Determination of Melamine Residue in Milk Powder and Egg Using Agilent SampliQ Polymer SCX Solid Phase Extraction and the Agilent 1200 Series HPLC/UV

Application Note

Food Safety

Authors

Chen-Hao Zhai and Yun Zou
Agilent Technologies Co. Ltd.
412 Ying Lun Road
Waigaoqiao Free Trade Zone
Shanghai, 200131
China

Carol Ball
Agilent Technologies, Inc.
200 Regency Forest Drive, Suite 330
Cary, NC 27511
USA

Abstract

This method was developed for the determination of melamine in milk powder and egg. Solid phase extraction (SPE) and HPLC/UV are used consistent with the Chinese regulatory method. The sample preparation is performed using a polymeric mixed mode strong cation exchange resin. The separation and detection are performed by HPLC/UV. The limit of detection is 10 µg/kg. Linear calibration curves were obtained over the calibration range of 1 to 20 mg/kg. Overall recoveries range from 84 to 96 percent, with RSD values below 3.0 percent.

Agilent Technologies

Introduction

Melamine made headlines in September 2008 because it was found to be the contaminant responsible for the deaths of several infants and making many more sick. Melamine is used as an adulterant in milk and milk products because it causes a false positive value in a protein content measurement. The presence of melamine in foods is strictly regulated worldwide. Because milk is used in many other products for human and animal consumption, melamine is now detected in other food products in China and many other countries. The analysis for melamine is proceeding in many labs all over the world.

The Chinese government released a government regulation (GB/T 22388-2008) that established analytical methods for melamine in raw milk and dairy products and set a maximum residue limit (MRL) for melamine in food of 2 mg/kg. This application note describes the implementation and optimization of the solid phase extraction (SPE) method used for milk powder and egg described in GB/T 22388-2008.

Experimental

Reagents and Chemicals

Melamine was obtained from Sigma-Aldrich (Shanghai, China). A 1 mg/kg melamine stock solution was prepared in methanol and kept in the freezer (–4 °C). Appropriate dilutions using mobile phase generated working solutions that were prepared fresh daily.

Sample Extraction for Milk Powder or Egg

For egg, homogenize, place in a clean, sealed container, and store in the freezer at –4 °C.

For homogenized egg and milk powder, weigh 2.0 ± 0.01 g of sample and add to a 50-mL centrifuge tube. Add 15 mL of 1% trichloroacetic acid in water and 5 mL of acetonitrile, then cap. Sonicate for 10 minutes and then place samples on a vertical shaker for 10 minutes. Centrifuge the samples for 10 minutes at 4,000 rpm. Wet a filter paper with 1% trichloroacetic acid in water, then filter the supernatant into a 25.0-mL volumetric flask and bring to volume with 1% trichloroacetic acid in water. Transfer a 5.0 mL aliquot of the extract into a glass tube, and then add 5.0 mL purified water. Mix thoroughly on a vortex mixer.

SPE Purification

Agilent SampliQ SCX SPE cartridges (p/n 5982-3236, 3 mL, 60 mg) were used to clean up sample extracts. All SPE steps, including conditioning, sample load, wash, and the final elution, are performed without vacuum with a flow rate between 0.5 and 1 mL/min. For the drying step, vacuum is applied to quickly dry the cartridges. The procedure used for the SPE extraction is shown in Figure 1. Cartridges were conditioned with 3 mL of methanol, then 5 mL of water. A 10-mL prepared sample solution (equivalent to a 0.4-g sample) was passed through the cartridge. After the sample effused completely, the cartridge was washed with 3 mL of water and 3 mL of methanol. The entire effluent was discarded. The cartridge was dried under negative pressure below 2.0 kPa for 3 minutes. Finally, the cartridge was eluted with 6 mL of 5% ammonium hydroxide in methanol. The eluent was collected and dried under nitrogen at 45 °C. The resulting residue was resuspended and made to a constant volume of 1 mL using the mobile phase. Then the residue was filtered through a 0.45-µm filter membrane (p/n 5185-5836) and analyzed.

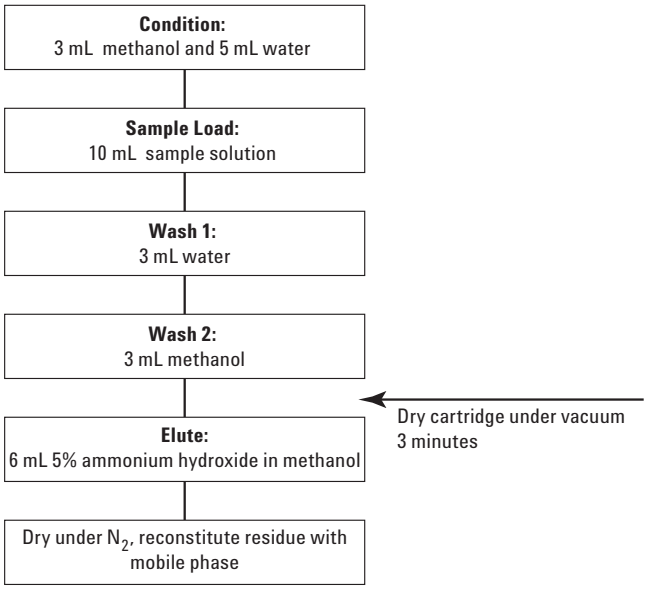

Figure 1. Melamine SPE procedure.

HPLC Conditions

Samples were analyzed on an Agilent 1200 Series HPLC with a diode array detector.

Column:	Agilent ZORBAX SB-C8 5 µm, 250 mm × 4.6 mm id (p/n 880975-906)
Flow rate:	1.0 mL/min
Column temperature:	40 °C
Detector wavelength:	240 nm
Injection volume:	20 µL
Mobile phase:	Acetonitrile-Buffer (15:85)
Buffer:	10 mmol/L citric acid and 10 mmol/L sodium octanesulfonate solution with a pH 3.0
Chromatography:	Isocratic

Results and Discussion

Linearity, Limit of Detection

Working standards were prepared at concentrations of 1, 5, 10, and 20 mg/kg by dilution of the stock solution with mobile phase. Linear regressions were calculated for melamine using the areas and the solution concentrations. The limit of detection (LOD) was the injection concentration whose signal-to-noise ratio was between 2 and 3. The linear range was between 1 and 20 mg/kg. The linearity and LOD results are shown in Table 1. The analysis of matrix blanks spiked with melamine shows no difference in area compared to solution based standards.

Table 1. Linearity and LOD of Melamine

Compound	Regression equation	Correlation coefficient	LOD (µg/kg)
Melamine	Y = 77.4698x + 0.2117	0.9999	10

Recovery and Reproducibility

The precision of the method is expressed as recovery of spiked melamine standard in milk or egg at concentrations of 2, 5, and 10 mg/kg levels. The analysis was performed in replicates of six at each level. Concentrations in the samples were calculated based on the external standard calibration curves. The chromatograms of the blank and spiked standard (2 mg/kg) are shown in Figures 2 to 5. The recovery and reproducibility data are shown in Table 2.

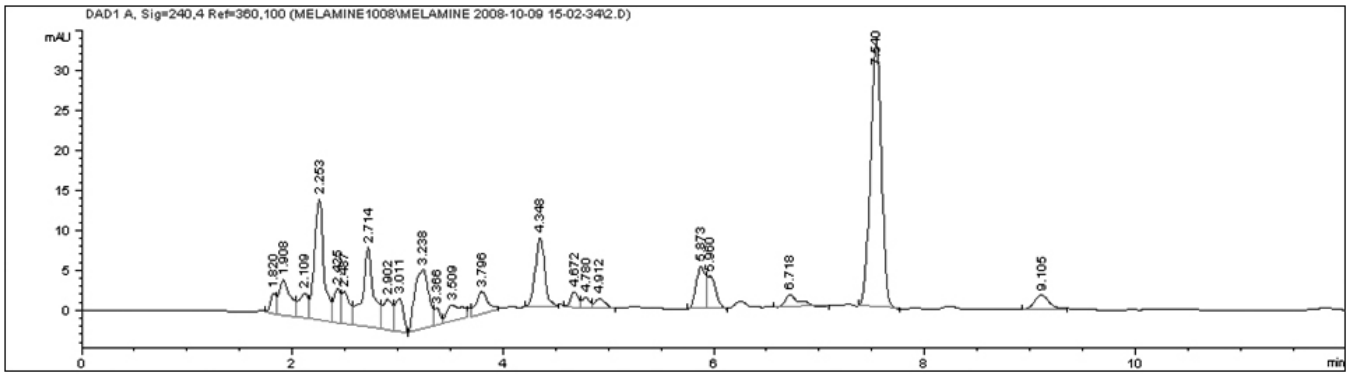

Figure 2. Chromatogram of a milk powder blank.

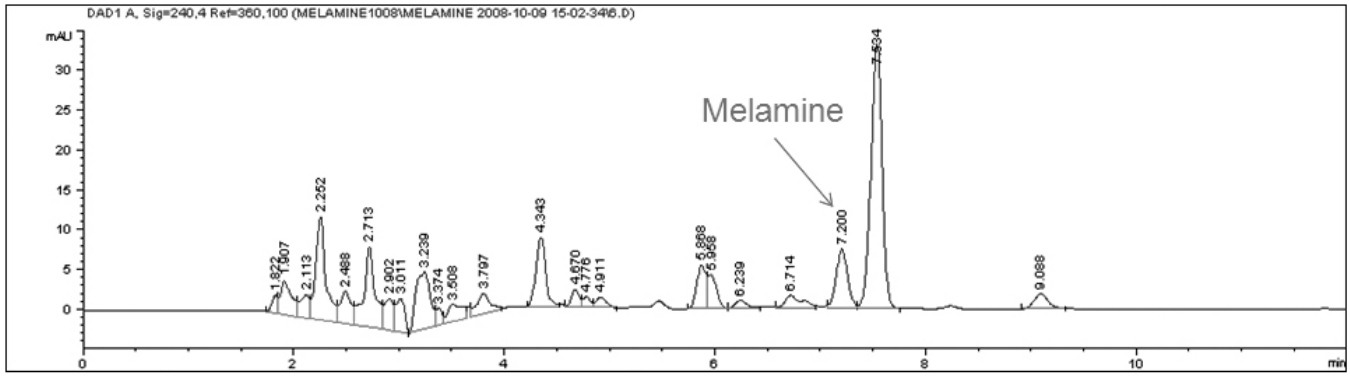

Figure 3. Chromatogram of a milk powder sample spiked at 2 mg/kg.

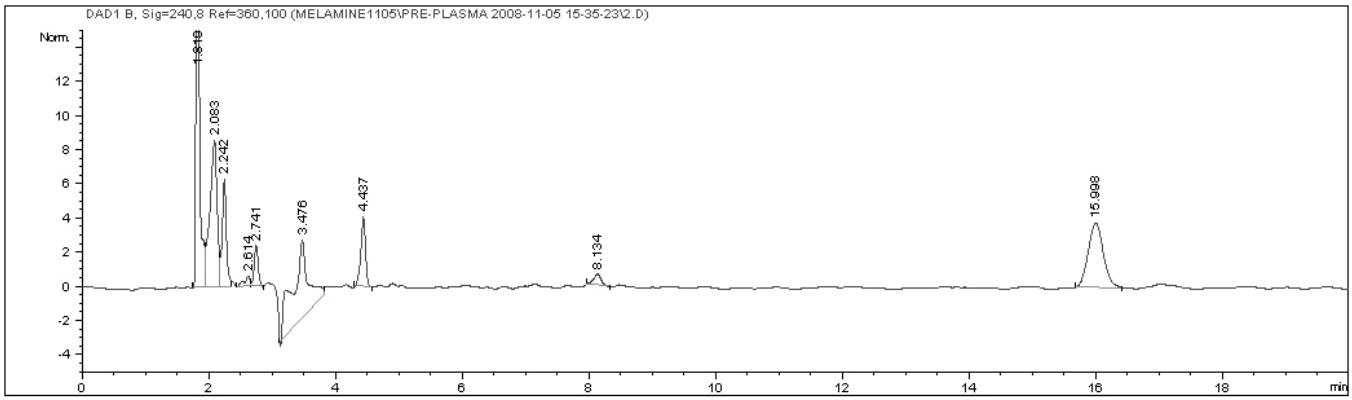

Figure 4. Chromatogram of an egg blank.

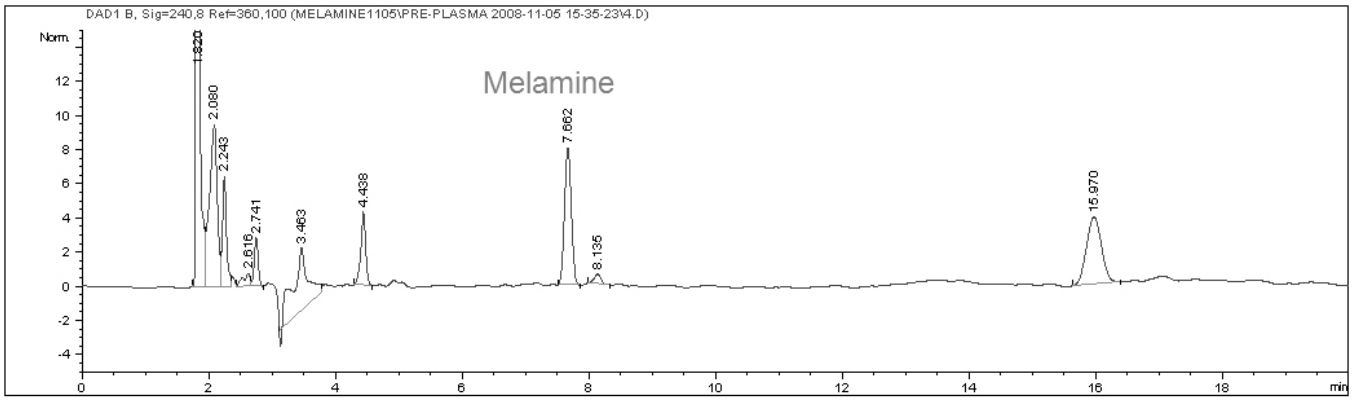

Figure 5. Chromatogram of an egg sample spiked at 2 mg/kg.

Table 2. Recoveries and Relative Standard Deviations of Melamine in Milk Powder and Egg Using Agilent SampliQ SCX SPE

Compound	Sample	Spiked level (mg/kg)	Recovery (%)	RSD (%)
Melamine	Milk powder	2	84.5	2.83
		5	85.3	2.56
		10	86.7	1.18
	Egg	2	95.2	3.00
		5	93.0	2.01
		10	95.7	2.89

Conclusions

Agilent SampliQ SCX solid phase extraction provides an effective single cartridge method for the purification and enrichment of melamine in milk powder and egg. The method is simple and complements any analytical procedure, such as LC/MS/MS or GC/MS. The recovery and reproducibility results, based on solution standards, are acceptable for melamine residue determination in milk powder and egg under the Chinese regulation. The impurities from milk and egg were minimal and did not interfere with the detection of melamine. The LOD (0.01 mg/kg) of melamine was significantly lower than the MRL (2 mg/kg).

Reference

GB/T 22388-2008, Determination of Melamine in Raw Milk and Dairy Products

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Published in the USA
December 16, 2008
5990-3365EN

Agilent Technologies