

Extraction of Melamine from Milk (Infant Formula) using EVOLUTE[®] CX Columns and RapidTrace⁺ Workstation

Introduction

This application note describes for the extraction of Melamine from infant formula milk using EVOLUTE CX and RapidTrace⁺ Workstation.

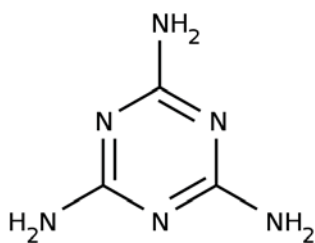


Figure 1. Structure of Melamine

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. Sustained melamine exposure can result in kidney stones and renal failure, with the young being most susceptible.

This application note has been optimized on the RapidTrace⁺; an updated version of the current RapidTrace software which allows the system to process smaller bed heights than were previously possible. The application note shows recoveries of 88-90% with RSDs <10%.

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

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).

RapidTrace procedure

EVOLUTE CX 100 mg/3 mL 50 µm part number: 611-0010-B

	Step	Source	Destination	Volume (mL)	Flow (mL/min)
1	Condition	Methanol	Organic Waste	3.0	2.00
2	Condition	Ammonium acetate	Organic Waste	3.0	2.00
3	Load	Sample	Biological Waste	2.0	1.00
4	Purge-Cannula	Ammonium acetate	Organic Waste	2.0	30.00
5	Rinse	Ammonium acetate	Organic Waste	3.0	2.00
6	Rinse	Methanol	Organic Waste	3.0	2.00
7	Collect	Methanol:NH ₄ OH (95:5)	Fraction 1	3.0	1.00
8	Purge-Cannula	Ammonium acetate	Organic Waste	6.0	30.00
9	Purge-Cannula	Methanol	Cannula Waste	6.0	30.00
10	Purge-Cannula	Water	Cannula Waste	2.0	30.00

Evaporate to dryness and reconstitute in acetonitrile/water (90:10, v/v, 500 µL) prior to analysis. Biotage recommends TurboVap Workstations post elution to evaporate your samples.

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 Spectrographic 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).
Quantifier ion transition: 127 > 85 (collision energy 12 eV).
Qualifier ion transition: 127 > 68 (collision energy 14 eV).

Results

As shown in figure 2 average analyte recoveries were all above 88% (n=3) based upon six injections per run with RSDs <10% for all recoveries. Figure 3 shows an example mass chromatogram of extracted melamine.

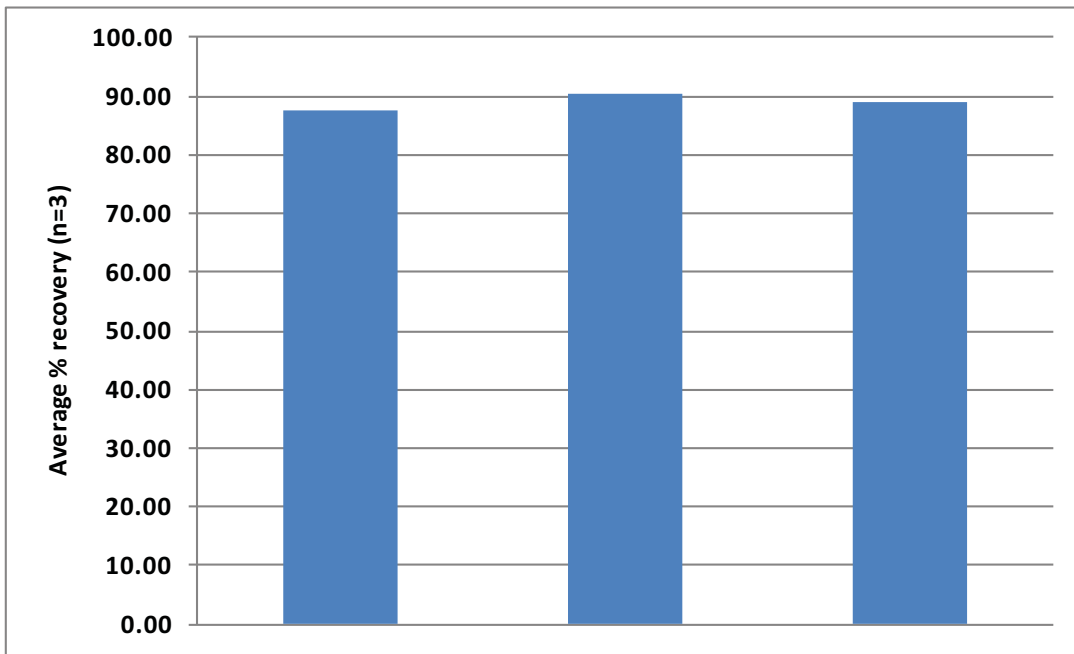


Figure 2. Average analyte recoveries of Melamine from milk (n=3)

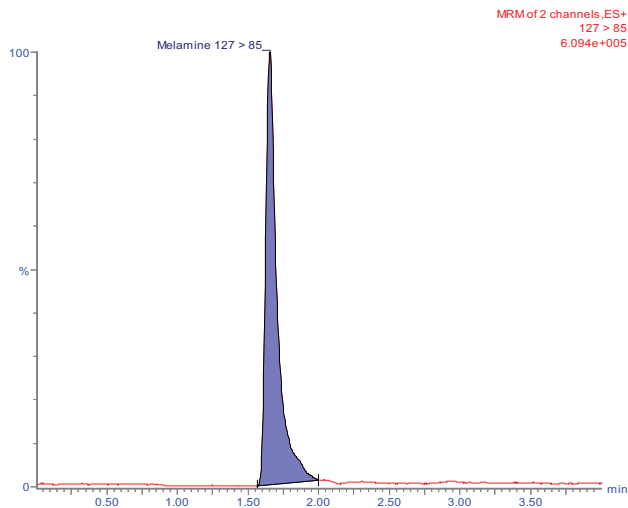


Figure 3. Example mass chromatogram for extracted melamine from milk at 400 pg on column

Reagent lines

Line 1: Water (HPLC grade)

Line 2: Methanol (HPLC grade)

Line 3: Ammonium acetate

Line 4: Methanol:NH₄OH (v/v, 95:5)

Ordering information

Part number	Description	Quantity
611-0010-B	EVOLUTE CX 100 mg/3 mL 50 µm	50
C50000	RapidTrace ⁺ 3 mL Workstation	1
C133966	RapidTrace ⁺ Software Upgrade 2.1	1
C50974	Tube Rack (13 x 100 mm Sample Tubes & 12 x 75 Fraction Tubes)	1
C40707	Test Tubes-13 x 100 mm, Uncap	1000
C44651	Test Tubes-12 x 75 mm, Uncap	1000
C52006	RapidTrace Start-Up Kit	1
C52689	RapidTrace Notebook Controller	1

RapidTrace⁺ Overview



The RapidTrace⁺ has been designed to eliminate sample preparation bottlenecks and it is a modular, highly scalable, automated platform designed for high throughput. Units are available to accommodate 1 mL, 3 mL and 6 mL SPE cartridges. Up to 10 modular units can be connected together and controlled through a simple, easy-to-use software package. The systems are widely used within analytical industry and are ideal both for industrial settings and for efficient SPE

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