SIMULTANEOUS EXTRACTION AND ANALYSIS OF MELAMINE AND CYANURIC ACID FROM BABY FORMULA WITH USING SOLID PHASE EXTRACTION (SPE)

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Abstract
In this research we present a new strategy for analysis of melamine and cyanuric acid that utilizes solid phase extraction (SPE) to selectivity retain both melamine and cyanuric acid (CYA) using a single SPE sorbent. The SPE procedure allows melamine and cyanuric acid to either be eluted together for simultaneous detection or eluted in separate fractions allowing for independent analysis of each compound. The method presented had a limit of detection for melamine of 2 µg/kg with a limit of quantitation below 20 µg/kg. Detection limits for cyanuric acid (CYA) were slightly worse than melamine due to acidic nature of the compound and the lower response by the LC/MS/MS in negative ion mode, but detection limits were still far below required levels (LOD 4 µg/kg, LOQ 20 µg/kg). The extracts were suitable for either LC/MS/MS or GC/MS depending on the capabilities of the lab.

Introduction
As the world faces its second melamine scare in two years, there has been global concern over international food product imports. At concentrations >2mg/kg, melamine and cyanuric acid form a crystal that is highly insoluble in most solvents and causes kidney failure in animals. In this research we present a new strategy for analysis of melamine and cyanuric acid that utilizes solid phase extraction (SPE) to selectivity retain both analytes using a single SPE sorbent. The SPE procedure allows melamine and cyanuric acid to either be eluted together for simultaneous detection or eluted in separate fractions allowing for independent analysis of each compound. The use of LC/MS/MS provides undisputable confirmation at levels far below the WHO cut off.

Materials and Method
Baby formula samples are diluted with water and spiked with melamine, cyanuric acid, and internal standards. Protein precipitation is performed and the supernatant loaded on an SPE sorbent containing a strong cation and weak anion exchange functionality. The samples are washed with ACN/water to remove matrix interferences. If melamine and cyanuric acid were to be eluted in one fraction, samples were eluted with 5%NH4OH in Methanol. When selective elution was done, a second wash step was used using MeOH/water. Analysis was performed in HILIC mode using an API 3000 LC/MS/MS operated in positive mode for melamine and negative mode for cyanuric acid.

Results & Discussion
The World Health Organization has set a cut off for melamine in food products at 1 mg/kg. The method presented had a limit of detection for melamine of 5 µg/kg with a limit of quantitation below 50 µg/kg. Detection limits for cyanuric acid were slightly worse than melamine due to lower response in LC/MS/MS for the negative ion, but detection limits were still far below required levels.

The use of a new blended SPE sorbent allowed melamine and cyanuric acid to be retained simultaneously on the same sorbent. By changing elution conditions, we were able to choose whether to elute both compounds together in the same fraction for simultaneous analysis or to selectively elute the compounds for analysis independent analysis of each compound.

Absolute recovery of melamine was lower than expected at 67%. No loss in any steps of SPE steps, suggesting melamine most likely had some protein binding interactions that were not disrupted using the protein precipitation method. Cyanuric acid was lost in the load step at concentrations below 50 µg/kg by

about 15-20% because it was not strongly retained via ion-exchange mechanisms. Absolute recovery in the selective elution was slightly better for cyanuric acid because an additional acidified MeOH elution could be applied after NH4OH/MeOH to further dislodge any trace amounts. Selective elution was better for melamine was similar, but selective elution allowed for removal of matrix contamination using a 100% MeOH wash before elution.

Relative recoveries for QC samples spiked at 200 ug/kg using the non-selective elution method were 115% (3.05% CV) and 64.5% (4.10 %CV) for melamine and cyanuric acid respectively. Using the selective elution method, the recoveries were 106% (5.39 %CV) and 102% (8.7 %CV).

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Table 1: Recovery Data

	LC/MS/MS						GC/MS	
	1. Simultaneous Elution		2. Separate Elution		3. Independent Elution		1. Simultaneous Elution	
	Absolute Recovery	Relative Recovery	Absolute Recovery	Relative Recovery	Absolute Recovery	Relative Recovery	Absolute Recovery	Relative Recovery
Melamine	67% (CV=3.10)	115% (CV=3.05)	72% (CV=2.12)	106% (CV =5.39)	87% (CV=1.56)	109 (CV=3.3%)	48.7%* (CV=2.45)	100% (CV=3.5)
Cyanuric Acid	68% (CV=2.02)	94.5% (CV=4.10)	96% (CV=5.20)	102% (CV=8.70)	96% (CV=5.20%)	104 (CV=1.8%)	73.8% (CV=6.7)	98.1% (CV=5.8)

% CV based of N = 4 extracted samples at 200 µg/kg

*Low absolute recoveries due to problems with derivatization procedure

Figure 2: LC/MS Analysis

Column: Luna 3 µm HILIC 200Å
Dimensions: 100 x 2.0 mm
Part No: 00D-4449-B0
Mobile Phase: Isocratic
Eluent A: Isocratic
90/10 ACN/100 mM Ammonium Formate, pH 3.2 Pct. A: 100
Flow Rate: 0.4 mL/ min
Detection: API 3000 LC/MS/MS with ESI (TurboIonSpray®) Switch from Negative Ion Mode (Cyanuric acid) to Positive Ion (for Melamine)at 1.7 minutes until 3.5 minutes; MRM; heater gas flow 7000 cc/min; heater temperature 450 ˚C

App ID: 18287

Sample:
1. Cyanuric acid (negative ionization mode)*
2. Melamine (positive ionization mode)*

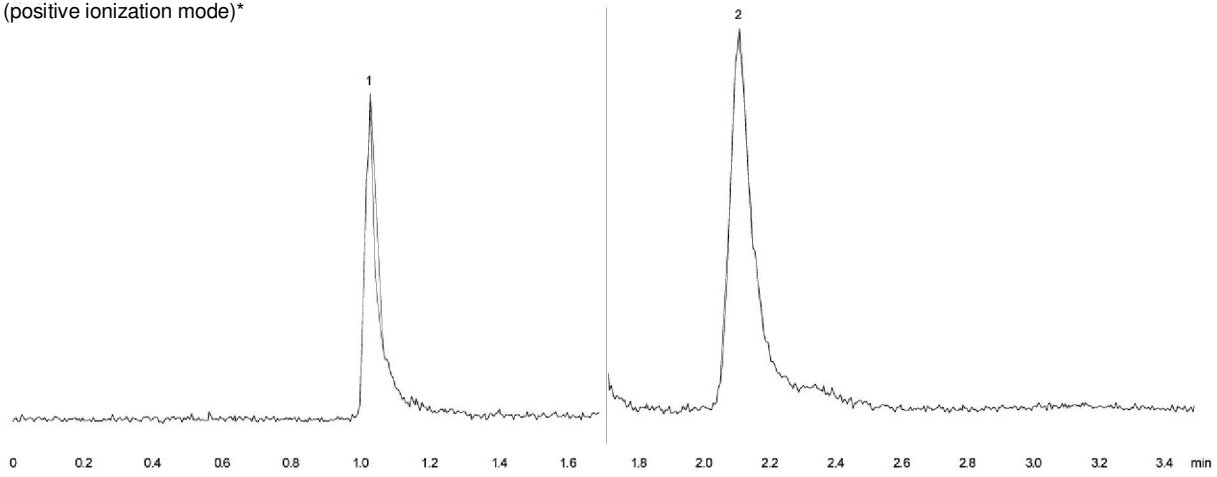

Figure 5: GC/MS Analysis

Column: Zebron™ ZB-XLB-HT Inferno™
Dimensions: 15 meter x 0.25 mm x 0.25 µm
Part No.: 7EG-G024-02
Injection: On-Column 0.1 µL @ 103 °C
Carrier Gas: Helium @ 1.4 mL/ min (constant flow)
Oven Program: 100 °C for 0.5 min to 320 °C @ 25 °C/ min
Detector: MSD @ 325 °C
Sample: Note: Analytes are 200 ng/ 100 µL in BSTFA/ Pyridine (1:1)
1. Cyanuric Acid $^{13}C_3$ (IS)
2. Cyanuric Acid
3. Melamine $^{13}C_3$ $^{15}N_3$ (IS)
4. Melamine

App ID: 18265