

# Extraction of Melamine from Milk (Infant Formula) Using EVOLUTE® EXPRESS CX Solid Phase Extraction Columns with Analysis by LC-MS/MS

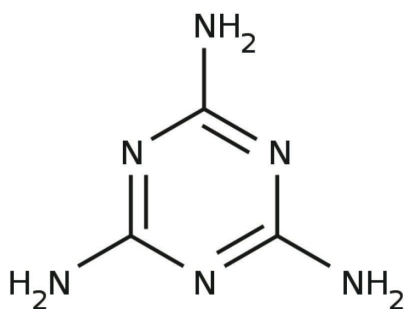


Figure 1. Structure of Melamine.

## Introduction

This application note describes the extraction of melamine from infant formula using EVOLUTE® EXPRESS CX polymeric mixed-mode strong cation exchange SPE columns.

Melamine is traditionally used in making plastics, however, its 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® EXPRESS CX SPE columns and plates extract a wide range of basic analytes from biological fluids and other aqueous matrices. EVOLUTE EXPRESS CX removes matrix components such as proteins, salts, non-ionizable interferences and phospholipids, delivering cleaner extracts with reproducible recoveries for accurate quantitation.

## Analytes

Melamine

## Sample Preparation Procedure

### Format

EVOLUTE® EXPRESS CX 60 mg/ 3 mL (Tablets) SPE columns, part number 611-0006-BXG.

[Note: This application was originally developed using EVOLUTE® CX 50 mg/3 mL SPE columns, p/n 611-0005-B. This format has now been replaced by the EXPRESS format, which provides improved flow characteristics].

### 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

Equilibrate 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 at 40 °C 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<sup>-3</sup> mbar

Positive ions were acquired in the multiple reaction monitoring mode (MRM).

### Quantifier 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 a range of infant formula products were above 85% with <10% 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 31<sup>st</sup> June 4<sup>th</sup>, 2009.

## Ordering Information

Part Number	Description	Quantity
611-0006-BXG	EVOLUTE® EXPRESS CX 60 mg/3 mL (Tablets) SPE Columns	50

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