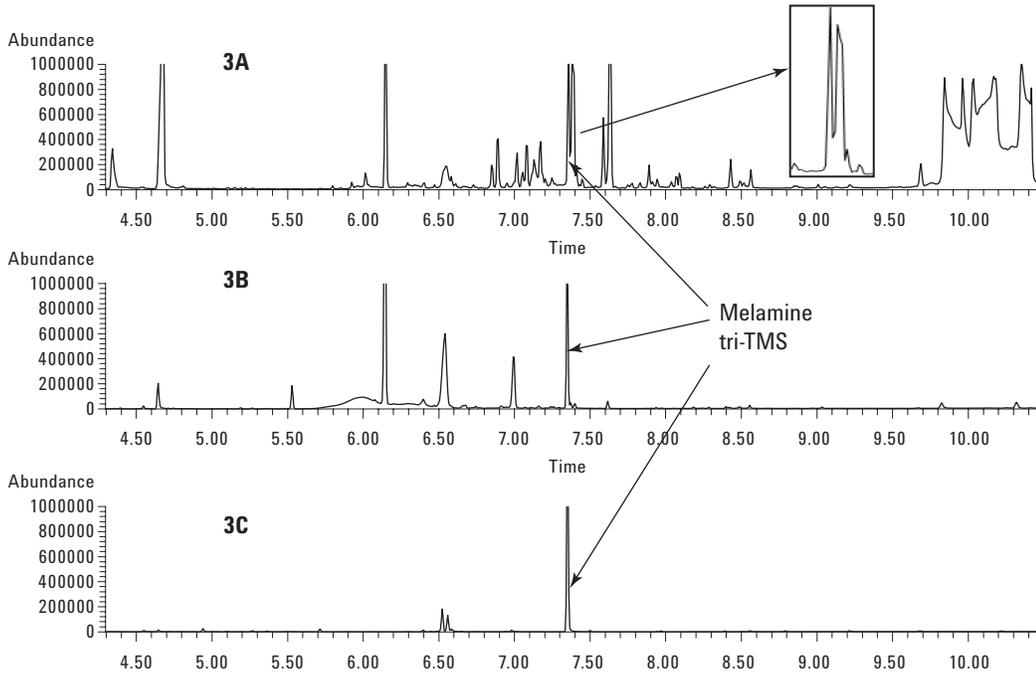


Figure 3. GC/MS SIM chromatogram of melamine tri-TMS. (3A: Sample without SPE cleanup; 3B: Sample with SPE cleanup; 3C: Standard)

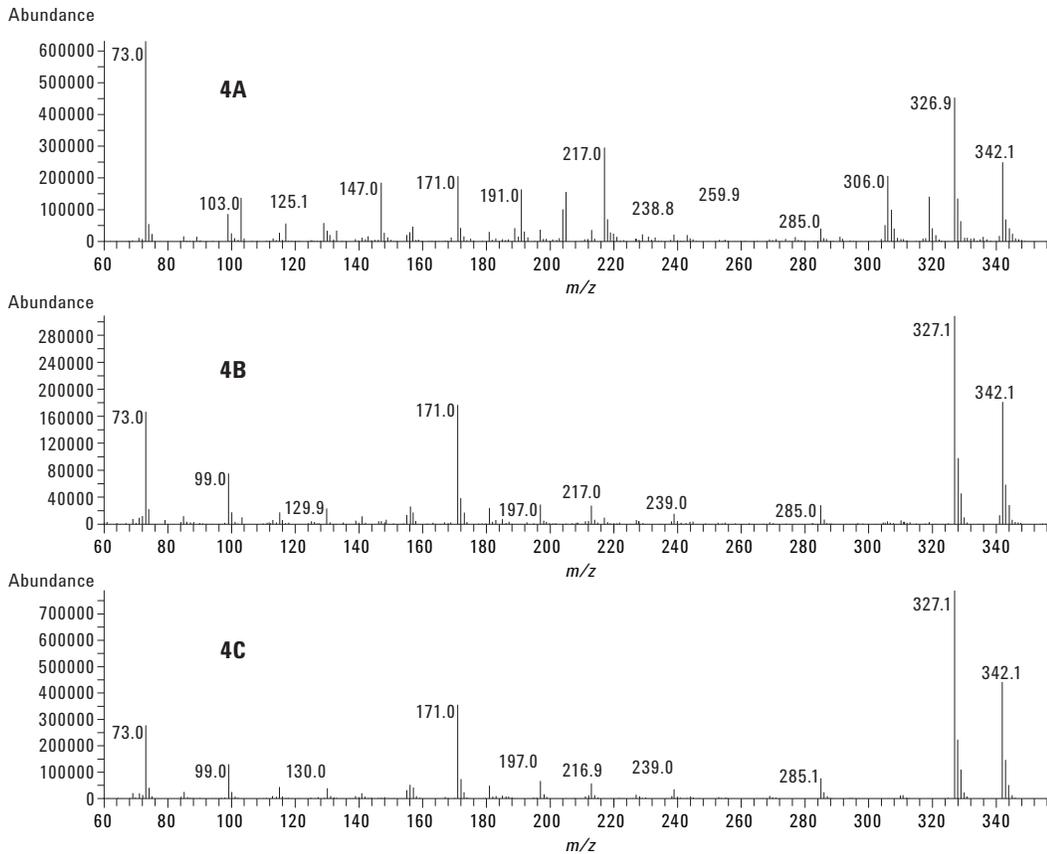


Figure 4. GC/MS mass spectrum of melamine tri-TMS. (4A: Sample without SPE cleanup; 4B: Sample with SPE cleanup; 4C: Standard)

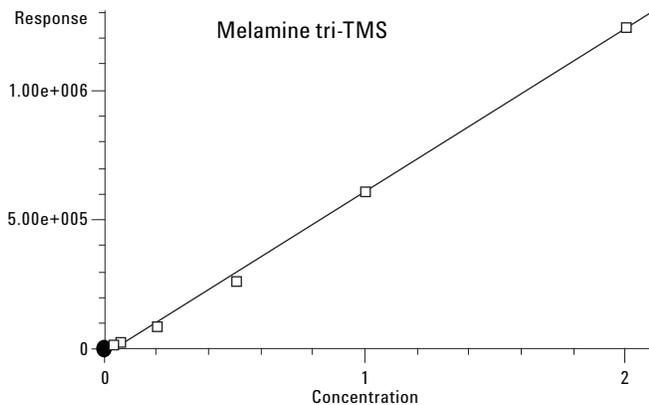


Figure 5. Calibration curve for melamine tri-TMS.

The recovery of the method was evaluated for melamine spiked at 0.080, 0.800, and 1.600 mg/kg levels. For each concentration level, the repeatability was evaluated on six duplicate samples. The spiked samples were treated according to the sample-preparation procedure described above. The recovery and repeatability data are listed in Table 3. The method resulted in good recoveries (over 80 percent) and repeatability with RSDs less than 5 percent at three levels. The LOD is calculated from the level of 0.08 mg/kg with a signal-to-noise ratio of 3. The LOD is 0.0025 mg/kg in the milk matrix, which is applicable for the trace-level analysis of melamine.

Table 3. Recovery and Repeatability of Spiked Samples

Compound	Spiked level (mg/g)	Recovery (%)	RSD (%) (n = 6)
Melamine	0.080	82.1	2.04
tri-TMS	0.800	82.8	4.88
	1.600	80.8	3.58

Conclusions

This application demonstrates a sensitive GC/MS method for melamine analysis in milk products using an Agilent J&W DB-5ms Ultra Inert GC column and SampliQ SCX SPE cartridges. It is suitable for trace-level analysis of melamine in raw milk and dairy products.

References

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