Extraction of Melamine from Milk (Infant Formula) using EVOLUTE® CX Columns

Application Note

Introduction

This application note describes the extraction of melamine from biological fluids using EVOLUTE CX.

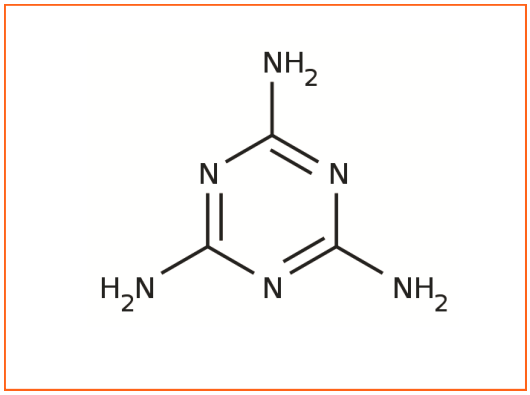

Figure 1. Structure of Melamine

Melamine is traditionally used in making plastics, however, it's low cost and high nitrogen content has led to exploitation in various sections of the food industry, most notably involving dairy products. The standard test for estimating protein content is based on measurement of nitrogen levels, therefore, addition of melamine to sub standard or watered down milk results in the protein levels appearing higher. Sustained melamine exposure can result in kidney stones and renal failure, with the young being most susceptible. EVOLUTE CX 50µm is a resin-based mixed-mode cation exchange SPE sorbent. The chemistry is identical to standard EVOLUTE CX; however, the mean particle size has been increased to accommodate extraction of more viscous samples and/or increased sample volumes.

EVOLUTE CX mixed-mode resin-based SPE sorbent extracts a wide range of basic analytes from biological fluids and other aqueous matrices using a generic procedure which minimizes method development time. EVOLUTE CX removes matrix components such as proteins, salts, non-ionizable interferences and phospholipids, delivering cleaner extracts with reproducible recoveries for accurate quantitation.

Analytes

Melamine.

EVOLUTE CX Configuration

EVOLUTE CX 50 mg/3 mL part number 611-0005-B.

EVOLUTE CX Procedure

Sample Pre-treatment:	For powdered formula: make up as per manufacturer's instructions, and cool prior to extraction. Dilute all milk samples (1 mL) with ammonium acetate buffer (1:1, v/v, 50 mM, pH 5).
Column Conditioning:	Condition column with methanol (3 mL).
Column Equilibration:	Equlibrate column with ammonium acetate buffer (50 mM, pH 5, 3 mL).
Sample Load:	Load pre-treated sample (2 mL) at a flow rate of 3 mL / min.
Interference Elution 1:	Remove polar and ionic interferences with ammonium acetate buffer (50 mM, pH 5, 3 mL).
Interference Elution 2:	Remove non-polar interferences with methanol (3 mL).
Analyte Elution:	Elute melamine with methanol containing ammonium hydroxide (95:5, v/v, 3 mL).
Post extraction:	Evaporate to dryness and reconstitute in acetonitrile/water (90:10, v/v, 500 µL) prior to analysis.

HPLC Conditions

Instrument:	Waters 2795 Liquid Handling System.
Column:	Phenomenex Luna HILIC 3 µm analytical column (100 x 2.0 mm id).
Guard Column:	Phenomenex Luna Phenyl-Hexyl security guard column.
Mobile Phase:	Isocratic, acetonitrile:20mM Ammonium Formate pH 3.2 (75:25, v/v), flow rate of 0.3 mL/min.
Injection Volume:	20 µL.
Temperature:	Ambient.

Mass Spectrometry Conditions

Instrument:	Waters Ultima Pt triple quadrupole mass spectrometer equipped with an electrospray interface.
Desolvation Temperature:	350 °C.
Ion Source Temperature:	100 °C.
Collision Gas Pressure:	2.4 x 10^{-3} mbar.
	Positive ions were acquired in the multiple reaction monitoring mode (MRM).
Quantfier ion transition:	127 > 85 (collision energy 12 eV).
Qualifier ion transition:	127 > 68 (collision energy 14 eV).

Results

From the results it can be seen that recoveries from both plasma and urine were above 90% with % relative standard deviations below 10.

Matrix	Powdered baby milk	Liquid baby milk 1	Liquid baby milk 2	Liquid baby milk 3
Melamine %recovery	84	97	89	98
% RSD (n=6)	<10	<10	<10	<10

References

This application note is based on the poster 'Extraction of melamine from various matrices using resin based mixed-mode cation exchange SPE and analysis with LC-MS/MS', L Williams et al, presented at ASMS, Philadelphia, May 31st-June 4th, 2009.

Ordering information

Part number	Description	Quantity
611-0005-B	EVOLUTE CX 50 mg/3 mL	50
C103198	TurboVap LV 120V	1
C103199	TurboVap LV 230V	1

Additional assistance

The TurboVap LV Concentration Evaporator Workstation provides simultaneous evaporation of up to 50 samples. This evaporation system offers many interchangeable tube racks giving you the flexibility for automated low volume sample preparation ranging in volumes size from 1.5 mL to 30 mL. The microprocessor-control provides monitoring of the timed operation and water bath temperature. It also provides automatic gas shutoff and operational diagnostics.

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