



MELAMINE IN FOOD MATERIALS USING CSDAU020

LC-MS/MS

September 30, 2009

1. PREPARE SAMPLE:

To 1-5 g of sample add 10-25 mL of CH₃CN/ DI H₂O (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 CH₂Cl₂

Shake for 5 minutes.

Centrifuge

Transfer upper layer to clean glass tube

Add 2 mL of DI H₂O to CH₂Cl₂

Shake for 5 minutes

Centrifuge

Add upper layer to previous aqueous portion

Apply to conditioned SPE column

2. CONDITION COLUMN:

1 x 3 mL CH₃OH

1 x 3 mL DI H₂O

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 CH₃OH.

Dry column (5 minutes at > 10 inches Hg)

5. ELUTE MELAMINE

1 x 2 mL of CH₃OH containing 5% NH₄OH

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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 CH₃CN

*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

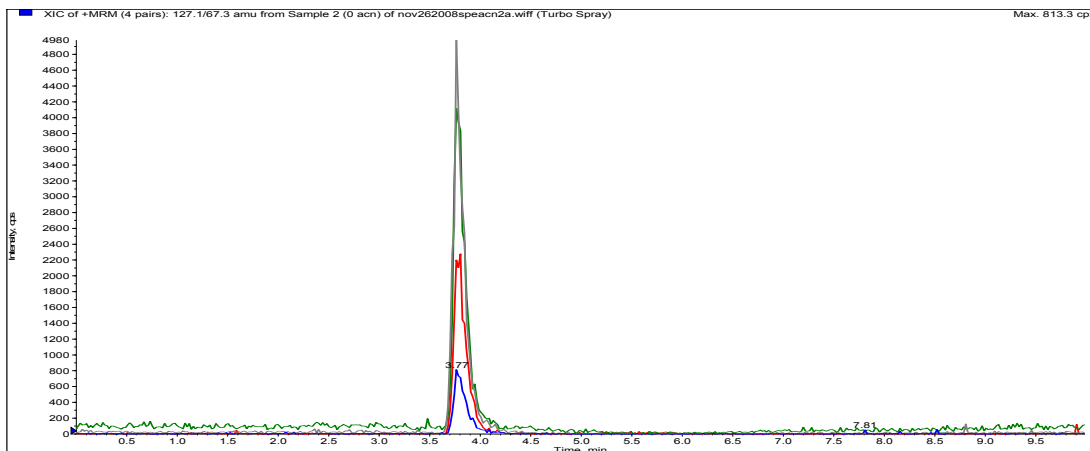
Mobile phase:

<u>Time:</u>	<u>% Acetonitrile</u>	<u>% 0.1% Formic acid</u>
0	90	10
3	20	80
3.5	90	10
10	90	10

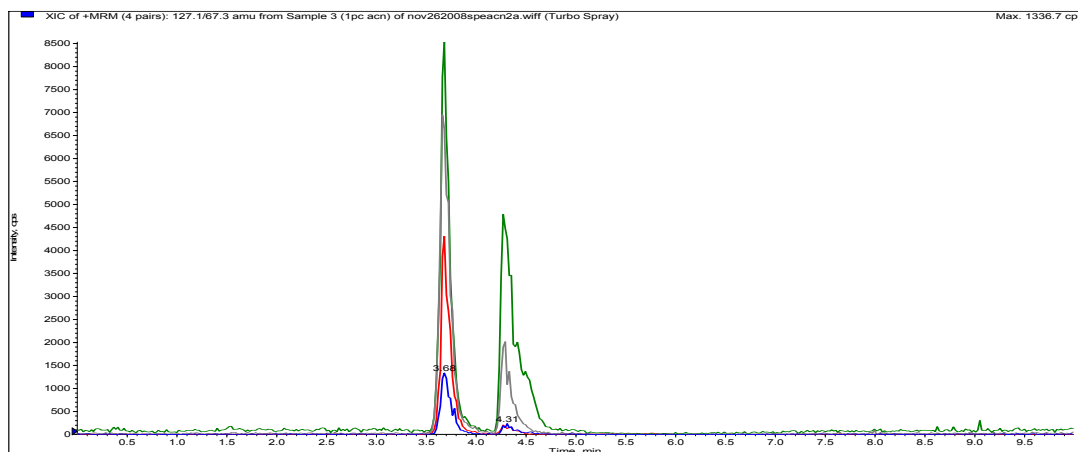
Column Temperature: ambient

<u>Compound</u>	<u>MRM</u>
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)

DCN-900390-29