

High Sensitivity Melamine GC/MS Analysis of Cat Food

Modified Conditions Save Costs and Reduce Maintenance

By Michelle Long, Innovations Chemist and Julie Kowalski, Ph.D., Food Flavor and Fragrance Innovations Chemist

- Excellent results in pet food matrix; lower pyridine background for better sensitivity.
- Easy sample preparation; reduced derivatization reagent volume lowers costs and keeps inlet and column clean.
- Modified conditions reduce maintenance and extend filament lifetime.

A large pet food recall occurred in 2007 when animals became ill or died after eating food contaminated with melamine and related compounds. Melamine is an industrial chemical used in the production of plastics, adhesives, flame retardants, fabrics and other materials. It is not a food ingredient, but since melamine and related compounds are high in nitrogen content—and protein testing methods are based on nitrogen levels—these compounds were used as additives to generate artificially high label values for protein content.

Procedure

The procedure for this experiment was adapted from the U.S Food and Drug Administration (FDA), GC/MS Method for Screening and Confirmation of Melamine and Related Analogs, Version 2, May 7, 2007. Standards were diluted to 10µg/mL and 1µg/mL with 10:40:50 diethylamine:water:acetonitrile. Three 0.5g matrix samples (dry cat food) were prepared: one control, one spiked at 50µg/g and one at 10µg/g.

Two modifications were made to the derivatization procedure in the FDA method. The amount of derivitizing reagent was reduced from 200µL to 50µL of BSTFA with 1% TMCS (which is still a molar excess of 50:1). Incubation time was subsequently increased from 45 min. to 120 min.

Analyses were performed on a Shimadzu QP-2010 Plus gas chromatograph mass spectrometer (GC/MS) using a 30m x 0.25mm ID x 0.25µm Rtx®-5MS column. The mass spectrometer data was acquired in SIM acquisition mode with selected ions for each analyte of interest (Table I).

Results

The original method conditions resulted in a significant initial baseline elevation due to the presence of pyridine, which is necessary for the derivatization reaction (Figure 2). Pyridine can increase ion signal background over a long period of time. To combat this, pyridine can be evaporated and the remaining analytes can be dissolved in a more GC amenable solvent, but this is time consuming and can result in analyte loss. A simpler solution is to eliminate the pyridine ion signal by changing the mass range to be scanned. All of the analytes have characteristic ions of interest well above m/z 79 which is associated with pyridine. Therefore,

Figure 1 Melamine and related compounds are nitrogen-rich and can artificially raise labeled protein content when used as an additive.

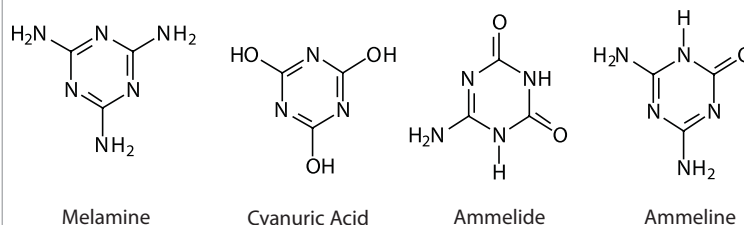


Figure 2 Original method produces an elevated baseline, compromising integration and reducing sensitivity (10µg/mL standard).

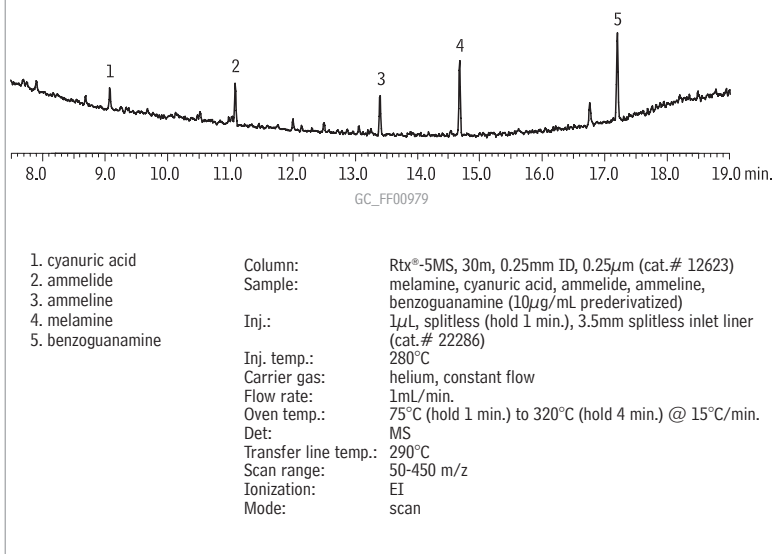
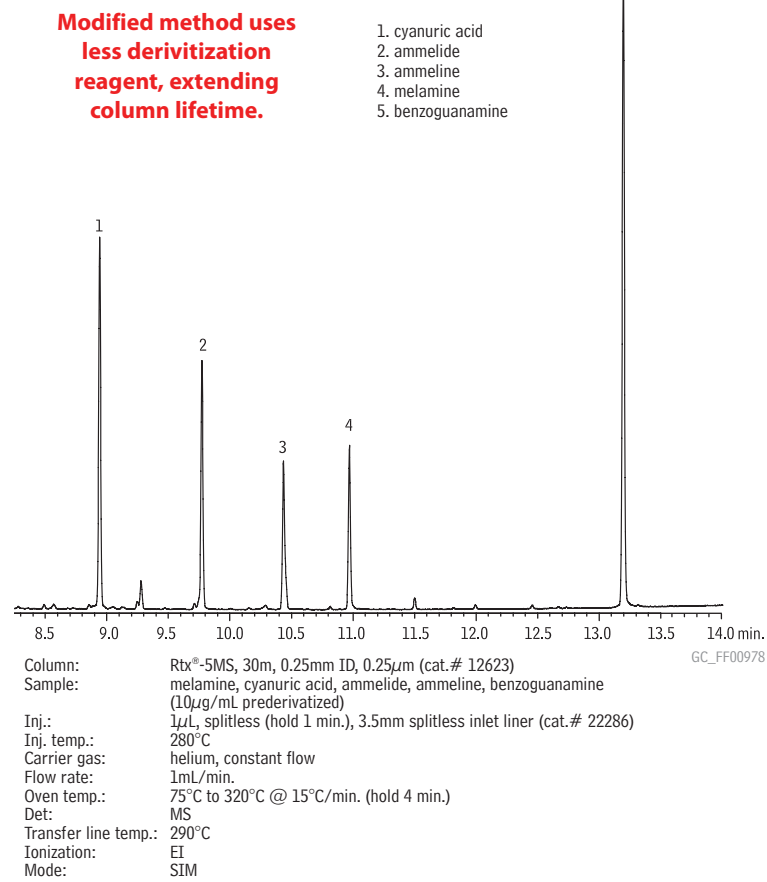


Table I MS conditions (SIM mode).

Compound	Retention Time (min.)	Target Ions	Reference Ions	Reference Ions	Reference Ions
Cyanuric Acid	8.97	345 (100)*	330 (36)	346 (30)	347 (15)
Ammelide	9.79	344 (100)	329 (30)	345 (58)	330 (16)
Ammeline	10.44	328 (100)	343 (79)	329 (29)	344 (24)
Melamine	10.97	327 (100)	342 (53)	328 (30)	343 (17)
Benzoguanamine	13.18	316 (100)	331 (68)	332 (20)	330 (9)

Figure 3 Excellent separation of melamine and related compound using modified conditions (10µg/mL standard).



the scan method was modified to begin scanning at m/z 85. The solvent delay was also increased to approximately 8 min. due to the high background levels. This extra time helps increase the filament lifetime and ensures all the analytes will be detected.

This method provides excellent separation of melamine and cyanuric acid, the suspected toxic compounds, as well as ammelide and ammeline (Figure 3). Reproducible and reliable retention times were obtained for matrix spikes; this, along with SIM mass spectrometric detection, allows easy identification of analytes at both the high and low spike levels (Figure 4).

Conclusions

This work demonstrates that the FDA method is a valuable guideline for analysts screening melamine and related analogs. Using an Rtx®-5MS column and modifying the original method provides additional benefits: 1) decreasing the derivitization reagent volume results in longer column lifetime and less inlet maintenance, and 2) increasing the solvent delay decreases pyridine ion background, resulting in higher sensitivity, approximately 5 times higher, for the analytes of interest.

References

GC-MS Method for Screening and Confirmation of Melamine and Related Analogs, Version 2, May 7, 2007, U.S Food and Drug Administration, <http://www.fda.gov/cvm/GCMSscreen.htm>.

Rtx®-5MS—Low-bleed GC/MS Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	12623

Splitless Liners for Shimadzu 17A, 2010, and 2014 GCs

ID* x OD & Length (mm)	qty.	cat.#
3.5mm Splitless		
3.5 ID x 5.0 OD x 95	ea.	22286
3.5 ID x 5.0 OD x 95	5-pk.	22287

*Nominal ID at syringe needle expulsion point.

Silylation Derivatization Reagents

Compound	CAS#	cat.#
BSTFA w/1% TMCS (N,O-bis[trimethylsilyl]trifluoroacetamide) w/1% trimethylchlorosilane)		
10-pk. (10x1g)	25561-30-2	35606
25g Flex Tube	25561-30-2	35607

Figure 4 Melamine production analytes are easily identified in cat food using SIM analysis (50µg/g spike).

