Extraction of Melamine from Various Matrices using Resin-based Mixed-mode Cation Exchange SPE and Analysis with LC-MS/MS

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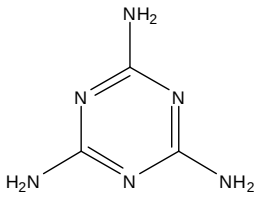
Introduction

Melamine (structure shown) 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 CX 50µm is a new 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. This poster will demonstrate the use of EVOLUTE CX 50µm for the extraction of melamine from various dairy products and human biological fluids, comparing generic mixed-mode cation exchange SPE protocols for optimum results.



Melamine Mwt 126.12

Experimental Procedure

Reagents

Formic acid, acetic acid, ammonium formate, ammonium acetate, and melamine were purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). Human urine was obtained from a healthy human volunteer and all milk products purchased from a local supermarket. All solvents were HPLC grade from Fisher Scientific (Loughborough, UK).

EVOLUTE CX 50µm Sample Preparation Procedure

Extractions were performed in the 50 mg/3 mL format using 1 mL of matrix. The matrices investigated were human plasma, human urine, pasteurized milk (semi skimmed, cow), powdered baby milk and various pre-mixed liquid baby milk formulations. Melamine was spiked at 100 ng/mL and extracted using three SPE protocols utilizing various buffers for pH control. The generic extraction protocol for EVOLUTE CX 50µm is based on a 50mM ammonium acetate buffer at pH 5. The other protocols used were: 50mM ammonium acetate buffer at pH 6; and a 2% formic acid protocol. Powdered baby milk was reconstituted with boiling water as per the suggested procedure and left to cool before pre-treatment.

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Sample Pre-treatment: Matrix (1 mL) diluted 1:1 (v/v) with:
a) 50mM ammonium acetate buffer at pH 5;
b) 50mM ammonium acetate buffer at pH 6;
c) 2% formic acid aq

SPE Column: EVOLUTE CX 50µm (P/N 611-0005-B)
Column Conditioning: Methanol (2mL)
Column Equilibration: 50mM ammonium acetate buffer at pH 5, pH 6 or 2% formic acid aq (2 mL)
Sample Loading: Pre-treated plasma sample (2 mL)
Interference Elution 1: 50mM ammonium acetate buffer at pH 5, pH 6 or 2% formic acid aq (2 mL)
Interference Elution 2: Methanol (2 mL)
Analyte Elution: 5% (v/v) NH_4OH in methanol (2 mL)

Post Extraction: The eluate was evaporated to dryness and reconstituted in 500 µL of 90:10 (v/v) MeCN/H_2O prior to analysis.

HPLC Conditons

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

Mass Spectrometry

Instrument: Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Position ions were acquired in the multiple reaction monitoring mode (MRM). The quant ion MRM transition, 127 > 85 (collision energy 12 Ev), and qualifier ion transition, 127 > 68 (collision energy 14 Ev) were monitored in all experiments.

Desolvation Temperature: 350 °C
Ion Source Temperature: 100 °C
Collision Gas Pressure: 2.4 x 10^{-3} mbar

Results

Melamine is a weak polar base with a reported pK_a of between 5-8.95 (depending on source). The polarity (LogP -1.37) makes it difficult to extract using traditional silica or resin-based non-polar SPE. Extraction with resin-based strong cation exchange is therefore the primary strategy, however, good pH control is required to avoid substantial breakthrough. Three SPE protocols utilizing varying pH control have been evaluated. **Table 1**. shows the various matrix pH's attained for these three protocols.

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Table 1. Matrix pH control using various SPE protocols

Buffer 1:1 v/v	Plasma pH	Urine pH	Pasteurized Milk pH	Powdered Baby Milk pH	Liquid Baby Milk pH
50mM NH_4OAc pH 5	6.59	5.66	6.00	5.66	5.61
50mM NH_4OAc pH 6	7.31	6.87	6.57	6.91	6.68
2% Formic acid aq	3.24	2.68	2.97	2.80	2.73

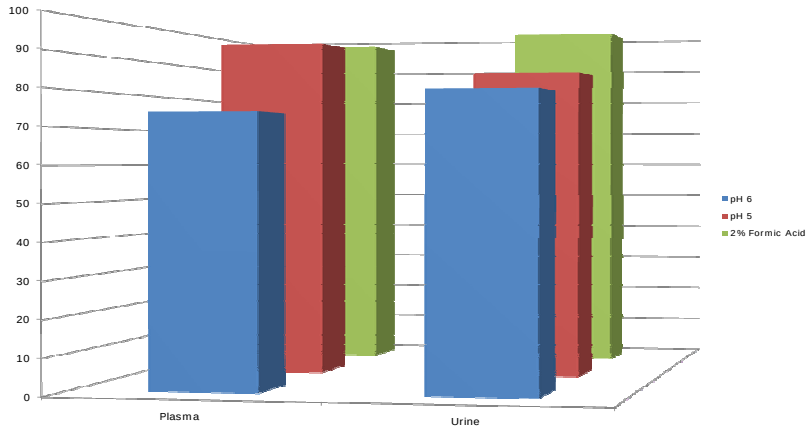

Figure 1. *Recovery comparison of human biological samples with different SPE protocols (n=6).*

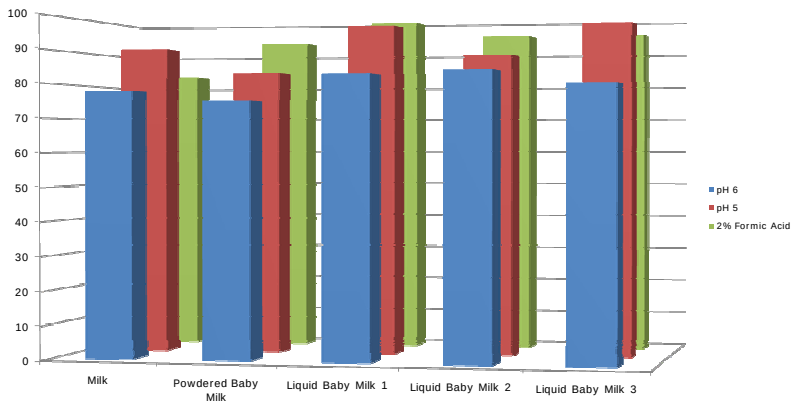

Figure 2. *Recovery comparison of milk samples with different SPE protocols (n=6).*

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Figure 1. shows the recoveries obtained for the SPE protocols when extraction human urine and plasma. **Figure 2**. shows the corresponding recoveries from various milk extractions. **Figure 3**. shows the quant/qualifier ion integrity obtained for the various matrices. **Figure 4**. shows the quant/qualifier ion TIC comparing signal intensity between SPE protocols for the extraction of pasteurized milk.

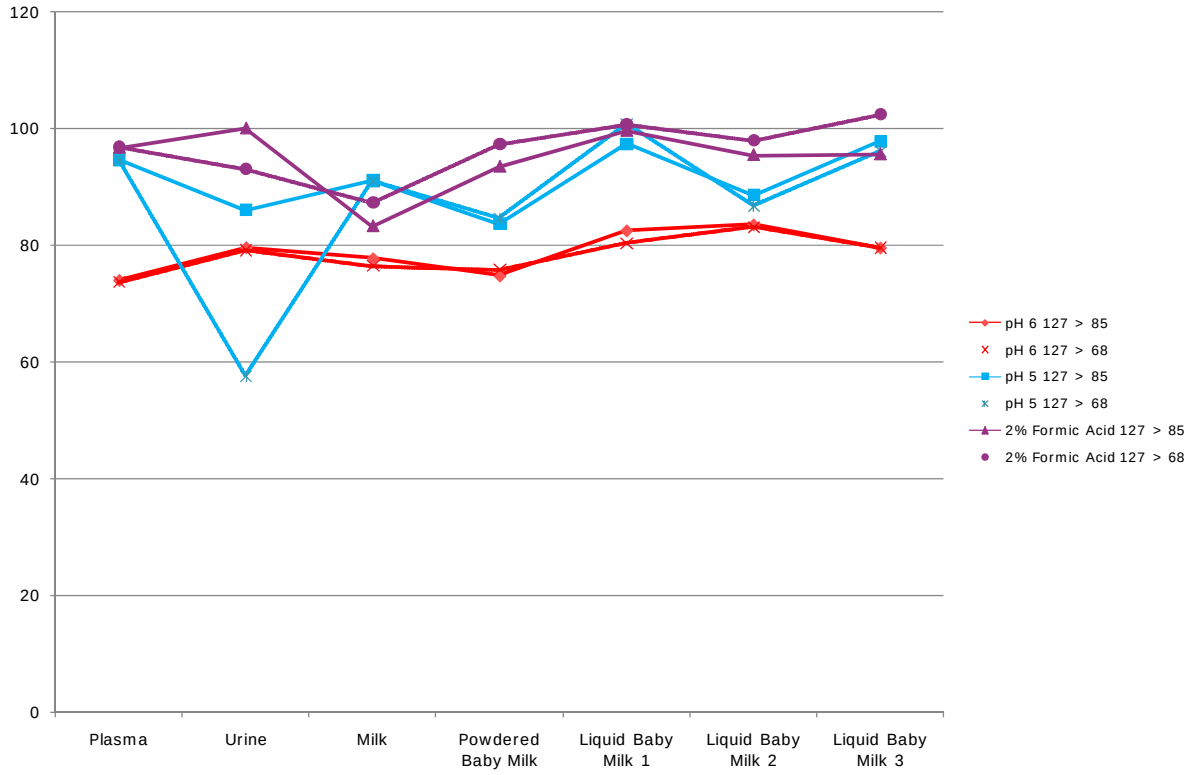

Figure 3. *Quant/Qualifier ion data integrity for all matrices.*

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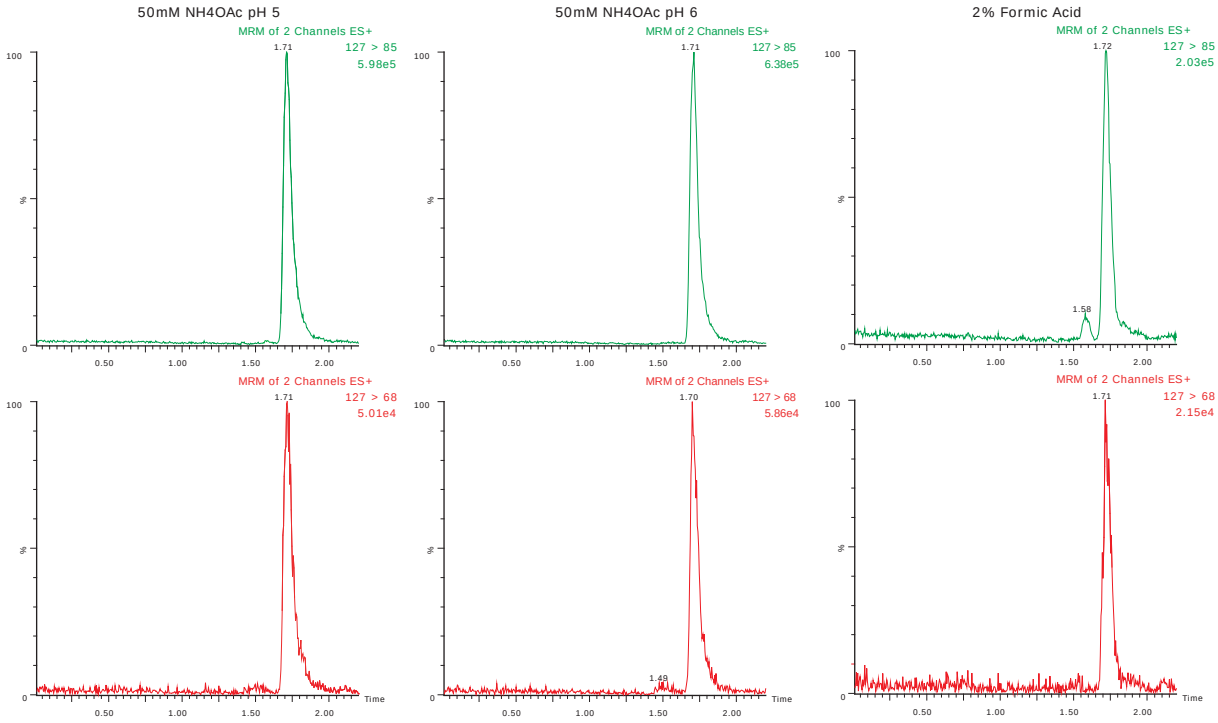

***Figure 4**. Quant/Qualifier ion TIC comparison for spiked pasteurized milk.*

Conclusions

- Overall the 50mM ammonium acetate pH5 method gives optimum combination of recoveries and suppression.
- Extractions with 50mM ammonium acetate buffer at pH 6 showed lower recoveries for all matrices. This may be due to insufficient pH control coupled with analyte polarity, ultimately leading to breakthrough.
- RSD's were below 10% for all SPE protocols.
- Good consistentcy of quant/qualifier ion results were obtained for all matrices apart from urine, where signal suppression was an issue.
- Far greater signal suppression was observed when using the 2% formic acid SPE protocol for all matrices. Estimated LoQ's are 2-3 times higher using this method.

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