Melamine Adulteration in Protein-Based Foods by GC/MS and LC/MS/MS

GC/MS Method:

Instrumentation: GC/MS with Programmable Split/Splitless EPC Injector (Ctrl + Click to follow link)
Column: Elite-5MS (30 m x 0.25 mm x 0.25 µm)
Injection Mode: Programmable Split/Splitless
Injection Type: Splitless
Carrier Gases: Helium
Col./Oven Temp: 75 °C hold 1 min, to 320 °C at 15 °C/min, then hold 2.67 min
Inlet temperature: 280 °C
Injection volume: 1 µL
Instrument Timed Events:

Time (min)	Flow (mL/min)
-0.5	0
1.0	50

Detection: MSD (Ctrl + Click to follow link)
Inlet temperature: 280 °C
Ion Source temperature: 230 °C
Function Type: Full scan
Full Scan Range: m/z 50-450
Solvent Delay: 6 min
Full Scan Time: 0.2 sec
InterScan Delay: 0.05 sec

Sample Preparation:
1. Dilute 5 mL of spiked milk with 5 mL 100 mM phosphate buffer (pH 2.5) and 1 mL acetonitrile
2. Sonicate for 5 minutes in an ultrasonic water bath
3. Centrifuge at 3500 rpm for 10 minutes
4. Isolate the middle supernatant layer for SPE processing
5. Process 2.2 mL of the middle supernatant layer (equivalent to 1 mL milk sample) using SPE.

The SPE was carried out on a strong cation exchange cartridge, Discovery® DSC-SCX (500 mg/6 mL, Sigma-Aldrich). The cleanup procedure is as follows:

- 5.1 Condition and equilibrate SPE cartridge with 3 mL methanol followed by 3 mL 0.1% formic acid
- 5.2 Load sample (2.2 mL)
- 5.3 Wash SPE cartridge with 3 mL 0.1% formic acid followed by 3 mL methanol
- 5.4 Elute melamine from SPE cartridge with 4 mL
- 5.5 5% ammonia diluted in methanol
- 5.6 Evaporate 1 mL SPE eluent to dryness, in an Autosampler vial, with nitrogen at 5 psi and 50 ˚C
- 5.7 Sample is ready for derivatization.

The dry sample is reconstituted in an autosampler vial with 200 µL of pyridine. Melamine is converted to trimethylsilyl (TMS) derivatives with the reagent Sylon-BFT (Supelco®) consisting of bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS). 300 µL of this solution is added and the sample is incubated at 70 ˚C for 45 minutes.

Results:

This method provides sensitivity down to 1 ppb melamine. The analysis of a 5-ppb standard achieved a signal to noise (RMS) of greater than 25:1. The average peak area measured in the analysis of 1 ppm

melamine extracts was 2.7 x 107 when compared to an average peak area of 2.3 x 107 for a 0.5 µg/mL standard (equivalent to 1 ppm in milk sample). Extractions of the same melamine sample yielded a precision of 3.45% RSD when comparing the measured peak area for the summed ions of m/z 327+342.

LC/MS/MS Method:

Instrumentation: Agilent HPLC system with AB/MDS Sciex 3200 Q Trap MS-MS
Column: Ascentis® Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm, or Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm
Elution Type: Gradient
Mobile Phase A: 10 mM ammonium formate in 90:10 acetonitrile:water
Mobile Phase B: 10 mM ammonium formate in 70:30 acetonitrile:water

Gradient:

Min	Flow Rate	%A	%B
0	0.2 mL/min.	100	0
5	0.4 mL/min.	0	100
10	0.4 mL/min.	100	0
15	0.2 mL/min.	100	0

Detection: MS/MS ; MRMs (127/85 & 127/68 m/z) – MRM 127/85 was used for quantitation

Q1	Q3	Declustering Potential (DP)	Entrance Potential (EP)	Collision Energy (CE)	Exit Potential (CXP)
127	85.0	46	4	31	4
127	68.0	41	4	31	4

Curtain gas: 20
Gas 1: 20
Gas 2: 40
Ion spray voltage: 5000
Temp.: 350 °C
LC temp.: 30 °C
Inj.: 2 µL

Sample Preparation: Same as GC/MS method

Results:
HILIC chromatography of melamine provided good peak shape and retention coupled with a short analytical run time of less than 4 minutes.
Based on a signal-to-noise ratio of 10:1, the estimated lower limit of quantitation is 4 ng/g (ppb) for dry milk powder and 4 ng/mL for whole milk. Note that background levels of melamine can be found in plasticware, solvents, and reagents; and should be monitored carefully. For example, up to 2-3 ng/g melamine was found when processing 1 g of blank sample. In addition, the FDA has reported up to 40 ng/g of background melamine when analyzing infant formula using a polymeric SPE phase.
Recovery and reproducibility was high across all the sample matrices and spike levels tested. The average recovery for whole milk and infant formula were 89% and 82%, respectively. RSDs were less than 11% for all the spike levels tested. Recovery for dry milk powder was 81% at the 1000 ng/g spike level tested.

References:
http://www.perkinelmer.com/pdfs/Downloads/APP_MelamineInDairyGCMS.pdf
http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/Application_Notes/t408188-melamine-analysis.pdf