GC/MS/MS Analysis of Melamine in Milk Powder with Agilent 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 (Figure 1, right) to generate a crystalline polymer, which is toxic to humans.

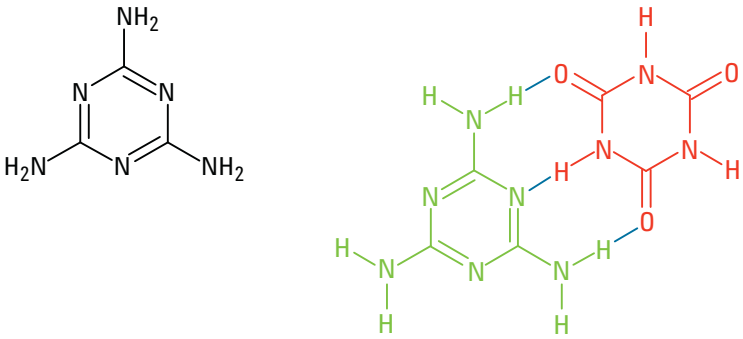

Figure 1. Molecular structure of melamine (left). Melamine bound to isocyanuric acid (right).

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A simple, rapid, sensitive, and robust method for melamine that is free of matrix interference is essential for quantitative analysis. Agilent Bond Elut Plexa PCX sample preparation, in combination with an Agilent J&W VF-5ms GC column and GC/MS/MS, is a complete solution for melamine analysis 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 was weighed into a centrifuge tube and mixed with 5 mL methanol. The solution was mixed for 10 minutes using an Ultra Turrax mixer. The sample was then centrifuged for 10 minutes at 4,500 rpm. The extract was filtered through a 0.45-µm membrane and then subjected to solid phase extraction (SPE) as described below.

1. Condition Bond Elut Plexa PCX with 3 mL methanol followed by 3 mL H_2O.
2. Add 3 mL supernatant fluid and extract by gravity.
3. Wash with 3 mL H_2O, followed by 3 mL methanol, and dry the cartridges for 5 minutes.
4. Elute with 5 mL 5% ammonia:95% methanol (v/v).
5. Evaporate to dryness with N_2 stream at 50 °C.
6. Reconstitute with 200 µL pyridine.

Derivatization of melamine

A 200 µL solution of bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added to the extract generated in step 2 above. The solution was mixed for 30 seconds using an Ultra Turrax mixer and then allowed to react for 30 minutes at 70 °C. The derivatized sample (Figure 2) was ready for analysis after cooling to room temperature.

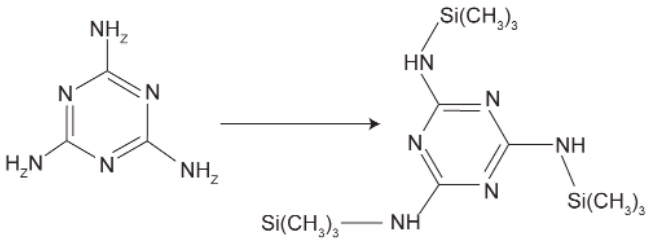

Figure 2. Formation of melamine derivative using BSTFA.

LC conditions

Column:	Agilent J&W VF-5ms, 30 m × 0.25 mm, 0.25 µm (p/n CP8944)
Sample prep:	Agilent Bond Elut Plexa PCX, 60 mg, 3 mL (p/n 12108603)
Temperature:	100 °C for 2 min, to 220 °C at 10 °C/min, hold for 5 min
Injection temperature:	260 °C, splitless
Injection volume:	1 µL

MS/MS conditions

Ion source temperature:	200 °C	
Transfer line:	280 °C	
MS/MS:	Precursor ion	Product ions
	327	171, 189, 285

Results and Discussion

Figure 3 shows the multiple-reaction-monitoring (MRM) trace of derivatized melamine spiked in milk powder.

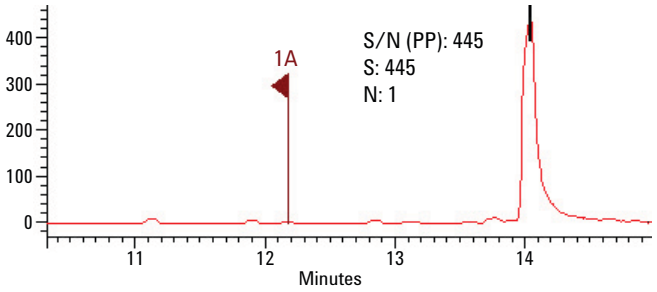

Figure 3. TIC of milk powder spiked with 50 µg/L melamine.

Figure 4 shows the product ion spectrum obtained from derivatized melamine. Product ion ratios can be used to obtain qualitative confirmation. Figure 5 demonstrates excellent linearity of the ion trap for the analysis, with an R^2 value of 0.9997.

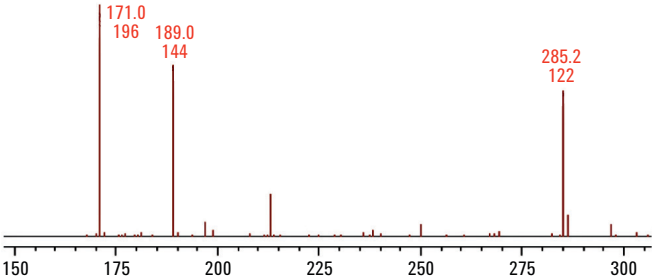

Figure 4. MS/MS product ion spectrum of melamine derivative in powdered milk

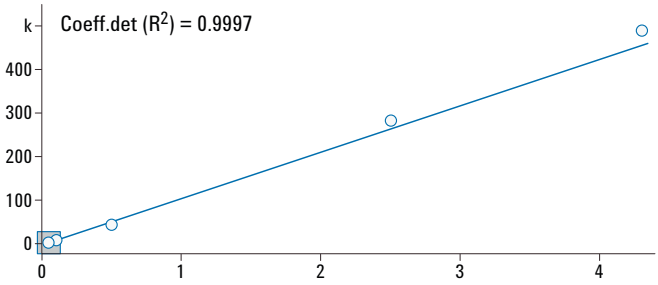

Figure 5. Calibration in powdered milk with 10, 50, 500, and 5,000 µg/L spiked with melamine.

Conclusions

This sample preparation method coupled with GC/MS/MS is cost-effective, fast, and sensitive for the analysis of melamine in powdered milk samples. Polymeric cation-exchange SPE removes neutral and acidic interferences from the matrix, and concentrates basic analytes. The method can quantitatively analyze melamine at µg/L levels and maintain accuracy, even in complex matrixes. MS/MS provides additional confidence in results by further eliminating matrix interference and providing product ion spectra for analytical quality control.

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Printed in the USA
June 5, 2013
5991-2495EN

Agilent Technologies