Fast and Quick Detection of Melamine and Its Analogues from Powdered Infant Milk

Application Note

Food Safety

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Introduction

Since the infant milk scandal in 2007, several methods have been released for the analysis of melamine from dairy products. However, a comprehensive method for the analysis of the related compounds (ammeline, ammelide, and cyanuric acid) is difficult to find. The US FDA has a comprehensive method, but the lengthy sample preparation steps may not be practical in a production laboratory where low sample preparation time is critical. The hydrophilic nature of the analytes, suggests a HILIC LC column would be the most promising and most procedures use a HILIC LC method. HILIC has many pitfalls concerned with reproducibility and high organic solvent usage.

An alternative simple method was developed using Captiva ND plates. This quick and reliable method uses a reversed phase LC column and limits the preparation time to a minimum, while maintaining quantification of melamine at regulatory levels. Using Captiva ND plates, a simple and quick analysis of melamine and its analogues was developed. Good linearity, accuracy, and precision was achieved for each analyte. A comparison of diluted samples to samples processed through the Captiva ND was investigated, demonstrating better sensitivity achieved by using the Captiva ND plate.

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Experimental

Standard Preparation

All compounds were ordered from ChromaDex

- Melamine ASB-00013163-100
- Cyanuric acid ASB-00003958-100
- Ammeline ASB-00001657-100
- Ammelide ASB-00001659-100

Melamine and cyanuric acid were prepared in water. Ammeline and ammelide were prepared in a 2% ammonium hydroxide solution. All four compounds were mixed together to a concentration of 100 µg/mL in 2% ammounium hydroxide. Ammonium hydroxide is required to keep all compounds in solution.

Calibration Curve

Powdered infant milk was prefortified to concentrations ranging from 0.1 µg/g to 50 µg/g of melamine and its analogues. Above 50 µg/g, a melamine:cyanuric acid complex forms in solution causing inaccurate results [1].

Sample Preparation

1. Weigh out 2 ± 0.01 g powdered infant milk.
2. Spike the milk with analytes to 1 µg/g or 2.5 µg/g.
3. Add 20 mL H_2O (10 mL per gram of powdered milk).
4. Vortex or shake, there should be no remaining powder.
5. Add 400 µL of ACN to a Captiva ND 0.2 µm PP (p/n A5969002) filtration plate.
6. Add 100 µL of spiked milk, followed by pipette mixing (5 cycles @ 300 µL).
7. Apply vacuum.
8. Transfer filtrate to a sample vial for analysis, or directly analyze from collection plate.

Note: Evaporation and reconstitution showed no added benefit.

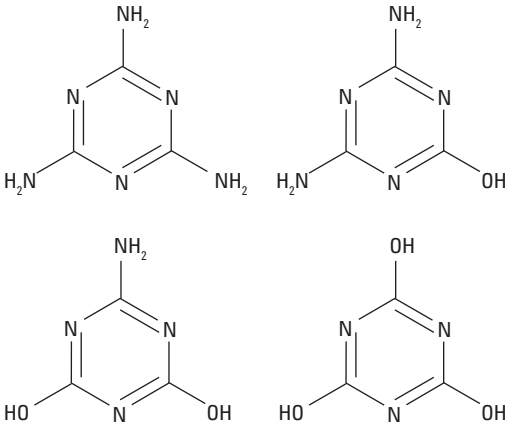


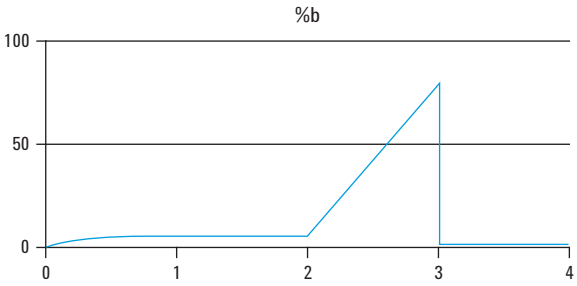
Figure 1. Chemical structures.
Top: melamine, ammeline
Bottom: ammelide, cyanuric acid

Instrumentation and Conditions

System	Agilent 1290 Infinity LC System Agilent 6460 Triple Quadrupole LC/MS system
Column	Pursuit XRs Ultra 2.8 Diphenyl 100 × 2.0 mm A7521100X020
Mobile Phase	A: 0.1% Formic Acid in H_2O B: MeOH

Gradient

time	%b
0.00	2
0.50	5
2.00	5
3.00	80
3.01	2



Flow	0.4 mL/min
Injection volume	5 µL
Temperature	ambient
Runtime	3 min
EMV	±300
Dwell	300
Cell Accelerator Voltage	7

Compound	Precursor ion	Product ion	Fragment	Collision energy	Polarity
Melamine	127	85.1	100	18	+
Cyanuric acid	128	42.1	60	14	-
Ammeline	128	69.1	140	34	+
Ammelide	127	84	100	6	-

Source

Gas temperature	300 °C
Gas flow	5 mL/min
Nebulizer	20 psi
Sheath gas temperature	275 °C
Sheath gas flow	7 mL/min
Capillary	+3500 -2000
Nozzle	+0 -500

Results and Discussion

Reversed phase chromatography resulted in baseline separation of the melamine-cyanuric acid pair and the ammeline-ammelide pair. Figure 2 is a chromatogram of 2.5 µg/g spiked milk after processing with Captiva ND. All compounds except for melamine were reliably detected down to 1.0 µg/g, whereas melamine was easily detected down to 0.5 µg/g.

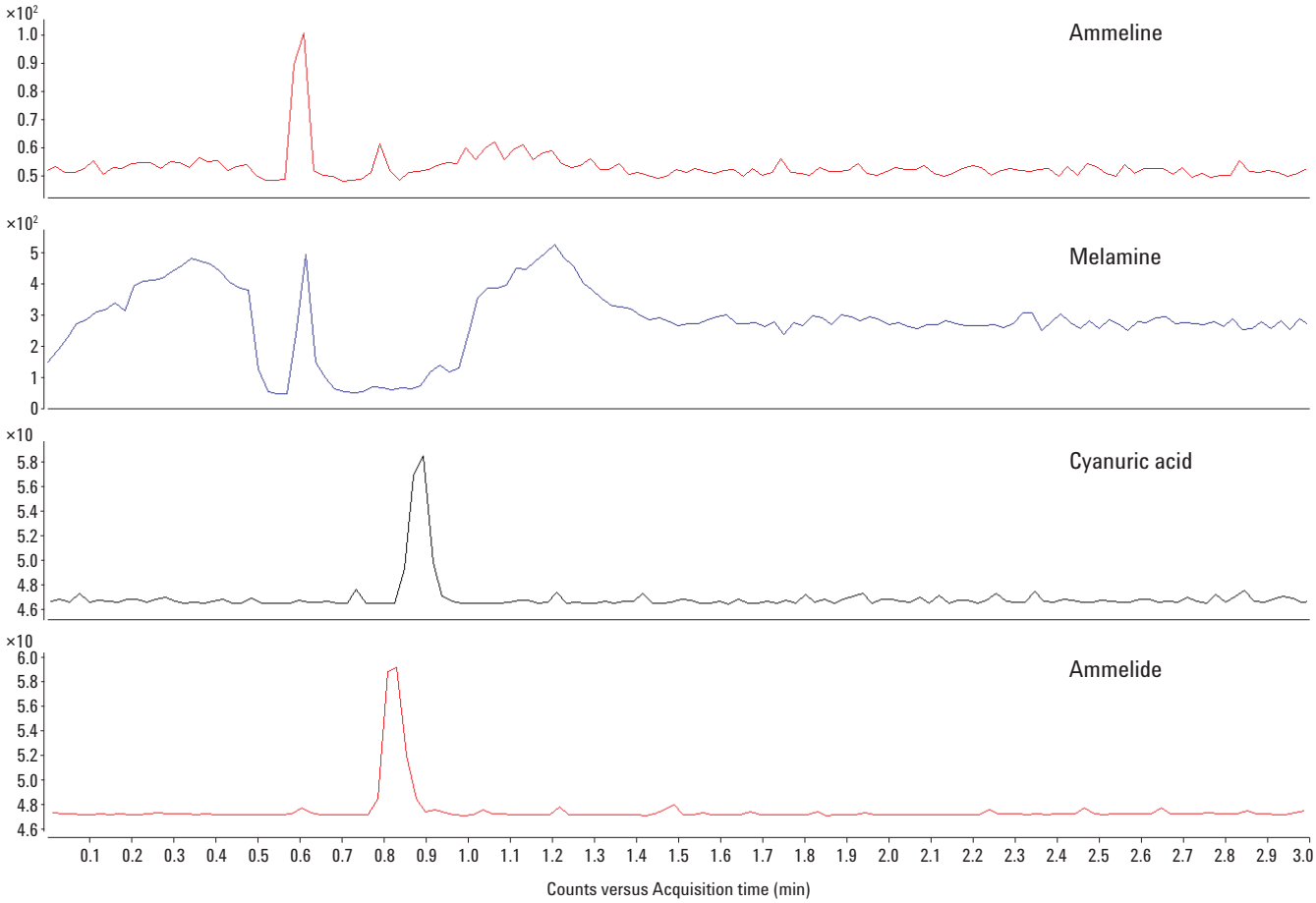


Figure 1. Separation of melamine and its analogues at 2.5 µg/g.

Figures 3 and 4 demonstrate analyte suppression caused by matrix interferences in powered infant formula. The top chromatograms show a sample which was processed through Captiva ND, whereas the bottom chromatograms were diluted 1:3 with water, to maintain the same concentration as the processed sample, and injected. The analyte of interest is completely suppressed by matrix interferences.

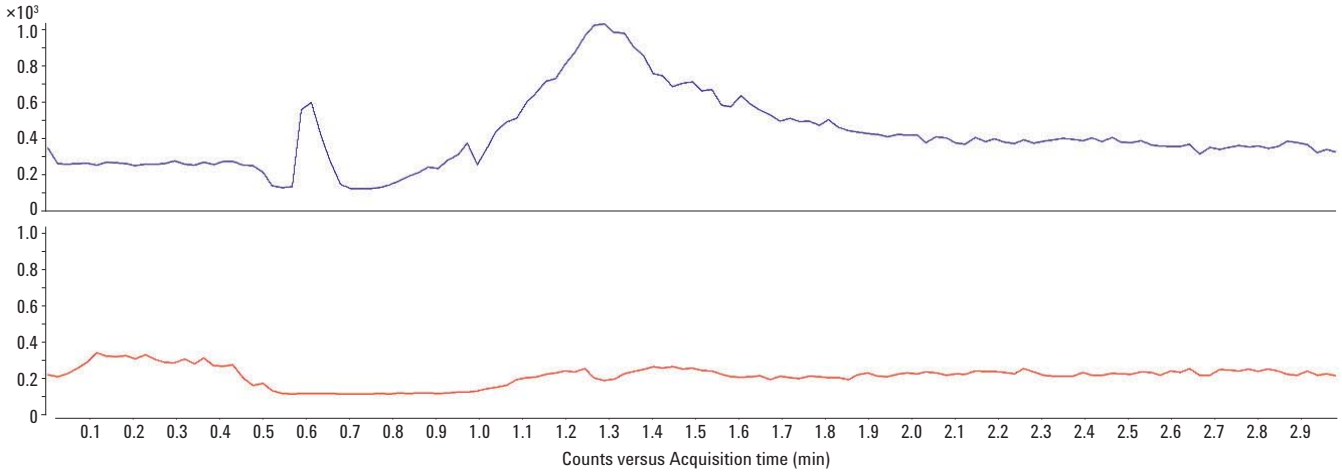


Figure 3. Top: Captiva ND processed spiked sample, Bottom: 1:3 diluted spiked sample. Melamine peak is completely suppressed by matrix interferences.

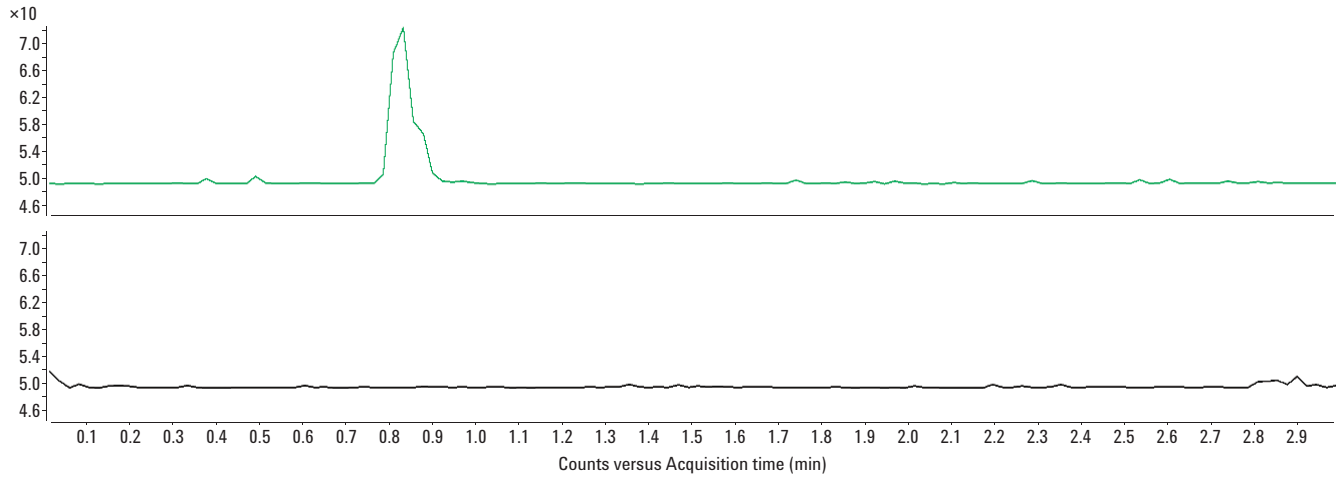


Figure 4. Top: Captiva ND processed spiked sample, Bottom: 1:5 diluted spiked sample. Ammelide peak is completely suppressed by matrix interferences.

All compounds showed linearity from 1 µg/g–50 µg/g with a R^2 above 0.995. Melamine was linear from 0.5 µg/g–50 µg/g with a R^2 of 0.999. A minimum of six levels were used for the calibration curves.

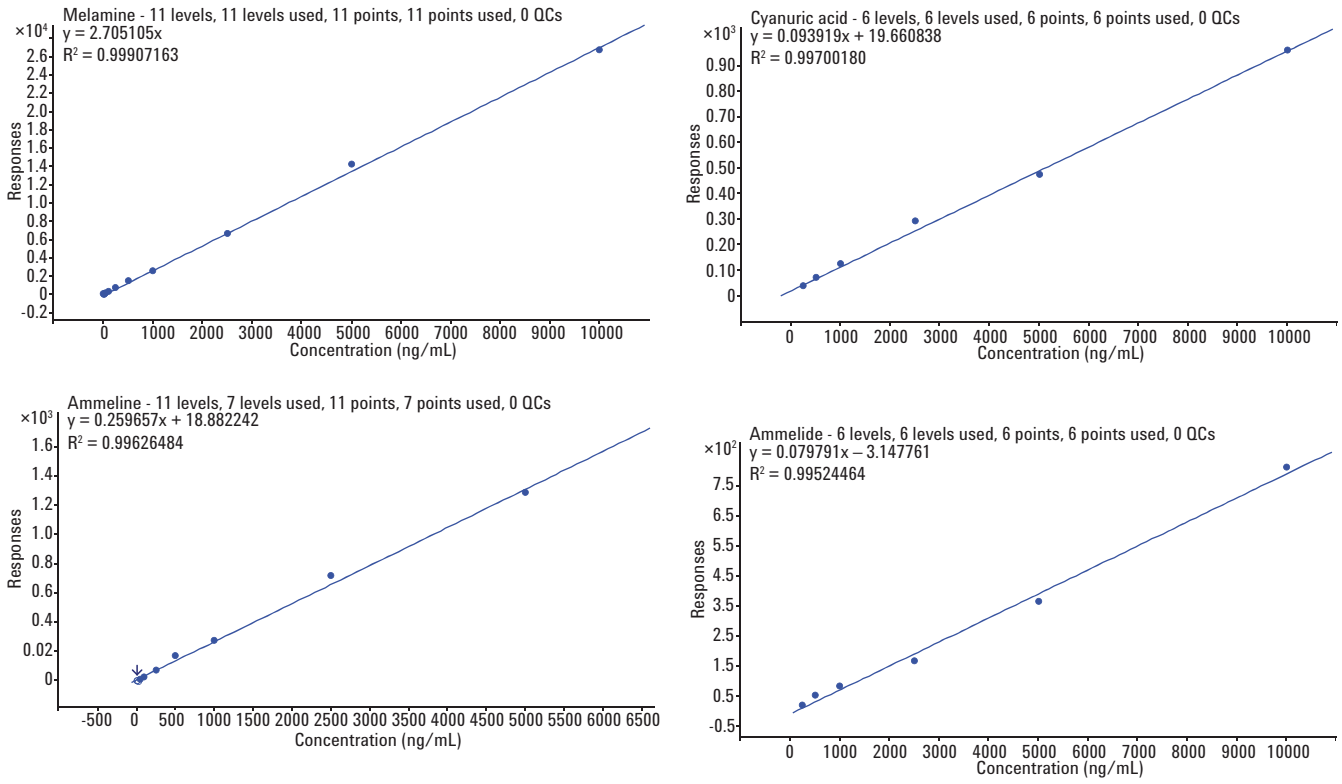


Figure 5. Calibration curves of melamine, cyanuric acid, ammeline, and ammelide, respectively.

Table 1 lists the relative recoveries of melamine and its analogues following sample processing with Captiva ND. Melamine showed relative recoveries within 11% of true value with RSDs below 13% at all levels. Cyanuric acid, ammeline, and ammelide at higher levels showed relative recoveries within 11% of true value and RSDs below 10%.

Table 1. Recoveries of Melamine and its Analogues from Fortified Powdered Infant Formula (n = 6)

	Average % Recovery ± RSD		
Compound	**1.0 µg/g**	**10 µg/g**	**25 µg/g**
Melamine	94 ± 12.4	89.5 ± 9.1	107 ± 9.9
Cyanuric acid	n/a	105 ± 8.3	102 ± 7.7
Ammeline	n/a	90.1 ± 5.6	110 ± 9.4
Ammelide	n/a	108 ± 9.4	92.4 ± 6.3

The minimum per sample processing time dramatically dropped with the use of Captiva ND. Single sample processing via the FDA method requires approximately 100 minutes versus 1 minute with the Captiva ND method presented. Parallel sample processing using the FDA method is limited by centrifuge size.

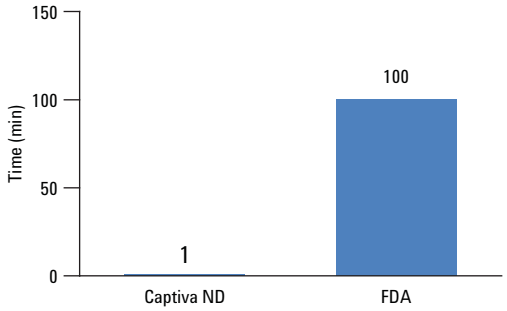


Figure 6. Minimum per sample processing time Captiva ND and FDA method.

Conclusions

The method employed in this application note provides a quick and easy method for the analysis of melamine and its analogues from powdered dairy products using a simplified sample preparation procedure and reversed phase chromatography. By using Captiva ND plates and Pursuit XRs Diphenyl column quick analysis for melamine was accomplished down to regulatory levels of 1 µg/g [2]. Linearity was demonstrated for all compounds with a linear regression coefficient greater than 0.995. This method is more sensitivity to melamine than its analogues, and has a signal-to-noise ratio > 5:1 required by the US FDA at the 1 µg/g level [1]. At the 1 µg/g level melamine's relative recovery was within 11% of true value with RSD less than 13%. At higher levels, all compounds had relative recoveries within 11% of true value with RSDs less than 10%. The combination of Captiva ND and Pursuit XRs Diphenyl allows for the quick and easy analysis of melamine from dairy products at a fraction of the sample processing time while still maintaining required sensitivity.

References

1. S.B. Turnipseed, C. Casey, C. Nochetto, and D.N. Heller. U.S. FDA Laboratory Information Bulletin, 2008:24:4421. Lib. No. 4421 [http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/DrugChemicalResiduesMethodology/ucm071637.htm] accessed September 1, 2011.
2. Chinese Ministry of Health (2011). China sets limits of melamine levels tolerable in food products, 21 April, 2011 (http://english.gov.cn/2011-04/21/content_1849392.htm)

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