Pharmaceutical Industry Guidance on Preventing Melamine Contamination

August 6, 2009 the U.S. FDA issued a Guidance for Industry - Pharmaceutical Components at Risk for Melamine Contamination. The events involving pet and livestock food products, and milk products for infants illustrate the potential for drug components to be contaminated with melamine. This guidance says that certain pharmaceutical ingredients used in the manufacture or preparation of drug products are recommended to be screened for melamine. Hence, it is important for drug manufacturers to assure that no component used in the manufacture of any drug is contaminated with melamine. FDA recommends that compounders who use at-risk components in drugs ensure proper testing.

The guidance for pharmaceuticals recommends the use of FDA-published methods based on equipment generally available to pharmaceutical manufacturers or contract testing labs. The test method used should be suitable to assay melamine contamination down to at least 2.5 parts per million (ppm).

Recommended methods are based on liquid chromatography triple quadrupole tandem mass spectrometry (LC-MS/MS) or gas chromatography/mass spectrometry (GC-MS). The LC MS/MS method is based on HILIC and also urge the need to prevent melamine degradation during sample handling, (see FDA methods). The compounds at risk may be, but are not limited to:

Adenine
Amino acids derived from casein protein hydrolysates
Calcium pantothenate
Chlorophyllin copper complex sodium
Copovidone
Dihydroxyaluminum aminoacetate
Glucagon
Hyaluronidase
Lactose
Povidone
Protamine sulfate
Taurine
Urea
Zein
Albumin
Ammonium salts
Caseinate or sodium caseinate
Colloidal oatmeal
Crospovidone
Gelatin
Guar gum
Imidurea
Melphalan
Povidone-Iodine
Protein hydrolysate (powder) for injection
Thioguanine
Wheat bran

This list was based on the FDA Inactive Ingredient Database (IID), and is not considered to be exhaustive. It is essential that manufacturers evaluate their drug components to determine whether they are vulnerable to melamine contamination.

Adulteration of Milk - India 2012

In January 2012, it was reported that more than 67% of Indian milk is adulterated. Everything from salt to detergents have been found. Among the substances found in milk were milk powder, fat, glucose and water. The Indian Food Safety and Standards Authority conducted a survey in 33 states and found that the problem is more severe in urban India, where nearly 70% of samples were found to be contaminated, compared with about 30% in rural areas.

Of these reasons, and considering the past scandals with pet, livestock food and infant formula milk powder, more and better testing is needed. Methods, not only for melamine and cyanuric acid is required as one can expect other nitrogen rich compounds to be used in economic adulteration to enhance the nitrogen content in milk products and bulk proteins.

•Risk Assessment/Safety Assessment

•Letter to the United States Food Manufacturing Industry, Regarding Melamine October 10, 2008

•Interim Safety and Risk Assessment of Melamine and its Analogues in Food for Humans October 3, 2008

•Update: Interim Safety and Risk Assessment of Melamine and its Analogues in Food for Humans November 28, 2008

In this application compilation, we present the latest analytical method from US FDA to be used as a powerful tool against economically motivated adulteration in protein-containing products. The new method have been developed to determine the presence of six nitrogen-rich compounds, cyromazine, dicyandiamide, urea, biuret, triuret, and amidinourea together with melamine. The method has been validated in skim milk, skim milk powder, soy protein, wheat flour, wheat gluten, and corn gluten meal matrices at concentrations as low as 1 ppm.

After acidic treatment of samples, acetonitrile is added to induce precipitation of proteins. Ready samples are analyzed using a SeQuant ZIC®-HILIC column and tandem mass spectrometry (HILIC–MS/MS) using electrospray ionization (ESI).

Determination of Nitrogen-rich Adulterants in Food using HILIC-MS/MS

FDA recommended column:
SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 150×2.1 mm (1.50454.0001)

Alternative column:
SeQuant® ZIC®-HILIC (3.5 µm, 200Å) PEEK 100×2.1 mm (1.50447.0001)

Recommended solvents and reagents

Acetonitrile: hypergrade for LC-MS LiChrosolv® (1.00029)

Water: Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q® water purification system

Formic acid: 98-100% for analysis EMSURE® ACS, Reag. Ph Eur (1.00264)

Ammonium formate: Use ACS grade or HPLC grade.

Recommended filtration tools:

Mobile phase filtration:
PTFE coated with funnel, base, stopper clamp (XX1004720)
Omnipore PTFE membrane filter 0.45µm (JHWP04700)

Sample filtration:
Millex-LG, 0.20 µm, Hydrophilic, PTFE, 13 mm, non-sterile (SLLGH13NL)
Samplicity™ starter bundle with filter 0.20µm (SAMPLG0BL)

Determination of Nitrogen-rich Adulterants in Food using HILIC-MS/MS

Mobile phase

prepare mixtures of 0.1% formic acid/10 mM ammonium formate in Milli-Q® water and acetonitrile
A: 95:5 ACN:0.1% formic acid/10 mM ammonium formate in Milli-Q® water
B: 50:50 ACN:0.1% formic acid/10 mM ammonium formate in Milli-Q® water

Gradient profile

Time (min)	Solution A (%)	Solution B (%)	Flow rate (mL/min)	Elution
0.0-5.0	100	0	0.400	isocratic
5.0-12.8	100→25	0→75	0.400	gradient
12.8-15.8	25	75	0.400	isocratic
15.8-16.0	100	0	0.400	equilibration
16.0-24.9	100	0	0.600	equilibration
24.9-25.0	100	0	0.400	equilibration

Sample preparation

Briefly:
- Mix 2 g sample with 18 mL 2% formic acid and shake immediately vigorously for 1 min
- Sonicate for 30 min and shake vigorously for 1 min
- Centrifuge at 4500 rpm for 30 min
- Take 50 µL supernatant and mix with 950 µL acetonitrile
- Centrifuge at 4500 rpm for 10 min
- Filter supernatant through 0.20 µm PTFE membrane
- (Milk samples only) Dilute 100 µL filtrate with 500 µL 95:5 acetonitrile:2% formic acid

For full details of the methods of sample preparation and HILIC-MS/MS, please refer to the original documents from FDA:

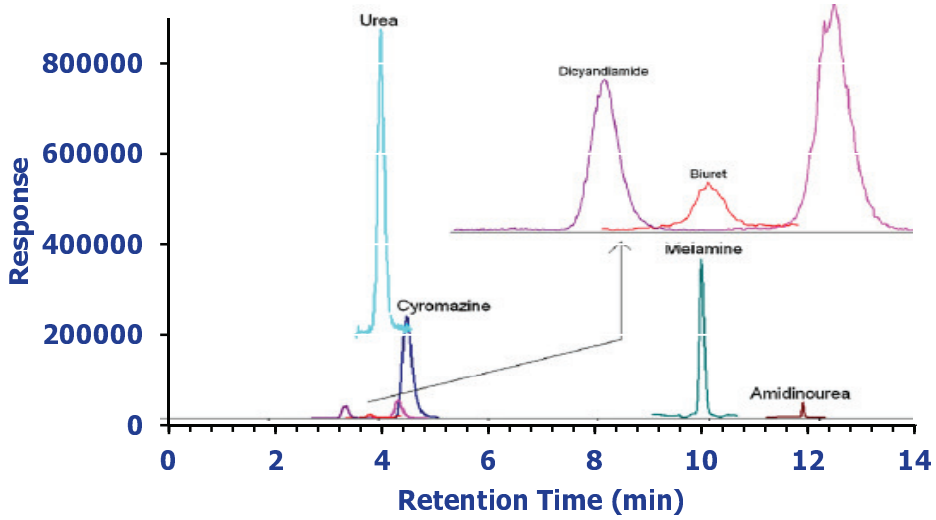
- FDA Laboratory Information Bulletin 4487 (not yet available online)
- Journal of Chromatography A 1220 (2012) 101–107
- FDA Laboratory Information Bulletin 4421

Determination of Nitrogen-rich Adulterants in Food using HILIC-MS/MS

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 150x2.1 mm	1.50454.0001
Injection:	20 µL	
Detection:	Individual LC–MS/MS quantitation ion chromatograms using a Shimadzu Prominence UFLC XR with AB Sciex 4000 QTRAP in ESI(+) mode $[M+H]^+$ m/z 85.0 for DC, 61.0 for urea, 104.1 for BU, 147.1 for TU, 167.1 for CY, 127.1 for melamine (MEL) and 103.0 for AU were the precursor ions for MS/MS;	
Flow Rate:	0.4 mL/min during separation, 0.6 mL/min during equilibration	
Mobile Phase (v/v):	A: 95:5 ACN:0.1% formic acid/10 mM ammonium formate in H2O B: 50:50 ACN:0.1% formic acid/10 mM ammonium formate in H2O	
Temperature:	Ambient	
Sample:	Extracted and spiked (1 ppm) wheat gluten sample	



Chromatogram reproduced from J. Chromatogr. A 1220 (2012) 101-107 , with permission from Elsevier Science and Shaun MacMahon, US FDA.

Chromatographic Data

No.	Compound	Time (min)	Transition (m/z)
1	Dicyandiamide (DC)	3.6	85.0→68.0; 85.0→43.1
2	Biuret (BU)	3.9	104.1→61.0; 104.1→44.0
3	Urea	4.0	61.0→44.0
4	Triuret (TU)	4.5	147.1→130.1; 147.1→104.1; 147.1→61.1
5	Cyromazine (CY)	4.7	167.1→85.1; 167.1→125; 167.1→68.0
6	Melamine (MEL)	9.9	127.0→85.0; 127.0→68.0
7	Amidinourea (AU)	11.7	103.1→60.1; 103.1→43.1