Rapid Screening of Melamine and Cyanuric Acid in Milk Products Using Agilent J&W HP-5ms GC Column and Agilent 7890A/5975C GC/MSD with Column Backflushing

Application Note

Food Safety

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Abstract

A rapid screening method for the determination of melamine and cyanuric acid in milk products is developed using Agilent 7890A/5975C GC/MSD along with Agilent J&W HP-5ms GC columns. With the backflushing by capillary flow technologies, this method eliminates the time consumption for column bakeout after elution of target compounds, so as to significantly shorten the GC run time from more than 70 minutes to 14.5 minutes. Good linearity was obtained within the range of 10 to 200 µg/g, with correlation coefficients greater than 0.9996. The recoveries of both target compounds are greater than 95%.

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Introduction

The contamination of food with melamine and cyanuric acid has attracted much attention all over the world. For the analysis of milk products, one of the major problems is the presence of less-volatile and nonvolatile matrix components, such as protein and fat. These components might contaminate the analytical system if the sample is introduced without selective sample preparation. The usual way to remove the matrix is to bake the column at high temperature, which often takes much longer than the sample run time for the analysis of interest. For example, the application of a milk extract of FDA method [1] usually takes about 70 minutes. Selective extraction or selective sample introduction is not easy, since the target compounds cover a broad volatility and polarity range. Moreover, for a routine QC analysis, laboratories want to reduce the typical cycle time by 25 to 60 minutes to improve productivity. The Agilent 7890A/5975C GC/MSD system meets this demand by using backflushing and faster cooling.

In this application note, a modified U.S. FDA method is developed using a standard split/splitless inlet and Agilent Capillary Flow Technology. A three-way splitter device with a makeup gas is placed at the end of the column and connected to MSD with a restrictor, thereby allowing column outlet pressure to be controlled with auxiliary electronic pneumatic control (EPC). By decreasing the column inlet pressure and increasing the column outlet pressure after the last peak of interest eluted from the column, the column flow is reversed, and the matrix interference, especially high boiler, can be removed out of the inlet end of the column. [2]

Experimental

Standards and Reagents

The standards and reagents used in the experiment are listed in Table 1. Stock solutions of melamine and cyanuric acid, each at a concentration of 1,000 µg/mL, were separately prepared in methanol. Stock solutions of standards are stored in the refrigerator.

Table 1. Standards and Reagents

Standard	Melamine	Sigma-Aldrich	>99% purity
	Cyanuric acid	Sigma-Aldrich	>99% purity
Solvent	Methanol	Fisher Scientific	HPLC grade
	Pyridine	Fisher Scientific	Certified A.C.S. Reagent
Silylating reagent	BSTFA with 1% TMCS*	Supelco	/

* BSTFA: bis(trimethylsilyl)trifluoroacetamide, TMCS: Trimethylchlorosilane

Instrument

The experiment was performed on an Agilent 7890A gas chromatograph equipped with a split/splitless capillary inlet, an Agilent 5975C GC/MSD with Triple-Axis Detector, and an Agilent 7683B automatic liquid sampler (ALS). The split/splitless inlet is fitted with a long-lifetime septum (P/N 5183-4761) and split injection liner (P/N 5188-4647). Injections are made using a 10-µL syringe (P/N 9301-0714). The instrument conditions are listed in Table 2.

Table 2. Gas Chromatograph and Mass Spectrometer Conditions

GC Conditions	
Column:	HP-5ms, 30 m × 0.25 mm × 0.25 µm (P/N 19091S-433)
Inlet temperature	EPC, split/splitless @ 250 °C
Injection volume	1 µL, split ratio at 3:1
Carrier gas	Helium, constant flow mode, 1.3 mL/min
Oven program	75 °C (1 min), 30 °C/min to 300 °C (1 min)
Post-run	280 °C, hold for 5 min (backflushing duration)
Transfer line	280 °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 (melamine: 342, 327*, 171, 99; cyanuric acid: 345*, 330, 188)
Backflush Conditions	
Device	3-way splitter (P/N G3183B)
Restrictor	0.706 m × 180 µm id
Outlet	PCM (P/N G1530-63309)
Outlet pressure	2 psi (60 psi for post-run)
Inlet pressure	2 psi (for post-run)

* Quantitative ion

Sample Preparation

Extraction

0.5 g of sample (powder or liquid) was weighed into a 20-mL polypropylene centrifuge tube; 5 mL of methanol was added. The sample was capped, vortex mixed, and then sonicated for 10 minutes. After the sample was centrifuged at 4,000 rpm for 6 minutes, the supernatant fluid was filtered through a 0.45-µm PTFE filter into a glass GC vial.

Derivatization

40 µL of the above extract was transferred into a glass GC vial. The extract was evaporated to dryness under a stream of nitrogen at approximately 70°C. 50 µL of pyridine and 50 µL of BSTFA were added. The sample was vortex mixed and incubated at 70 °C for 30 minutes.

Results and Discussion

7890A/5975C GC/MSD with a Backflush System for Milk Extracts

Milk extract usually contains many low-volatile or nonvolatile constituents. These compounds may stay near the front of the column until the oven temperature is high enough to move them through the column. In this application, a three-way splitter with makeup gas was employed to perform the backflush. The device has makeup gas supply tubing and four connectors, one connector for the analytical column and three connectors for the restrictor tube connecting to three available detectors. Since only an MSD is used as a detector in this application, the first two connectors were capped in the direction of makeup flow, the third connector was for the column in, and the last connector for the restrictor out to the MSD. This flow configuratin was used to avoid peak broadening due to improper flow sweeping. The length and internal diameter of the restrictor tubing is 0.706 m and 0.18 mm, respectively. The schematics of the GC/MS system configuration and tubing connection for the three-way splitter are shown in Figure 1.

First, a milk extract was analyzed in a typical mode – without backflush – by programming the oven to 300 °C to ensure that late eluters were eluted. It took more than 70 minutes to elute all the constituents in the extract (Figure 2A). Then, the extract was analyzed with a 5-minute backflush (Figure 2B). The backflush was accomplished by increasing the pressure in the outlet and decreasing the inlet pressure. The column flow was reversed to push the "heavy" constituents through the column inlet and out of the split vent. Figure 2C shows a 70-minute blank solvent run after the backflush. The blank run shows that the column was clean after backflushing, except for some peaks coming from the vial septa. Instead of baking the column at 300 °C for 55 minutes, the heavy matrix components were effectively removed from the column through backflushing. This reduced the run time from 70 minutes to 14.5 minutes, or a 4.8-fold increase in speed.

For a complex matrix, even baking for a long time cannot thoroughly remove the high boiler, which may result in peak retention time shift in the following injection. In Figure 3, two consecutive runs of the extract with 5-minute backflushing are shown. Excellent retention time and peak area repeatability were obtained, with no evidence of carryover, no emerging ghost peaks, and no increasing baseline. This demonstrated

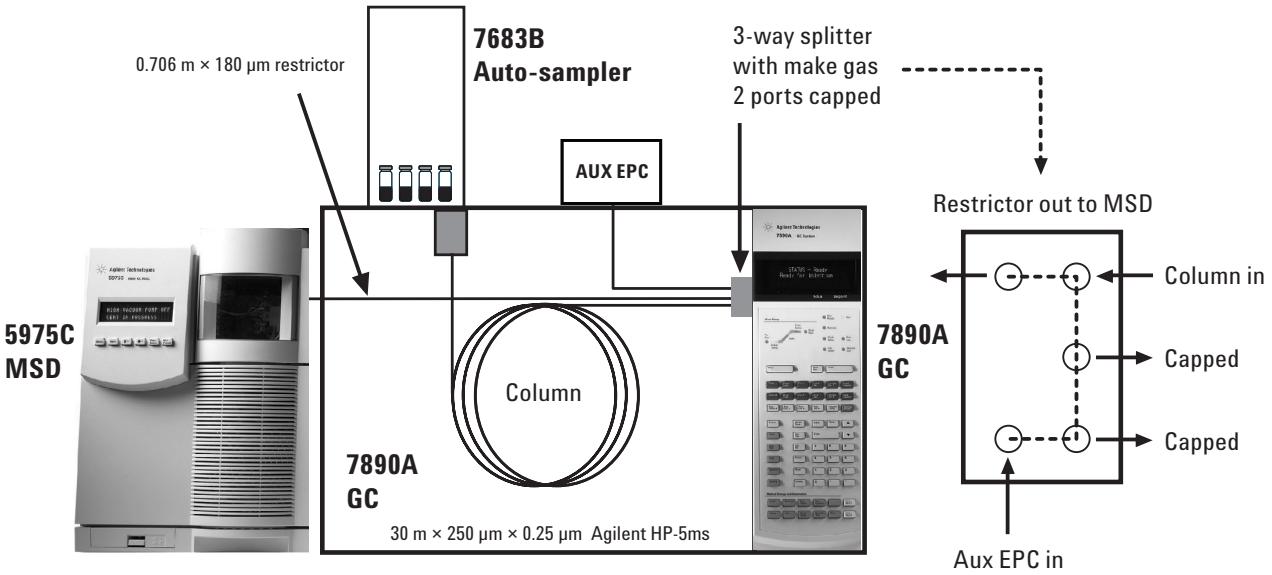

Figure 1. Schematics of GC/MS system configuration and tubing connection for three-way splitter.

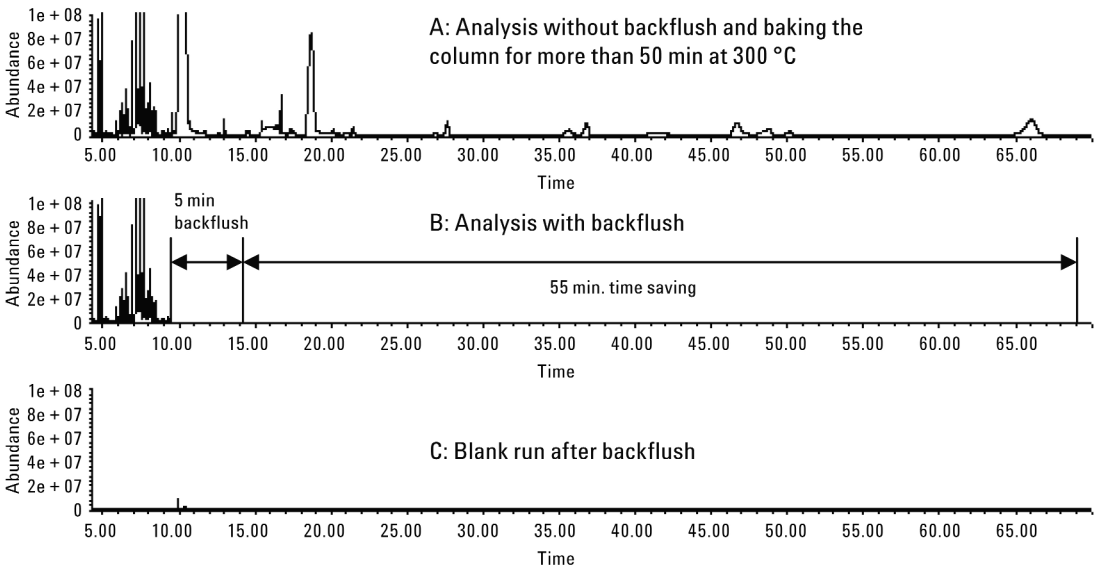

Figure 2. Time savings using backflush.

that backflushing is a perfect solution to avoid both high-temperature baking and retention time shift from run to run. Meanwhile, the faster oven cooling down capability of the Agilent 7890A allows for shorter cycle time. Additional time savings can be realized by using the three-way splitter used in this application, so that the liner and column can be changed without venting the MSD.

Figure 4 shows the total ion chromatograms (TIC) of a spiked milk powder sample. Synchronous SIM/scan was used to monitor ions of interest with high-sensitivity SIM mode and to simultaneously acquire library-searchable scan data in one run. This helped simplify the process of confirming positive or negative results. Figure 5 shows the mass spectra of cyanuric acid tri-TMS derivative (6.335 minutes) and melamine tri-TMS derivative (7.341 minutes), respectively.

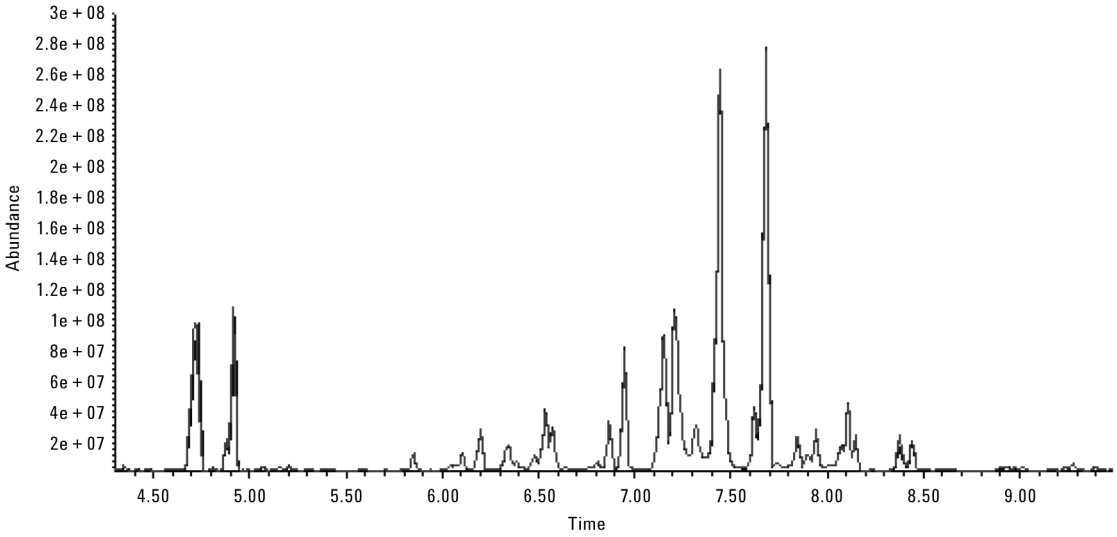

Figure 3. Overlay of total ion chromatogram spectra of two consecutive runs of a powdered infant formula extract.

Linearity and Recovery

A matrix blank milk sample is employed for a linearity experiment. 0.5 g of milk samples were spiked with four levels of cyanuric acid and melamine (10, 20, 80, and 200 µg/g). Excellent linearity was obtained for the two compounds within the range of 10 to 200 µg/g with a correlation coefficient higher than 0.9996.

To check the applicability of the method, a powdered infant formula (blank matrix) spiked with 40 µg/g of targeted analytes was analyzed. Excellent recoveries were obtained, with 96.1% for cyanuric acid and 95.6% for melamine (see Table 3).

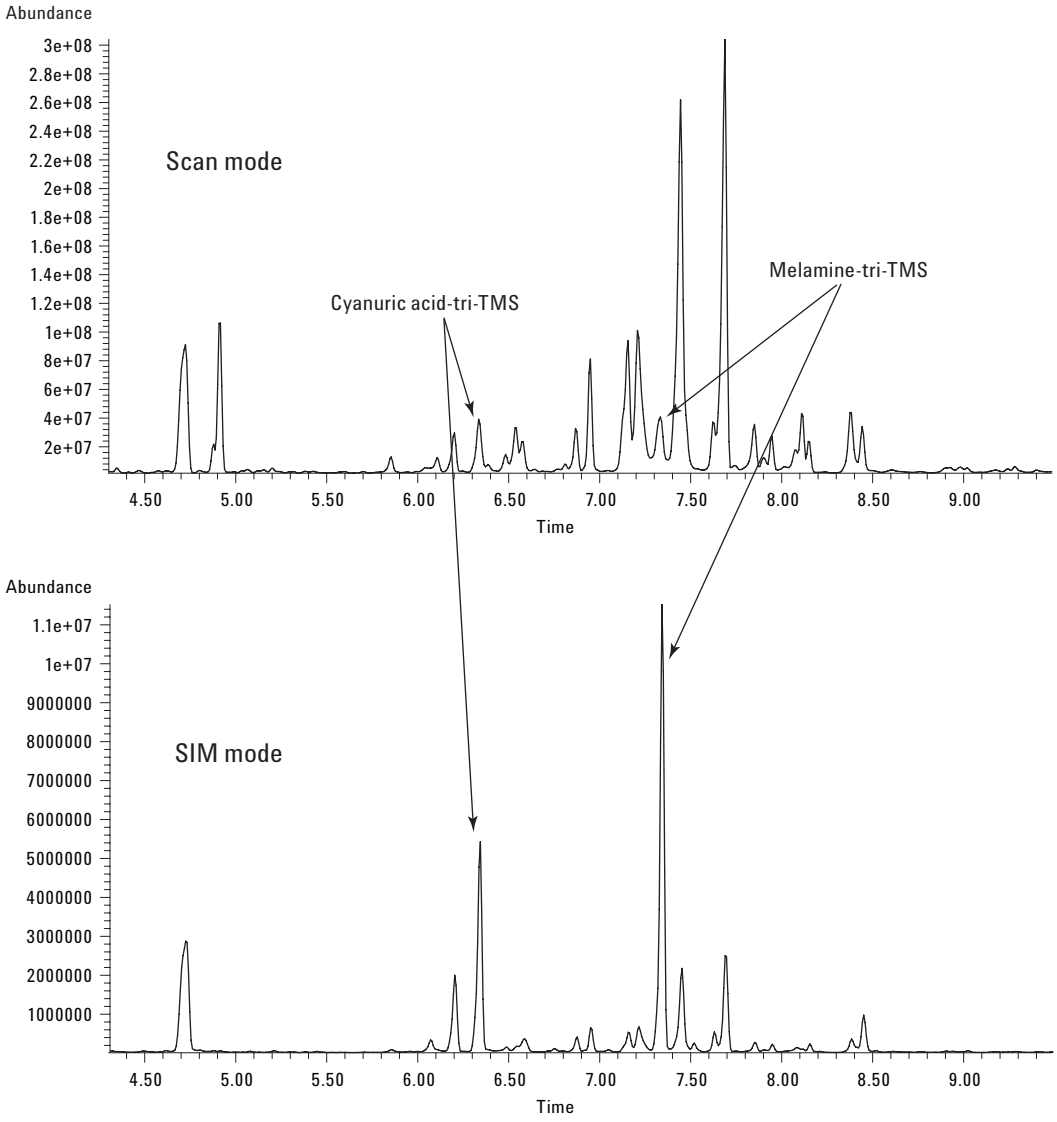

Figure 4. TICs of powdered infant formula sample.

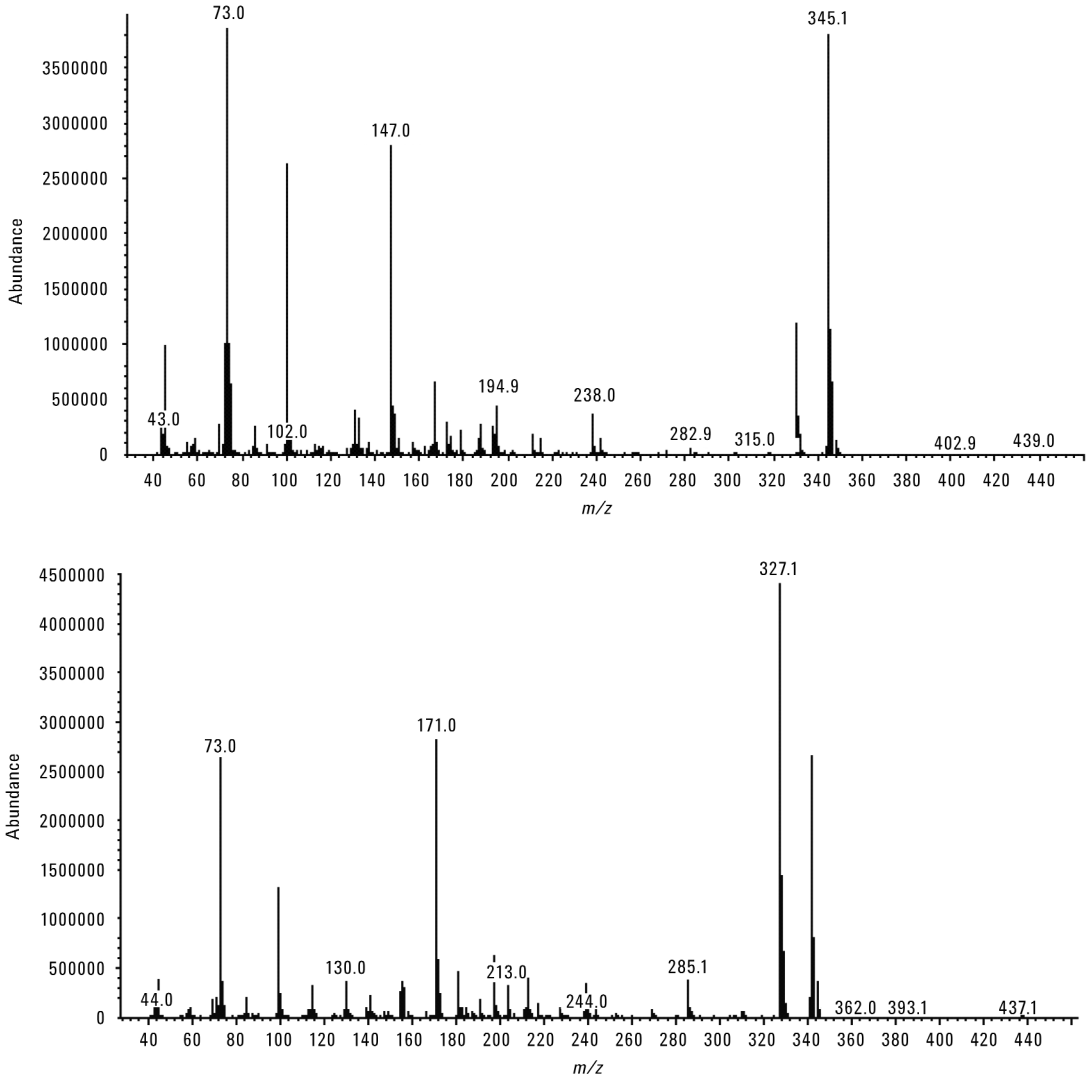

Figure 5. Mass spectra of cyanuric acid tri-TMS derivative (top) and melamine tri-TMS derivative (bottom).

Table 3. Recovery of Spiked Sample

Compound	RT (min)	Spiked level (µg/g)	Measured level (µg/g)	Recovery (%)
Cyanuric acid tri-TMS	6.319	40	38.42	96.1
Melamine tri-TMS	7.333	40	38.23	95.6

Real Sample Analysis

A brand of liquid milk was analyzed with backflush using the previously described method. The two targeted compounds were identified in less than 10 minutes, with cyanuric acid at 34.90 µg/g and melamine at 3.72 µg/g (see Figure 6).

Conclusions

The work described here is a rapid screening and quantitation method for the analysis of melamine and cyanuric acid in milk products that provides excellent linearity and recovery. Using Agilent 7890A/5975C GC/MSD combined with backflushing, the analysis time was cut down to five times shorter than conventional method. This method is fast and suitable for quality control of milk products for the determination of melamine and cyanuric acid.

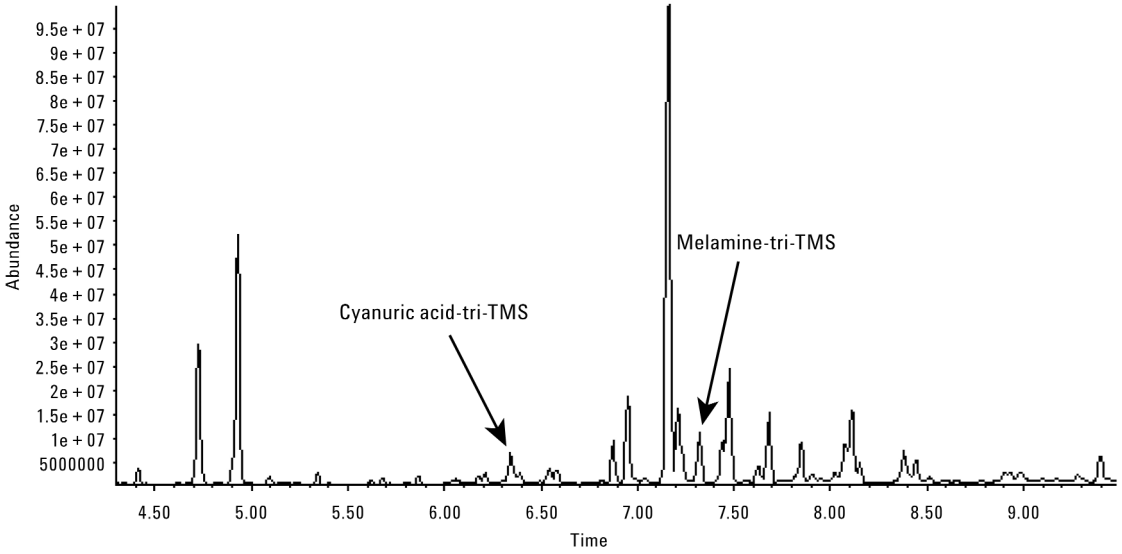

Figure 6. Total ion chromatogram of a contaminated brand of liquid milk.

References

1. U.S. Food and Drug Administration, “GC-MS Method for Screening and Confirmation of Melamine and Related Analogs,” Version 2, May 7, 2007.
2. Mike Szelewski, "New Tools for Rapid Pesticide Analysis in High-Matrix Samples," Agilent Technologies publication 5989-1716EN, Oct.13, 2004.

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