LC/MS/MS Analysis of Melamine in Liquid Milk and Milk Powder with Bond Elut Plexa PCX

Application Note

Food Testing & Agriculture

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Introduction

Standard quality control tests for food products estimate protein levels by measuring the nitrogen content. Melamine (Figure 1, left) is an additive that is typically used as a modifying agent for plastics, paints, and coatings. Food manufacturers or suppliers in China have added melamine to food or feed to deceive quality control analyses. The US Food and Drug Administration (FDA) determined that melamine caused widespread pet illness, death, or both, when illicitly added to pet food in 2007. A wave of illness among Chinese infants was attributed to melamine-tainted infant formula. The FDA found that melamine binds easily with isocyanuric acid to generate a crystalline polymer (Figure 1, right), which is toxic to humans.

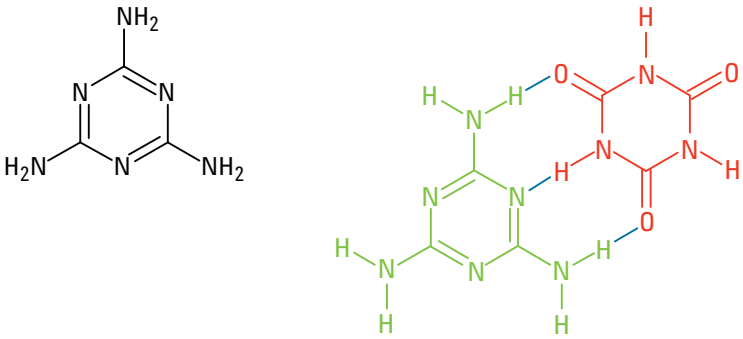

Figure 1. Molecular structure of melamine (left). Melamine bound to isocyanuric acid (right).

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A simple, rapid, sensitive, and robust method for melamine that is free of matrix interference is essential for its quantitative analysis. Agilent Bond Elut Plexa PCX sample preparation, combined with Agilent Pursuit C8 LC columns and LC/MS/MS, is a complete solution for melamine analyses in complex matrixes.

Materials and Methods

A 1,000-ppm stock solution was prepared by dissolving 50 mg of melamine into 20 mL methanol and diluting to 50 mL with laboratory reagent water. This stock solution was further diluted with extracts of melamine-free liquid milk or milk powder for instrument calibration.

Sample preparation

A 5 g amount of powdered milk or 10 mL of liquid milk was dissolved in 24 mL acetonitrile:H_2O (50:50, v/v) and 1 mL 1.0 M HCl. The solution was mixed for 1.5 minutes with an Ultra Turrax mixer. The sample was then centrifuged for 5 minutes at 4,000 rpm at 5 °C. The extract was filtered through a 0.45-µm filter and then subjected to solid phase extraction (SPE) as described below.

1. Condition the Bond Elut Plexa PCX cartridge with 5 mL methanol followed by 5 mL water.
2. Add sample supernatant and extract by gravity.
3. Wash with 5 mL 0.1 mol/L HCl followed by 2 mL methanol.
4. Dry the cartridge under vacuum for 1 minute.
5. Elute with 5 mL 5% ammonia in 95% methanol (V:V).
6. Evaporate to dryness under nitrogen at 50 °C.
7. Reconstitute with 95:5 acetonitrile:ammonium formate (20 nM), bring to 1 mL, centrifuge for 10 seconds, or filter through 0.45-µm membrane.
8. Inject sample into LC/MS/MS.

LC conditions

Column:	Agilent Pursuit C8, 4.6 x 50 mm, 5 µm (p/n A3030050X046)	
Sample prep:	Agilent Bond Elut Plexa PCX, 200 mg, 6 mL (p/n 12108206)	
Eluent:	A, 25 mM ammonium acetate in H_2O; B, 25 mM acetic acid in CH_3CN	
Injection volume:	10 µL	
Flow rate:	200 µL/min	
Gradient:	Time (min)	% B
	0.0	10
	2.5	95
	4.5	95
	5.0	10
	7.0	10
Temperature:	35 °C	

MS/MS conditions

Ionization mode:	ESI positive		
Needle:	3.6 kV		
Shield:	225 V		
API drying gas:	22 psi at 300 °C		
API nebulizing gas:	55 psi		
Collision gas:	1.8 mTorr argon		
MRM parameters:	Precursor ion	Product ion	Collision energy (V)
	126.9	67.9	21.5
		84.9 (qualifier ion)	13.5

Results and Discussion

MS/MS was used to analyze spiked matrix samples to increase the sensitivity and selectivity of the analysis, and minimize matrix interferences. The multiple-reaction-monitoring (MRM) chromatogram for the melamine transition *m/z* 127 > 85 is shown in Figure 2. Calibration was linear from 1 to 100 µg/kg, with R^2 greater than 0.998 (Figure 3).

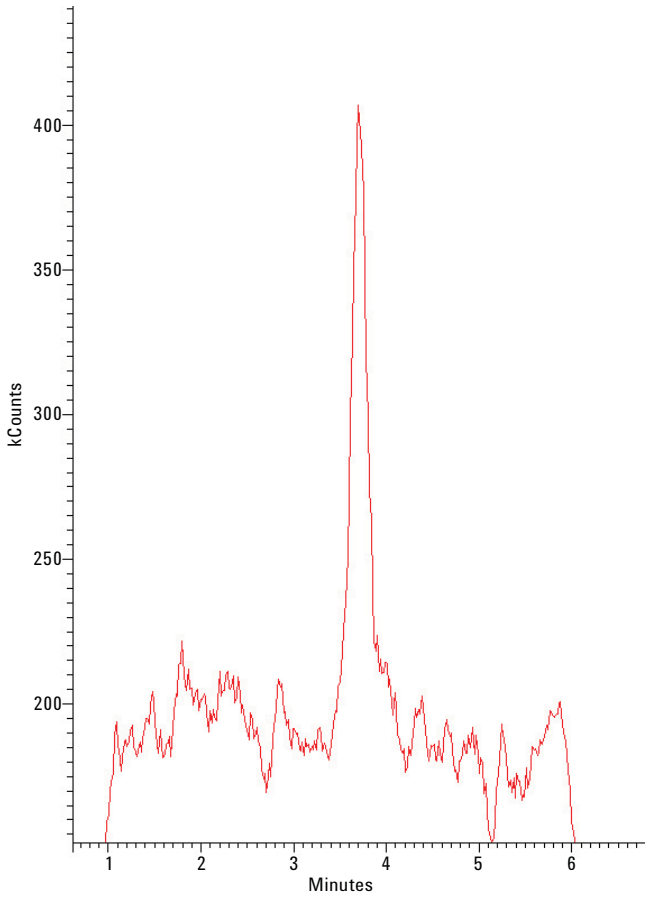

Figure 2. Chromatogram of melamine spiked at 1 µg/kg in a milk sample free of melamine contamination.

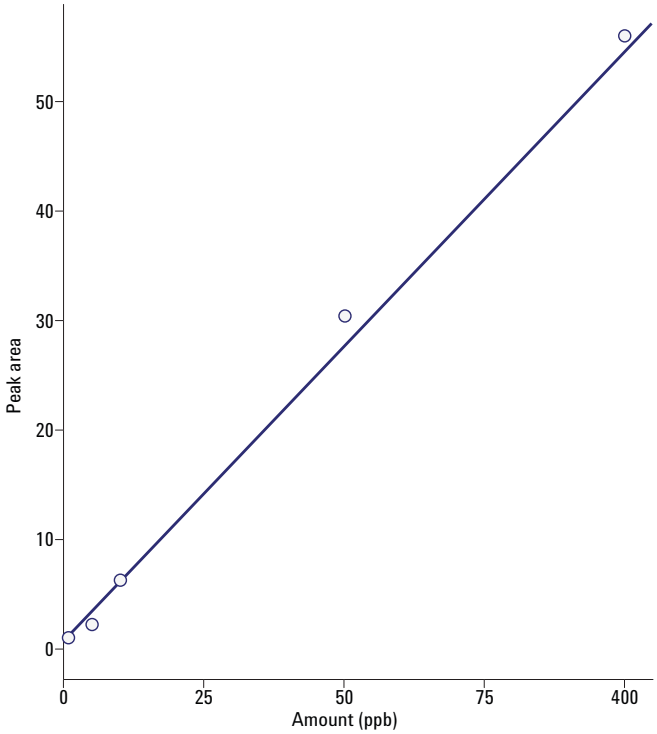

Figure 3. Calibration curve for melamine in liquid milk with calibration points at 1, 5, 10, 50, and 100 µg/L.

Conclusions

This sample preparation method, which combines LLE and SPE, is simple, fast, and sensitive for the LC/MS/MS analysis of melamine in milk. The polymeric cation-exchange technique removes neutral and acidic interferences from the matrix, and concentrates basic analytes. This results in improved sensitivity in the quantification of basic compounds such as melamine. The method can quantitatively analyze melamine at 1 µg/kg and maintain accuracy, even in complex matrixes.

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