DIONEX

Application Note 224

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Determination of Melamine in Milk Powder by Reversed-Phase HPLC with UV Detection

INTRODUCTION

Recent investigations of death and health problems of babies in China have revealed that some baby foods (milk powder) have been contaminated by melamine (structure shown in Figure 1). Some manufacturers illegally used melamine as an adulterant to increase the apparent protein content. Standard methods enacted by the Chinese government for determining melamine in raw milk and dairy products included HPLC-UV, LC-MS, and GC-MS methods.[1] However, the high cost of operation and maintenance of GC/LC-MS systems as well as the labor intensive derivatization that GC-MS requires limits their use in the milk product factories. The HPLC-UV method therefore is presently the popular choice for most factories. In this method, melamine is separated on a C_8 or C_{18} column using an ion pair buffer (mixture of citric acid and sodium 1-octane sulfonate) and acetonitrile mobile phase.

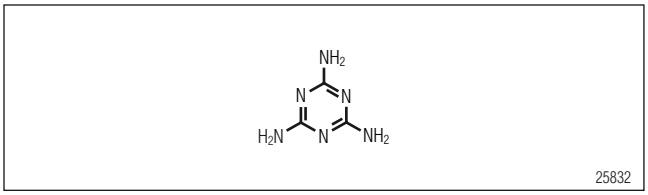

Figure 1. Structure of melamine.

In this Application Note (AN), we determined melamine in milk powder samples following the regulated method.[1] Melamine was separated from other components in the powdered milk samples using an Acclaim® 120 C18 column and an UltiMate® 3000 HPLC system with UV detection. The results of method detection limits (MDL), recovery, and permitted detection deviation match the requirements in the regulated method.

EQUIPMENT

Dionex UltiMate 3000 HPLC system consisting of:
- HPG 3400A pump
- WPS 3000TSL autosampler
- TCC-3000 thermostatted column compartment
- VWD-3400 UV-vis detector

Chromeleon® 6.80 SP5 Chromatography Data System

Kudos® SK3200LH ultrasonic generator, Kudos Ultrasonic Instrumental Co., Shanghai, China

Mettler Toledo AL-204 Electoral o balance, Mettler (Shanghai) Co., Shanghai, China

Anke® TGL-16B centrifuge, Anting Scientific Instrumental Factory, Shanghai, China

IKA® MS1 Minishaker, IKA Works, Guangzhou, China

Strata™-x-c SCX SPE (Phenomenex) column

SE-506 Nitrogen Purge Instrument, Shine Tech., Beijing, China

REAGENTS AND STANDARDS

Water, from Milli-Q® Gradient A 10

Methanol (CH_3OH), HPLC grade, Fisher

Acetonitrile (CH_3CN), HPLC grade, Fisher

Trichloroacetic acid, analytical grade, SCRC, China

Citric acid ($C_6H_8O_7 \cdot H_2O$), analytical grade, SCRC, China

Sodium 1-octane sulfonate (98%), Baker Analyzed @ HPLC Reagent, USA

Melamine (99.0%), HPLC grade, Fluka

Nitrogen (N_2, 99.999%), Lumin Gas Works, Shanghai, China

Ammonia solution (25%–28%), analytical grade, SCRC, China

CHROMATOGRAPHIC CONDITIONS

Guard Column:	Acclaim 120 C18, 5 µm, 4.3 × 10 mm, P/N 059446, with guard column holder, P/N 59526
Analytical Column:	Acclaim 120 C18, 5 µm, 4.6 × 250 mm, P/N 059149
Mobile Phase:	Buffer (dissolve 2.10 g citric acid and 2.16 g sodium 1-octane sulfonate in 980 mL H_2O, adjust pH value to 3.0 with 1 M NaOH solution, add water to the mark of 1000-mL volumetric flask)— CH_3CN (92 : 8, v/v)
Column Temp.:	40 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	20 µL
UV Detection:	Absorbance at 240 nm

PREPARATION OF STANDARDS

Stock Standard Solution

Accurately weigh ~100 mg of melamine, dissolve in a 100-mL volumetric flask with aqueous methanol (50%, v/v). The concentration of melamine is 1000 µg/mL.

Working Standard Solutions

Prepare seven working standard solutions for calibration by adding defined volumes of the stock standard solution and diluting with mobile phase. The concentrations of melamine are 0.2, 0.5, 2.0, 20, 25, 50 and 100 µg/mL, respectively.

SAMPLE PREPARATION

Sample Extraction

Put an accurately weighed ~2 g of dried sample to a 50-mL centrifuge tube, and then add 15 mL aqueous trichloroacetic acid (1%, v/v) and 5 mL acetonitrile. After 1 min of vortex shaking, put in an ultrasonic bath for 30 min, and then shake for 10 min. After 10 min of centrifugation (setting = rpm ≥ 10,000), move the supernatant to a 25-mL volumetric flask while passing through filter paper, and add the 1% aqueous trichloroacetic acid to the mark.

Cleaning Sample Extract on an SCX SPE Column

Prior to use, the SPE column should be activated by passing 3 mL CH_3OH and 5 mL H_2O in turn.

Mix 5 mL of the sample extract and 5 mL water, move them to the activated SCX SPE column. Wash the SPE column with 3 mL methanol and 3 mL water, respectively, then elute with 6 mL of aminated methanol solution (mixture of 5 mL ammonia solution and 95 mL methanol). Dry the collected eluent with N_2 at 50 °C, dissolve the residue in 1-mL mobile phase, and then vortex the solution for 1 min. Prior to injection, filter the solution through a 0.2-µm filter (Millex®-HV).

Spiked Milk Powder Samples

Add 20 µL of stock standard solution of melamine to the 50-mL centrifuge tubes together with the accurately weighed ~2 g of dried sample. The remainder of the sample preparation procedure is the same as that for the milk powder sample.

RESULTS

Method Reproducibility

Figure 2 shows an overlay of chromatograms of seven melamine standards with different concentrations. The RSD for retention time is 0.143%.

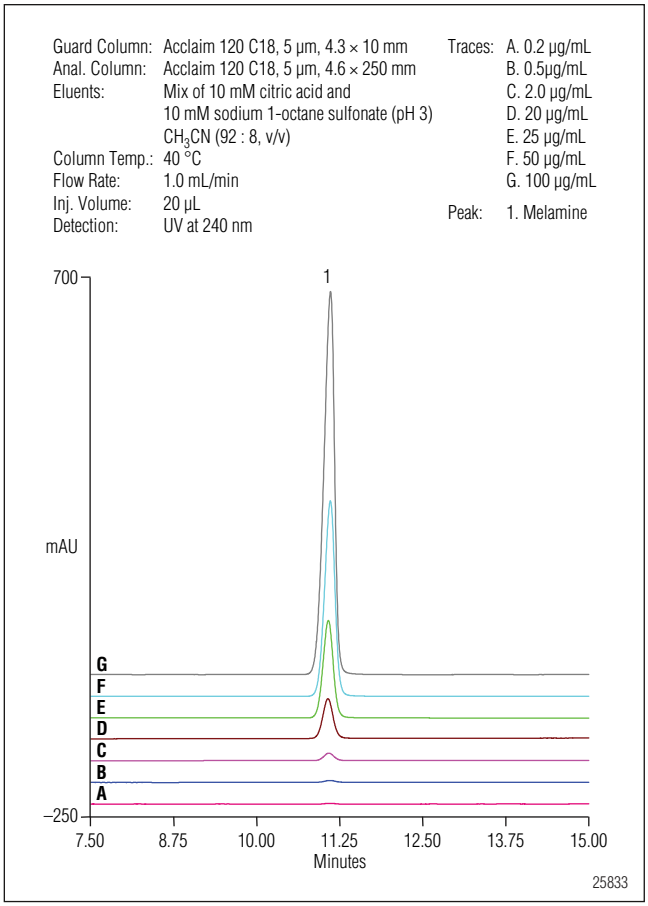

Figure 2. Overlay of chromatograms of seven melamine standards with different concentrations.

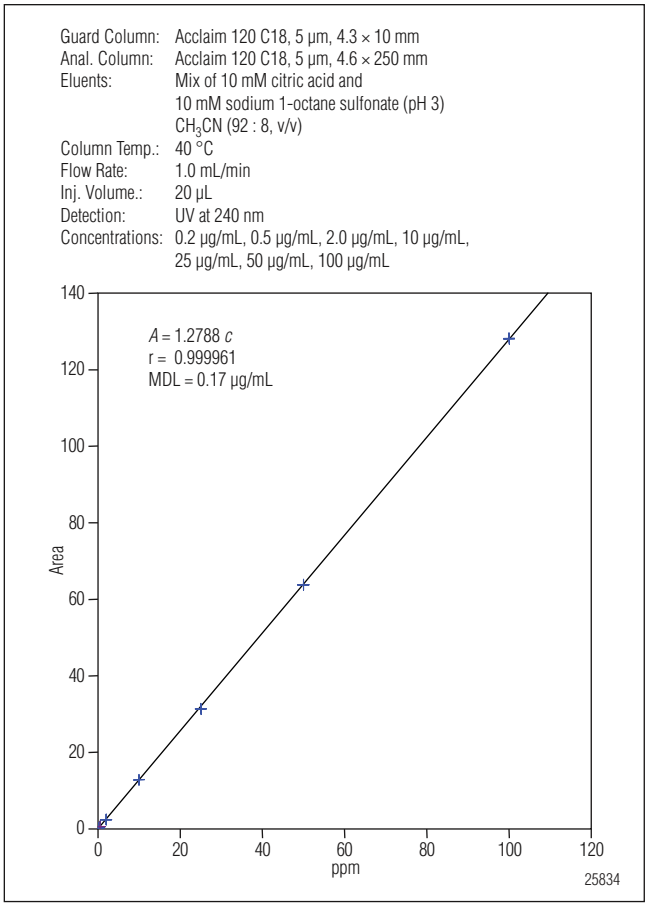

Figure 3. Calibration curve for melamine.

Linearity and Detection Limit

Calibration linearity for melamine was investigated by making three replicate injections of each standard prepared at seven different concentrations. The external standard method was used to establish the calibration curve and to quantify melamine in samples. As shown in Figure 3, excellent linearity was achieved throughout the range from 0.2 to 100 µg/mL. The linearity equation of melamine is as follows with the curve forced the through origin.

$$A = 1.2788\ c$$

Where, A stands peak area, and c stands melamine concentration (µg/mL). The correlation coefficient (r) is 0.999961.

Method detection limit (MDL) of melamine was calculated by using S/N = 3, where S = signal, N = noise. The calculated value of MDL is 0.17 µg/mL.

Sample Analysis

Five milk powder samples (1–5) obtained from a manufacturer were analyzed. Melamine was found in samples #1 through #4. An amount of melamine less than the MDL was detected (0.12 µg/mL) in sample 5 and therefore can not be reliably identified as melamine. The results are summarized in Table 1. Figure 4 is an overlay chromatograms of these samples.

Table 1. Sample Analysis Data								
Sample #	**1**				**2**	**3**	**4**	**5**
Melamine	**Detected (µg/mL)**	**Added (µg/mL)**	**Found (µg/mL)**	**Recovery (%)**	**Detected (µg/mL)**	**Detected (µg/mL)**	**Detected (µg/mL)**	**Detected (µg/mL)**
	0.83	4.0	3.2	80	0.58	0.61	3.6	0.12

Notes: 1. 3 injections were made for each sample
2. Spiked sample was prepared according to the description in the sample section.
3. The detected amount in the list is for the prepared sample (diluted sample).
4. The amount in original sample should be calculated by multiplying "Diluted fold", which is 2.5 in this Application Note.
5. Found = Measured Value of spiked sample - Measured Value of sample.

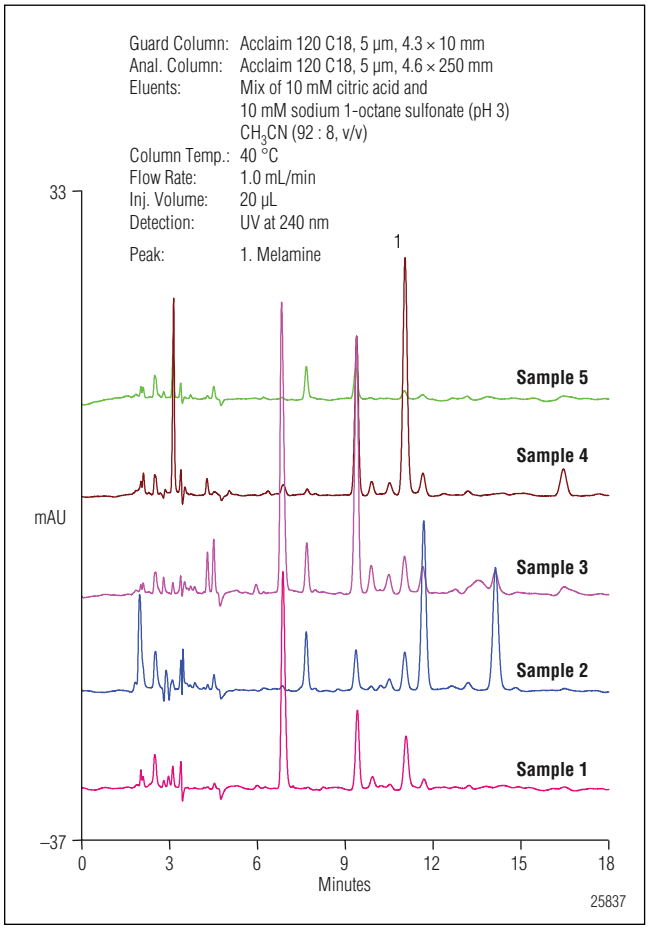

Figure 4. Chromatograms of five milk powder samples.

CONCLUSION

The determination of melamine in milk powder was performed on the Acclaim 120 C18 column and UltiMate 3000 HPLC system with UV detection following the regulated method.[1] Table 2 lists the comparison of chromatographic performance data obtained in this experiment to the requirements in the standard method, which demonstrates that the results match the requirements in the regulated method.

Table 2. Method Performance		
	Method in this Application Note	**Requirement in the Regulated Method**
Correlation coefficient (r)	0.999961	\
Linearity range (µg/mL)	0.2 ~ 100	0.8 ~ 80
Recovery (%)	80	80 ~ 110

Note: Regulated method, see Ref. 1.
\ Not specified.

REFERENCE

1. Determination of Melamine in Raw Milk and Dairy Products, *GB/T* 22388 **2008**.

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