APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Solid Phase Extraction and GC/MS Analysis of Melamine Adulteration in Dairy Products

Introduction

In September 2008, melamine again made global headlines with contamination and adulteration of dairy products in China. This incident occurred about 18 months after melamine contamination of pet foods. During the initial melamine scare, gas chromatography/mass spectrometry (GC/MS) analysis was used successfully in testing finished food products as well as raw materials. This analysis is presented in the PerkinElmer application note "Screening for Melamine Adulteration in Protein-Based Foods by GC/MS"[1].

When the melamine in milk crisis began, similar test methods were used to test baby formula and other dairy products. The sample matrix of milk and dairy products is, however, much different than that of pet foods, with a much higher content of fat and sugar. This difference in matrix required that sample preparation methods be modified from those used in GC/MS analysis of pet foods. The major modification necessary is solid phase extraction (SPE) of the sample extract to remove the matrix of the milk. This paper will present the modifications necessary to successfully analyze dairy products for melamine with GC/MS. Additionally, GC/MS analysis of the data will support the method modifications.

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Experimental

The analysis of milk and dairy products requires a specific sample preparation. The techniques used in this application are a combination of an extraction procedure by Sigma-Aldrich®[2] and a modified derivatization reaction and analytical procedure presented in an FDA method[3].

Melamine samples were created in the lab by spiking full-fat milk with a melamine standard (50 µg/mL 50:50 acetonitrile:water) to a concentration of 1 µg/mL. The extraction procedure used follows:

1. Dilute 5 mL of spiked milk with 5 mL 100 mM phosphate buffer (pH 2.5) and 1 mL acetonitrile
2. Sonicate for 5 minutes in an ultrasonic water bath
3. Centrifuge at 3500 rpm for 10 minutes
4. Isolate the middle supernatant layer for SPE processing
5. Process 2.2 mL of the middle supernatant layer (equivalent to 1 mL milk sample) using SPE.

The SPE was carried out on a strong cation exchange cartridge, Discovery® DSC-SCX (500 mg/6 mL, Sigma-Aldrich). The cleanup procedure is as follows:

1. Condition and equilibrate SPE cartridge with 3 mL methanol followed by 3 mL 0.1% formic acid
2. Load sample (2.2 mL)
3. Wash SPE cartridge with 3 mL 0.1% formic acid followed by 3 mL methanol
4. Elute melamine from SPE cartridge with 4 mL 5% ammonia diluted in methanol
5. Evaporate 1 mL SPE eluent to dryness, in an autosampler vial, with nitrogen at 5 psi and 50 °C
6. Sample is ready for derivatization.

The dry sample is reconstituted in an autosampler vial with 200 µL of pyridine. Melamine is converted to trimethylsilyl (TMS) derivatives with the reagent Sylon-BFT (Supelco®) consisting of bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS); 300 µL of this solution is added and the sample is incubated at 70 °C for 45 minutes.

Following derivatization, the samples are ready for GC/MS analysis. The GC/MS system used in this paper was the PerkinElmer® EcoAnalytix™ Melamine Analyzer, based on the Clarus® 600 GC/MS, and the instrument parameters are summarized in Tables 1 and 2.

Table 1. Gas Chromatograph Conditions for Melamine-TMS Analysis.

Gas Chromatograph:	PerkinElmer Clarus 600		
Analytical Column:	Elite-5MS (30 m x 0.25 mm x 0.25 µm)		
Injection Port Type:	Programmable Split/Splitless		
Injector Temperature:	280 °C		
Injection Type:	Splitless		
Syringe Volume:	5 µL		
Injection Volume:	1 µL		
Injection Speed:	Fast		
Carrier Gas Type:	He		
Carrier Gas Program:	1 mL/min		
Oven Program:	**Temperature**	**Hold Time**	**Rate**
	75 °C	1 min	15 °C/min
	320 °C	2.67 min	End
Instrument Timed Events:	-0.5 min	Spl1 = 0 mL/min	
	1.0 min	Spl1 = 50 mL/min	

Table 2. Mass Spectrometer Conditions for Melamine-TMS Analysis.

Mass Spectrometer:	PerkinElmer Clarus 600 T
GC Inlet Line Temperature:	280 °C
Ion Source Temperature:	230 °C
Function Type:	Full scan
Full Scan Range:	*m/z* 50-450
Solvent Delay:	6 min
Full Scan Time:	0.2 sec
InterScan Delay:	0.05 sec

Results

Previously, it was demonstrated that the GC/MS method for melamine analysis can easily detect and quantify melamine below 0.1 µg/mL, 25 times less than the 2.5 ppm level established for melamine in food[1]. Earlier applications work confirmed the sensitivity of the method with the analysis of low-level standards between 1 and 10 ppb. Figure 1 demonstrates the analysis of a 5-ppb standard achieving a signal to noise (RMS) of greater than 25:1 (note: this analysis was run with a slightly different GC oven program and resulted in a later elution of the melamine peak).

The extraction procedure was carried out 8 times on a single batch of milk spiked with melamine at 1 ppm – Figure 2 demonstrates a chromatogram generated in this analysis. The samples were spiked at 1 ppm to test the precision and recovery of the SPE method at a level close to the regulatory level. The average RMS measured for the extracted melamine samples spiked at 1 ppm in milk was approximately 13,700:1 (n=8). This verifies that the method achieves sensitivity that will far surpass regulatory testing needs.

Additionally, the analysis of 8 different extractions of the same melamine sample yielded a precision of 3.45% RSD when comparing the measured peak area for the summed ions of *m/z* 327+342. This data establishes that both the extraction and analytical methods are very reproducible. The average peak area measured in the analysis of 1 ppm melamine extracts was 2.7 x 10^7 when compared to an average peak area of 2.3 x 10^7 for a 0.5 µg/mL standard (equivalent to 1 ppm in milk sample). The percent recovery of this extraction is approximately 120%. This recovery is on the high side of acceptable, but similar to the 112% recovery demonstrated in reference 2.

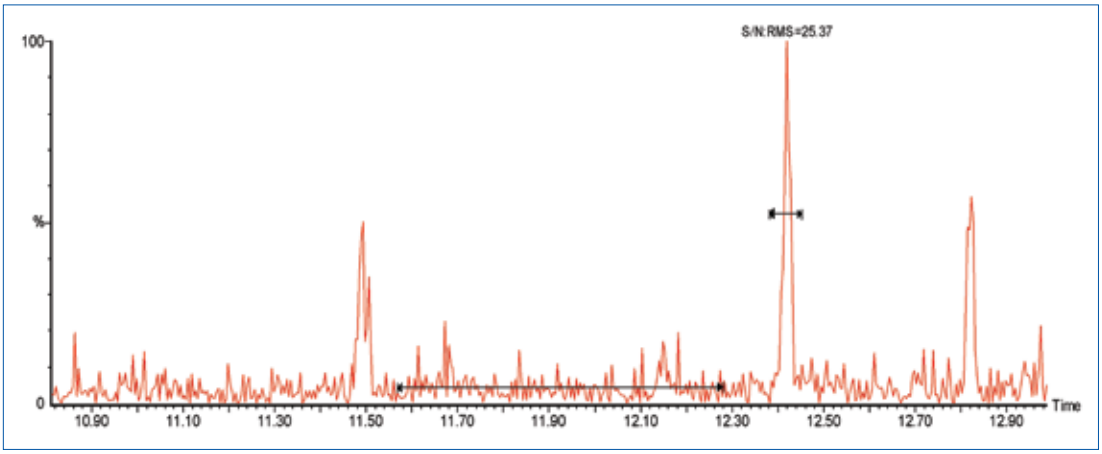

Figure 1. Chromatogram (extracted ion *m/z* 327+342) of the GC/MS analysis of a 5-ppb standard of melamine, demonstrating the sensitivity of the method.

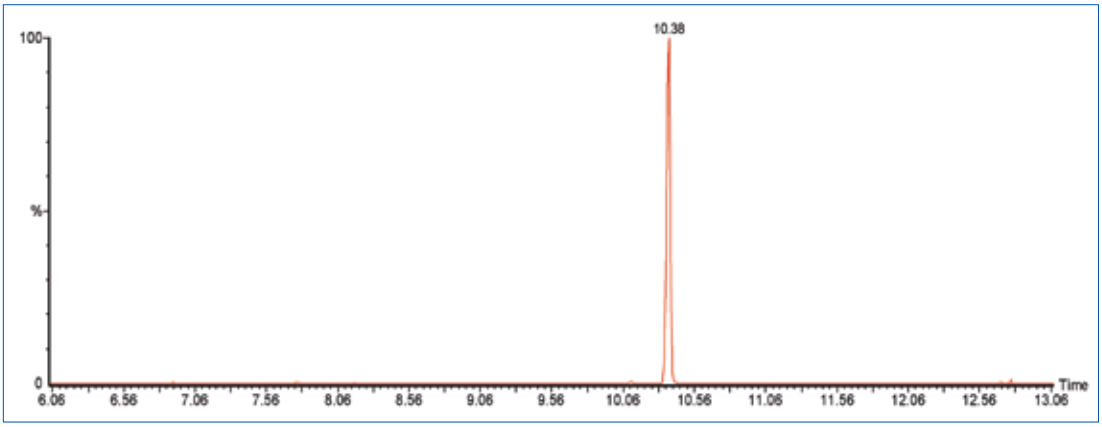

Figure 2. Chromatogram (extracted ion *m/z* 327+342) of the analysis of a milk sample spiked at 1 ppm with melamine.

Conclusion

The analysis of milk and dairy products for melamine requires the use of SPE to remove interferences caused by the high fat and sugar content of the matrix. A method including strong cation exchange, SPE, derivatization, and GC/MS analysis has demonstrated that melamine in dairy products can successfully be analyzed by GC/MS well below the limits required by regulation.

References

1. "Screening for Melamine Adulteration in Protein-Based Foods by GC/MS" James Neal-Kababick, Flora Research Laboratories, William Goodman, PerkinElmer (007969A_01).

2. "The Extraction and Analysis of Melamine in Milk-Based Products using Discovery DSC-SCX SPE and Ascentis Express HILIC LC-MS/MS" Olga Shimelis, Carmen T. Santasania, and An Trinh. Sigma-Aldrich.

3. "GC-MS Method for Screening and Confirmation of Melamine and Related Analogs" V2 May 7, 2007 FDA LIB 4423 Jonathan J. Litzau, Gregory E. Mercer, Kevin J. Mulligan. Forensic Chemistry Center (FCC), Food and Drug Administration, 6751 Steger Drive, Cincinnati, OH 45237, Pacific Regional Laboratory Northwest (PRLNW), Food and Drug Administration, 22201 23rd Drive SE, Bothell, WA 98021.

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