MELAMINE IN FOOD MATERIALS USING EUBCX22Z

LC-MS/MS

September 30, 2009

1. **PREPARE SAMPLE:**
   To 1-5 g of sample add 10-25 mL of CH_3CN/ DI H_2O (50:50).
   Shake for 5 minutes.
   Centrifuge
   Transfer 5 mL of supernatant to clean glass screw top tube.
   Add 1 mL of 100 mM HCl.
   Add 1 mL of CH_2Cl_2
   Shake for 5 minutes.
   Centrifuge
   Transfer upper layer to clean glass tube
   Add 2 mL of DI H_2O to CH_2Cl_2
   Shake for 5 minutes
   Centrifuge
   Add upper layer to previous aqueous portion
   Apply to conditioned SPE column

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL DI H_2O
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute

4. **WASH COLUMN:**
   1 x 3 mL 100 mM HCl
   1 x 1 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg)

5. **ELUTE MELAMINE**
   1 x 2 mL of CH_3OH containing 5% NH_4OH
   1 x 3 mL of CH_3OH containing 5% NH_4OH
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40°C

7. **RECONSTITUTE** sample in 1000 µL of CH_3CN
   *Add external standard
   Inject 5 µL

INSTRUMENT CONDITIONS:

AP1 2000 MS/MS (ESI Mode)

Column: 150 x 2.1 mm (4 µm) Diamond Hydride (MicroSolv)

Flowrate: 0.50 mL/ minute

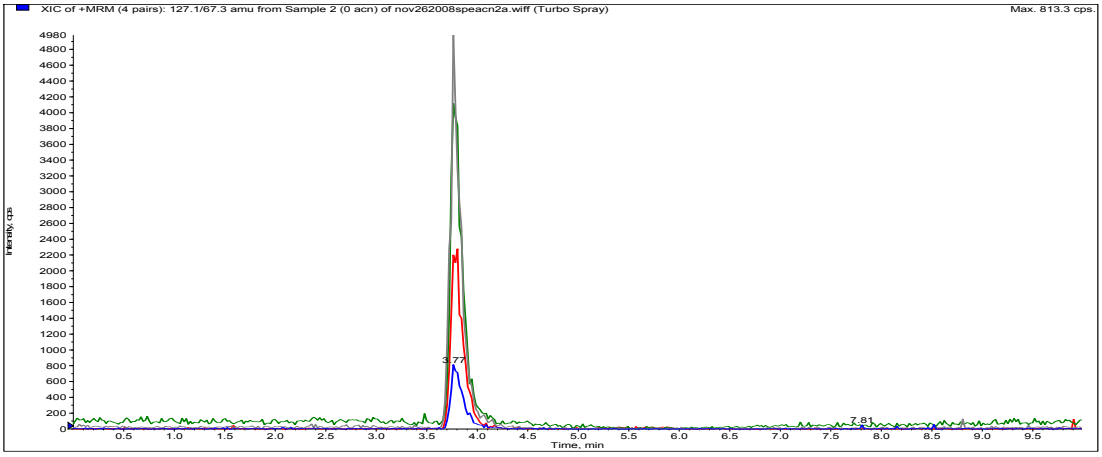
Mobile phase:

Time:	% Acetonitrile	% 0.1% Formic acid
0	90	10
3	20	80
3.5	90	10
10	90	10

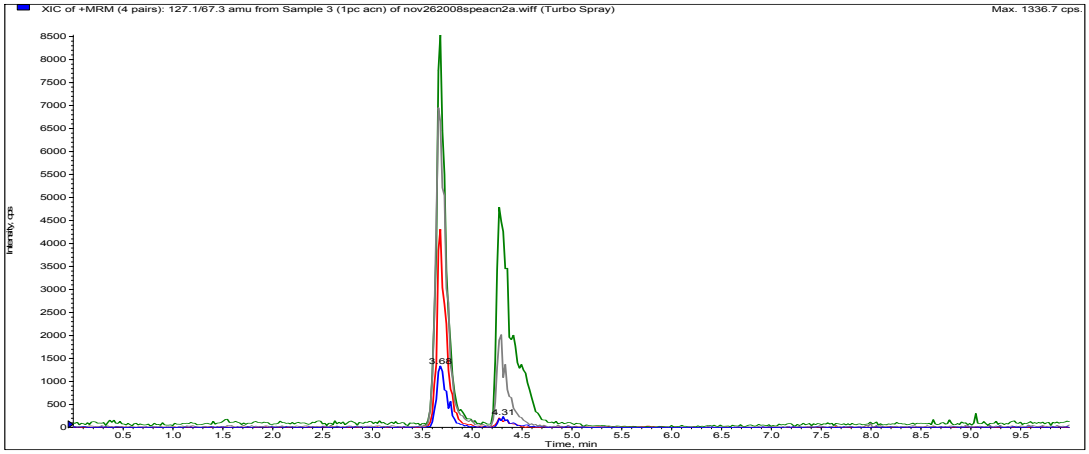
Column Temperature: ambient

Compound	MRM
Melamine	127.1/85.1
*2, 4 Diamino 6-hydroxy pyrimidine	127.1/ 67.0

LC-MS/MS Chromatogram of: Blank Milk Powder (upper)



Spiked Milk Powder (lower)



Recovery > 90% (N=10)

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