

Introduction

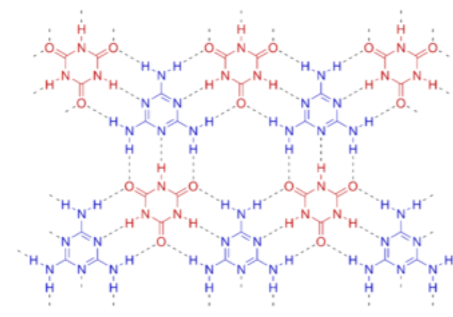
The adulteration of food with melamine has quickly become an international problem as it has been detected in baby formula produced in the US, chocolates distributed in Canada, biscuits sold in the Netherlands, condensed milk in Thailand and eggs in Hong Kong.

The Threat

Melamine has a LD₅₀ similar to that of table salt, but in the presence of cyanuric acid, another low toxicity compound, a crystalline complex forms. The crystalline melamine cyanurate can cause the formation of kidney stones and potentially kidney failure and death [2] – as seen in dogs and cats during the 2007 pet food contamination and in children during the 2008 Chinese milk scandal.

The Response

An FDA import alert, February 2009, requires that a testing method with a sensitivity of 250 ng/g for melamine and its analogs be used to assure compliance to the MRLs. To meet this new requirement a rapid method for the screening and confirmation of melamine, ammelide, ammeline and cyanuric acid in baby formula and soy meal was developed using the tandem quadrupole GC/MS with backflush.



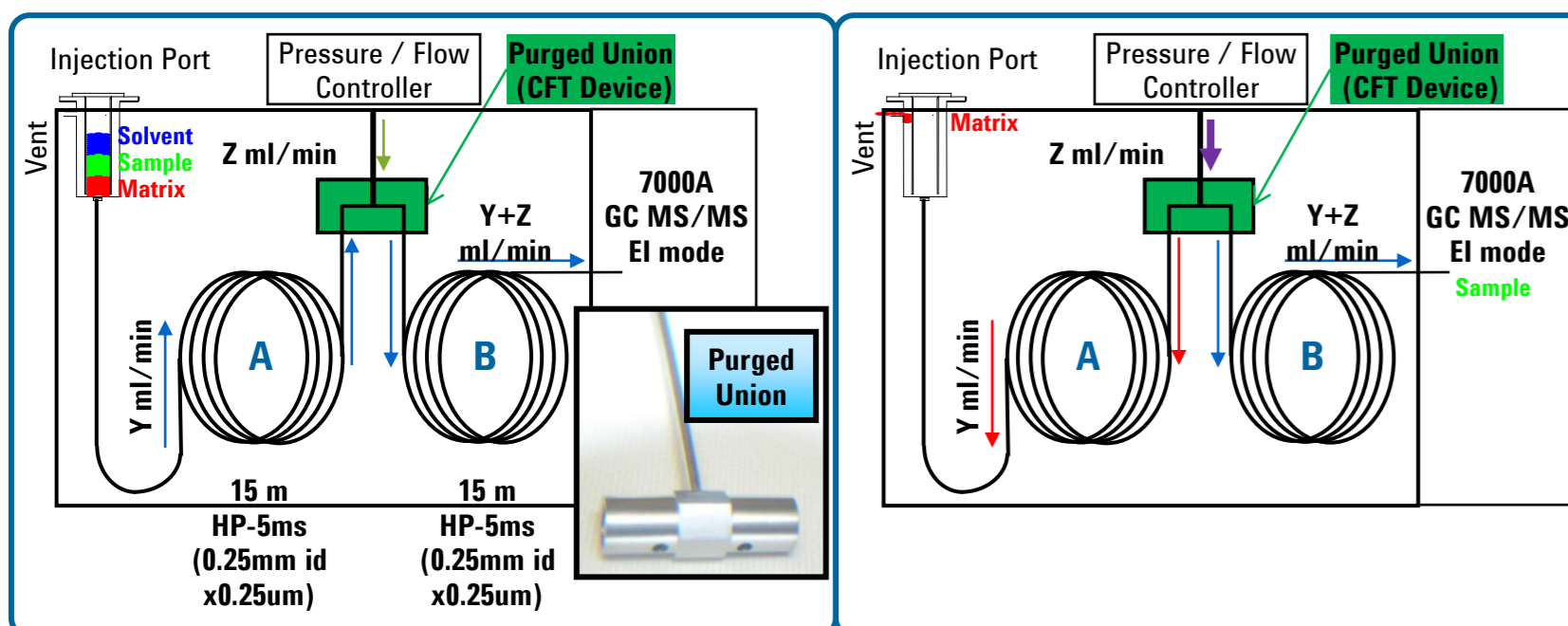
Melamine-Cyanuric Acid Complex

Instrument Configuration and Backflush

The extraction and derivatization procedures are the same as those used in the FDA GC/MS method [1]. The GC method included a splitless injection into a series of two 15 m DB-5ms columns. A deactivated, purged union with an auxiliary Electronic Pneumatic Control is added at the union of the two columns. This mode of backflush protected the ion source from high boiling matrix components and shortened the analysis cycle by enabling simultaneous separation on column B and backflush clean up on column A. By eliminating the need to bake out high boiling compounds the analytical method is reduced by 10 minutes.

We would like to acknowledge the guidance and material assistance of Dr. Greg Mercer at the FDA Pacific Regional Laboratory.

Mid-column Backflush



Analysis Mode: Column A & B flow is generated from Injection Port pressure. Z is set ≈ 0 ml/min. Flow to the MS (Column B) is not affected.

Backflush Mode: Increase pressure at Z and decrease injection port pressure. Flow Y is reversed, backflushing column A. Analytical flow in column B is not affected. Backflush Time is set when last target compound enters column B

Experimental^[3]

Sample Prep Method: Courtesy FDA – Pacific Regional Laboratory

- Weight out 0.5 g of sample.
- Add 20 mL of 50 % ACN / 40% Water / 10% DEA.
- Vortex for 30s and sonicate for 30 min.
- Centrifuge for 10 min at 5000 g or better.
- Filter the supernatant using a 0.45 μm nylon filter disk.
- Take a 160 μL aliquot for derivatization.
- Evaporate aliquot to dryness at 70 °C under a stream of dry nitrogen.
- Add 600 μL of internal standard solution in pyridine. ISTD = 57.7 ng/mL.
- Add 200 μL of Sylon BFT (99% BSTFA & 1% TMCS). ISTD = 43 ng/mL.
- Vortex to mix the sample.
- Incubate at 70 °C for 45 min before injecting.

Note: DEA is added to the extraction mixture to disrupt the melamine-cyanuric acid complex, reducing the risk of false negatives in the analysis.

GC Conditions:

Oven ramp: 100°C (1 min), 10°C/min to 210°C
 Injector : 280°C
 Carrier gas: helium
 Head pressure: 12.9 psi
 Injection volume: 1 μL
 Pulsed splitless: 25 psi @ 0.5 min
 Purge : 20 mL/min @ 0.75 min
 Column: DB-5ms μm
 30 m x 0.25 mm x 0.25
 Column flow: 1.2 mL/min
 Backflush: -3.6 mL/min @ 300°C for 1.3 min
 Transfer line temp: 290°C

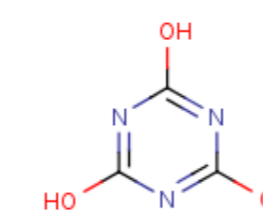
MS Conditions:

Tune: Autotune
 Delta EMV: 400V
 Acquisition: EI MRM
 Filament Delay: 6 minutes
 MS Source: 230°C
 MS Quad: 150°C

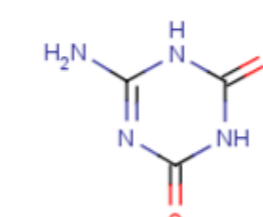
Three MS/MS transitions were optimized for each analyte. The MS/MS conditions are detailed below.

MS/MS conditions for Melamine and Related Compounds

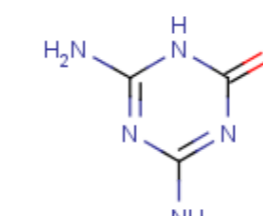
Compound	Start Time (min)	GC MS/MS			
		Tr	MRM	Dwell Time (ms)	Collision Energy (EV)
Cyanuric Acid	9	9.613	345→215	50	8
			345→188	50	12
			330→215	50	4
Ammelide	10	10.801	344→171	50	22
			329→171	50	20
DACP (ISTD) 2,6-Diamino-4-chloropyrimidine	10.85	11.185	273→237	150	12
			273→99	150	20
Ammeline	11.3	11.748	328→171	50	25
			343→214	50	20
			343→171	50	30
Melamine	12	12.467	327→171	20	17
			342→285	150	20
			342→213	150	22



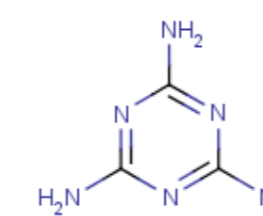
Cyanuric Acid



Ammelide



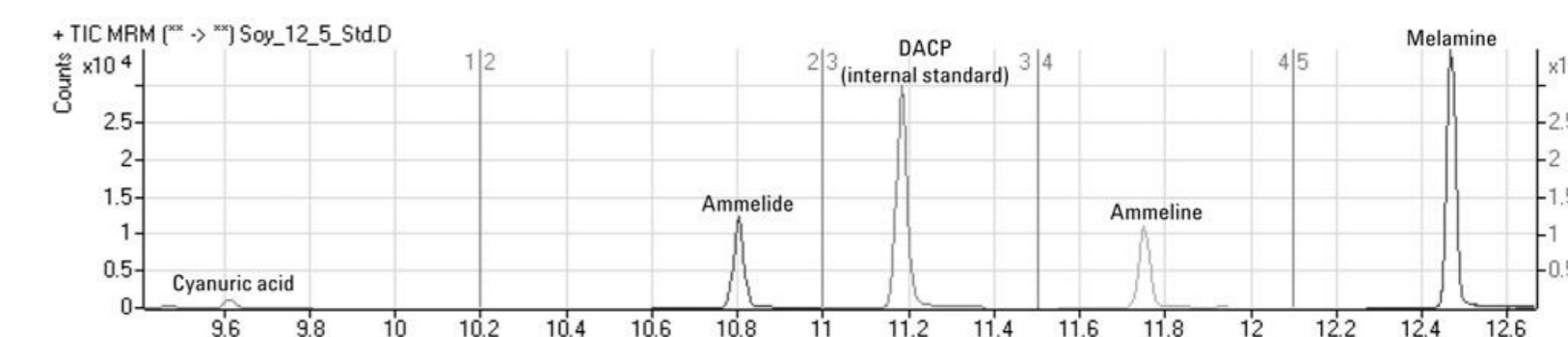
Ammeline



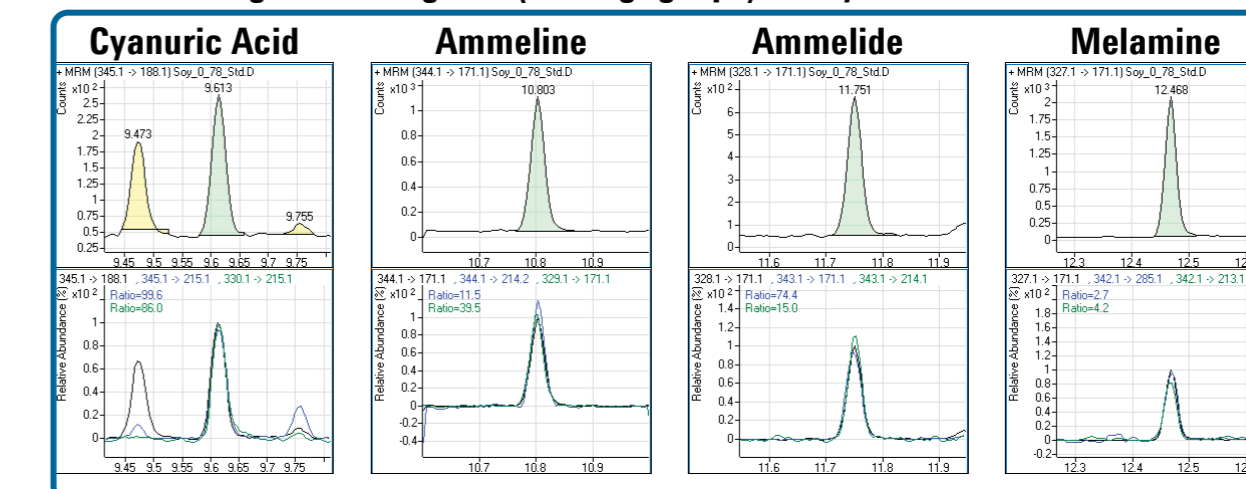
Melamine

Results and Discussion

Reconstructed Total Ion Current Chromatogram (RTICC) resulting from MRM analysis, illustrating the resolution of melamine and its analogs



Quantifying and normalized qualifying transitions for melamine and its analogs at 0.78 ng/mL (0.25 ng/g eqv.) in soy meal.



Conclusions

The FDA import alert applies to all items with entry dates after March 4th, 2009. All third-party laboratory testing need to employ a method that achieves 250 ng/g of sensitivity for melamine and analogs. Importers can access the import alerts via the FDA public site.

In response to the discovery of melamine and related compounds in milk products, the minimum reporting level of melamine has been set to 1 ng/g for infant formula and 2.5 ng/g for other milk products. By applying GC/MS/MS we were able to lower the method detection limits from 2.5 ng/g to 0.25 ng/g without changing the extraction and derivatization procedure. We generated full calibration data for soy meal and baby formula. We were also able to use backflushing to significantly reduce the instrument cycle time and improve productivity.

Future Concerns

Melamine and its analogs will eventually disappear from the food supply. Unfortunately, they may be replaced by other highly nitrogenous industrial chemicals in an effort to trick the prevailing procedures for protein analysis, the Kjeldahl and the Dumas methods

Fortunately, considering the heavy costs of product recalls many food suppliers will be more vigilant when they inspect their raw materials and audit their suppliers.

References

- [1] U.S. Food and Drug Administration, GC/MS Screen for the Presence of Melamine, Ammeline, Ammelide, and Cyanuric Acid, LIB No. 4423, Volume 4, October 2008.
- [2] "Melamine and Cyanuric Acid Interaction May Play Part in Illness and Death from Recalled Pet Food", American Veterinary Medical Association (AVMA), Press Release, May 1, 2007. Accessed 2008-09-27.
- [3] Rapid Screening and Confirmation of Melamine and its Analogs in Baby Formula and Soy Products Using Triple Quadrupole GC/MS and Backflushing. Stephan Baumann, Agilent Technologies Application note 5990-4071EN