TOSOH

Application Note

Analysis of Melamine and Cyanuric Acid in Milk by LC-MS/MS

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton. Melamine is combined with formaldehyde to produce melamine resin, a very durable thermosetting plastic, and melamine foam, a polymeric cleaning product. The end products include countertops, dry erase boards, fabrics, glues, house wares and flame retardants. Melamine is one of the major components in Pigment Yellow 150, a colorant in inks and plastics.

Melamine is nontoxic in low doses, but when combined with cyanuric acid it can cause fatal kidney stones due to the formation of an insoluble melamine cyanurate.

In 2007 a pet food recall was initiated by some pet food manufacturers who had found their products had been contaminated and caused serious illnesses or deaths in some of the animals that had eaten them. In March 2007, the US Food and Drug Administration reported finding white granular melamine in the pet food, in samples of white granular wheat gluten, imported from a single source in China. Melamine was added to the wheat gluten to artificially increase the apparent protein content. It was found in crystalline form in the kidneys and urine of affected animals as well.

In September 2008, several companies were implicated in a scandal involving milk powder and infant formula, which had been adulterated with melamine, leading to kidney stones and other renal failure, especially among young children. By end of September 2008, nearly 53,000 people had become ill, with more than 12,800 hospitalizations and four infant deaths.

Here, we report the investigation of simultaneous determination of melamine and cyanuric acid by HILIC-MS/MS on a TSKgel Amide-80 3 µm column (2 mm ID x 15 cm L, P/N 21865). Melamine can be detected by electrospray ionization mass spectrometry (ESI-MS) in positive mode (MRM 127/85), cyanuric acid by ESI-MS in negative mode (MRM 128/42) as shown in Figure 2. For milk spiked with melamine and cyanuric acid as a model sample, both melamine and cyanuric acid could be determined with high recovery after a simple deproteination pretreatment (Figure 3). Good linearity from 0.5 to 50 ppb was found for both compounds.

STRUCTURAL FORMULA

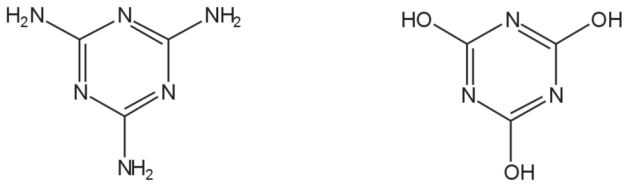


Figure 1

CHROMATOGRAPHIC CONDITIONS

Column:	TSKgel Amide-80 3 µm 2.0 mm ID x 15 cm L
Eluent:	A: 0.05% formic acid in water
	B: 0.05% formic acid in actetonitrile
	75% B
Flow rate:	0.2 ml/min
Injection vol.:	5 µl
Temperature:	40°C
Detection:	LC-ESI-MS/MS, QTrap (MDS SCIEX),
	127/85+ (Melamine); 128/42- (Cyanuric acid)

PRETREATMENT OF MILK SAMPLE

Milk + (water / acetonitrile = 20/80) =
10 + 90 (v + v)

↓

Mixture, Ultracentrifugation
(5,000 rpm, 5 min)

↓

Filtration (0.5 µm pore size)

SEPARATION OF MELAMINE AND CYANURIC ACID (10 PPB EACH)

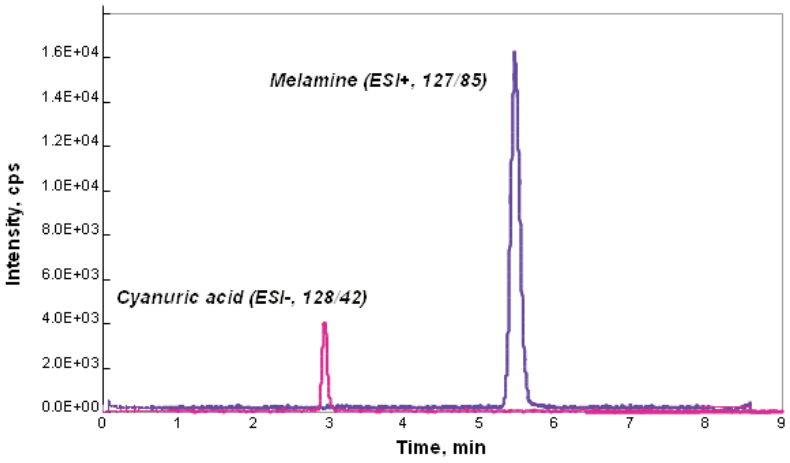


Figure 2

MRM CHROMATOGRAMS OF MILK AND SPIKED MILK SAMPLES (10 PPB EACH)

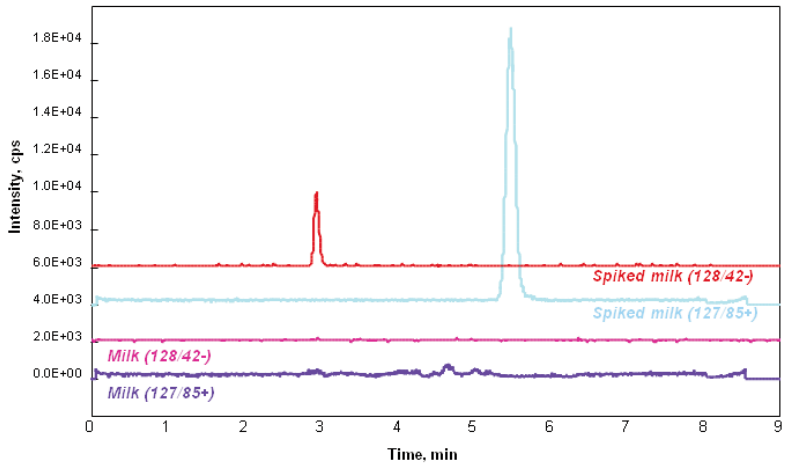


Figure 3

TSKgel Amide-80 HILIC Columns for the Analysis of Melamine and Cyanuric Acid in Milk by LC-MS/MS

TSKgel
APPLICATION NOTE

Introduction

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton. Melamine can react with formaldehyde to produce melamine resin, a very durable thermosetting plastic, and melamine foam, a polymeric cleaning product. Some end products made from melamine include countertops, dry erase boards, fabrics, glues, housewares, and flame retardants. Melamine is also one of the major components in Pigment Yellow 150, a colorant in inks and plastics.

In 2007 a pet food recall was initiated by Menu Foods and other pet food manufacturers when veterinary scientists in the US determined melamine to be the cause of hundreds of pet deaths, because of pet food contamination. Prior to these reports, melamine had been regarded as non-toxic or minimally toxic. Wheat gluten, a common ingredient in pet food, was found to contain residues of melamine and a related compound, cyanuric acid. Widespread pet illness and death was subsequently attributed to the formation of melamine-cyanurate crystals in the kidneys of these animals.

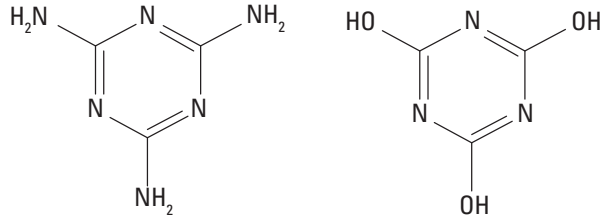
In September 2008 several companies in China were implicated in a scandal involving milk and infant formula which had been adulterated with melamine, leading to kidney stones and other renal failure, especially among young children. By September 22nd, nearly 53,000 people had become ill, with more than 12,800 hospitalizations and four infant deaths. Indications are that companies producing milk in China added melamine to mask the loss in nitrogen content from dilution of milk products.

On September 26 of this year, the FDA issued a statement saying that it had "broadened its domestic and import sampling and testing of milk-derived ingredients and finished food products containing milk, such as candies, desserts, and beverages that could contain these ingredients from Chinese sources. Milk-derived ingredients include whole milk powder, non-fat milk powder, whey powder, lactose powder, and casein."
(http://www.fda.gov/bbs/topics/NEWS/2008/NEW01891.html)

To aid chemists charged with the determination of melamine and related products in milk, Tosoh scientists developed a method for the simultaneous determination of melamine and cyanuric acid by HILIC-MS/MS using a three micron TSKgel Amide-80 column. Milk was spiked with melamine and cyanuric acid standards to serve as a model sample.

The structural formulas for melamine and cyanuric acid are shown in *Figure 1*, while *Figure 2* details the pretreatment of milk samples prior to the addition of the standards.

Figure 1. Structural formulas of melamine and cyanuric acid



Melamine *Cyanuric acid*

Figure 2. Pretreatment of milk

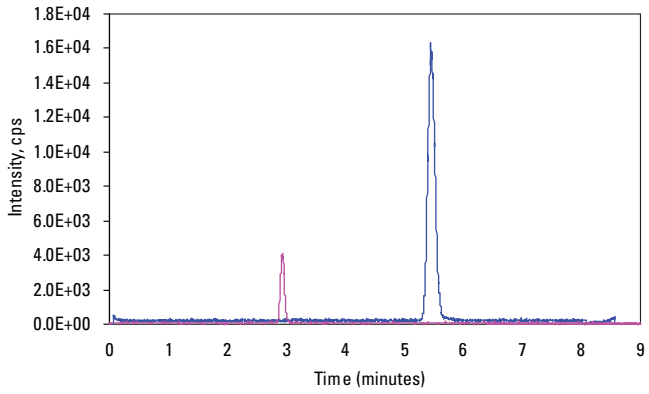
Milk + H_2O/ACN = 20/80 = 10 + 90 (v/v)
↓
Mix
↓
Ultracentrifugation @ 5,000rpm for 5minutes
↓
Filtration (pore size: 0.5 µm)

Results

Both melamine and cyanuric acid were determined with high recovery (data not shown). Pretreatment of the milk samples was achieved by simple deproteination with acetonitrile, as shown in *Figure 3*. Good linearity was obtained for both compounds for the range of 0.5 to 50ppb.

Multiple Reaction Monitoring is a mode of MS/MS that yields maximum sensitivity and selectivity for known target analytes. *Figure 4* shows the results of this type of mass analysis on unspiked and spiked milk samples. The figure demonstrates that the original milk sample did not contain any amount of either melamine or cyanuric acid. After adding the compounds to the milk sample, melamine and cyanuric acid were independently detected, with more than sufficient resolution between the compounds. Although not shown here, analysis time was reduced to 2 minutes by using a shorter TSKgel Amide-80 column (3µm, 2mm ID x 5cm).

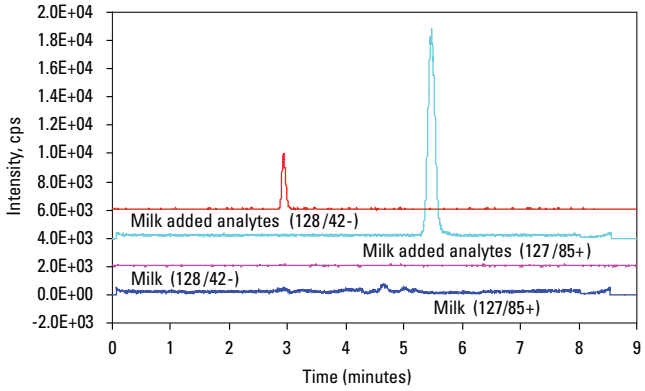
Figure 3. Separation of melamine and cyanuric acid in milk



TSKgel Amide-80, 3µm, 2.0mm ID x 15cm

Eluent:	A: 0.05% formic acid in H_2O
	B: 0.05% formic acid in ACN
	A/B=25/75
Flow rate:	0.2mL/min
Injection vol.:	5µL
Temperature:	40°C
Instrument:	QTRAP (MDS SCIEX)
Ion source:	ESI
	127/85+ (melamine)
	128/42- (cyanuric acid)

Figure 4. Multiple Reaction Monitoring (MRM) chromatograms of milk and spiked milk samples - 10ppb each



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	127/85+ (melamine)
	128/42- (cyanuric acid)

Conclusions

It has been demonstrated that a TSKgel Amide-80 column can simultaneously separate melamine and cyanuric acid in milk with high recovery and excellent resolution. This is a noteworthy application, since adding melamine, to mask the loss of nitrogen when diluting milk products, has resulted in serious health effects and even deaths.

TOSOH

TOSOH BIOSCIENCE

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