Adulterants

Definition

1. An adulterant is a chemical substance which should not be contained within other substances for legal or other reasons. The addition of adulterants is called adulteration.

2. Adulterants need to be relatively odorless, colorless and tasteless to avoid negative impact upon consumer acceptance of the fraudulent product.

3. If used as a protein substitute, the adulterant(s) also need to be commercially available in large quantities.

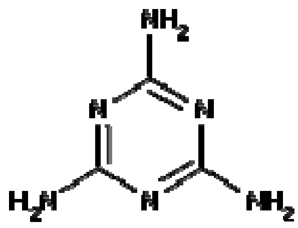
4. The ultimate goal with adulteration is economic fraud, not to injure consumers; hence food producers would be unlikely to deliberately use an acutely toxic chemical as an adulterant. However, as could be proven with melamine adulteration, even relatively non-toxic chemicals can cause unforeseen health effects.

5. Addition of adulterants must generally result in a product that costs less than the authentic food product, or otherwise there is no real economic incentive for adulteration.

6. Adulterants when used in illicit drugs are called cutting agents, while deliberate addition of toxic adulterants to food or other products for human consumption is known as poisoning.

Further reading on Food Contaminants & Adulteration:
http://www.fda.gov/food/foodsafety/foodcontaminantsadulteration

Economically Motivated Adulteration in Protein-containing Foods and Food Ingredients



Melamine

Incidents:	USA	2007
	China	2008

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton. It is a small polar compound which is very rich in nitrogen (67% by mass). It has been found in milk products and animal feed, where it have been added to give a false impression of high protein content. Melamine combined with cyanuric acid can cause fatal kidney stones due to the formation of an insoluble melamine-cyanurate complex. Determination of melamine and other small nitrogen-rich compounds, is therefore of large importance to ensure food safety.

Economically Motivated Adulteration in Protein-containing Foods and Food Ingredients

In 2012, US FDA published a new analytical methodology to be considered as a powerful tool against economically motivated adulteration in protein-containing products. After incidents with deliberate addition of melamine to pet food (2007) and milk powder (2008), public awareness on food safety has increased and more efforts are taken by official organizations in order to control quality of food and beverages consumed by humans and animals. Despite this it is likely that criminal ingredient suppliers or food producers are searching for, and testing out new alternatives to melamine (other poly-nitrogenous compounds) to artificially enhance the concentrations of protein detected in their products.

Kjeldahl Method

The traditional standard technique for measuring protein content in food is the Kjeldahl method; a quantitative determination of total nitrogen content. The method consists of heating a substance with sulfuric acid, which decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulfate. In this step potassium sulfate is added to increase the boiling point of the medium. Chemical decomposition of the sample is complete when the medium has become clear and colorless (initially very dark). The solution is then distilled with sodium hydroxide (added in small quantities) which converts the ammonium salt to ammonia. The amount of ammonia present (hence the amount of nitrogen present in the sample) is determined by back titration.

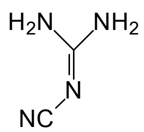
The Kjeldahl method is therefore a non-direct measurement of protein, hence it is possible to artificially enhance protein concentrations by adding nitrogen-rich chemicals, therefore any chemical compound having a high percentage of nitrogen, by weight, has the potential to be used in economically motivated adulteration of protein-containing food products.

The Canadian Food Inspection Agency's Food Safety Division has recently generated a list of potential adulterants. The Canadian list has also been shared with other food safety agencies. The US FDA has, based on the Canadian recommendations and their own intelligence, determined a number of potential compounds likely to be used in protein adulteration.

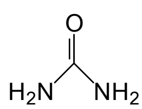
Economically Motivated Adulteration in Protein-containing Foods and Food Ingredients

FDA listed compounds most likely to be used in protein adulteration:

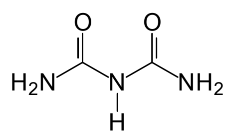
•Dicyandiamide (DC)	used in the production of melamine, as well as in fertilizers and as a fire-proofing agent
•Urea	found in fertilizers, as a non-protein nitrogen source in animal feeds, as well as in production of many commercial products
•Biuret (BU)	non-protein nitrogen source. It is the result of condensation of two molecules of urea and is a problematic impurity in urea-based fertilizers. May be used in some animal feeds
• Triuret (TU)	non-protein nitrogen source. May be used in some animal feeds
•Cyromazine (CY)	insect growth regulator. A cyclopropyl derivative of melamine produces melamine upon metabolism
•Amidinourea (AU)	used in fertilizers



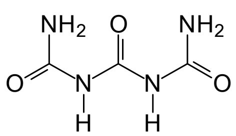
Dicyandiamide



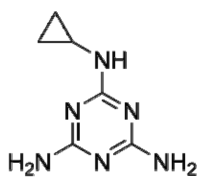
Urea



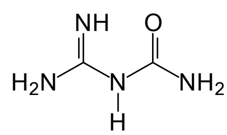
Biuret



Triuret



Cyromazine



Amidinourea

Melamine and Cyanuric Acid in Pet Food

In 2007 many brands of pet food was recalled in the United States, Europe and a few other countries, in response to reports of renal failure in pets. Initially, the recalls were associated with the consumption of mostly wet pet foods made with wheat gluten. A month after the first recall, rice protein was also identified as being contaminated, causing kidney failure in pets. On April 27, 2007, United States Food and Drug Administration (U.S. FDA) detained ALL vegetable proteins imported from China, intended for human or animal consumption. This meant wheat gluten, rice gluten, rice protein, corn gluten, soy protein, proteins (includes amino acids and protein hydrolysate), mung bean protein, and variants thereof.

It was found that melamine (MEL) and cyanuric acid (CYA), a hydrolysis by-product of melamine, had been deliberately added to pet food, to fool the Kjeldahl method for protein determination.

Melamine and cyanuric acid can cause serious health issues in human and pets. While each is relatively innocuous, they can form a complex (MEL:CYA) which is nearly insoluble and crystallizes in kidney tubules, leading to illness or death.

The determination of MEL and CYA, and the MEL:CYA complex is quite challenging because the compounds are very polar. A number of screening methods including GC and LC methods have been developed for the determination of MEL and CYA in foods in recent years. Most of these methods require complex derivatization and/or extraction procedures, and do not allow for simultaneous quantitation or provide sufficient identification confidence for regulatory action. In this compilation you will find links to the original validated ZIC®-HILIC method for the determination of melamine and cyanuric acid in animal feeds developed by scientists at the US FDA Center for Veterinary Medicine. The protocol was successfully employed for the analysis of melamine in commercial aquaculture, fish and shrimp feed. The method was developed to comply with regulatory analysis of animal feed.

http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Melamine/default.htm

Chinese Milk Scandal in 2008

In September 2008, several Chinese companies were involved in a scandal involving milk and infant formula adulterated with melamine, leading to kidney stones and other renal failure, especially among young children. By December 2008, nearly 300,000 people had become ill, with more than 50,000 infant hospitalizations and six infant deaths.

As late as July 2010, almost 2 years after the first reports, Chinese authorities were still reporting some seizures of melamine-contaminated dairy product in some provinces.
It is though unclear whether these new contaminations were a result of illegal reuse of material from the 2008 adulterations.

Food Testing

Until 2007, melamine had not routinely been tested in food, except in the perspective of plastic safety or insecticide residue. The U.S. FDA issued in October 2008 new methods for the analysis of melamine and cyanuric acid in infant formulations, see links below. Similar recommendations have been issued by other authorities, like the Japanese Ministry of Health, Labor and Welfare, both based on liquid chromatography – mass spectrometry (LC/MS) detection after hydrophilic interaction liquid chromatography (HILIC) separation.

Official Liquid Chromatographic Methodology from US FDA

Determination of Melamine and Cyanuric Acid Residues in Infant Formula using LC-MS/MS:
Laboratory Information Bulletin 4421, October 2008

Interim Method for Determination of Melamine and Cyanuric Acid Residues In Foods using LC-MS/MS:
Laboratory Information Bulletin 4422, October 2008

After the Chinese scandal, the Joint Research Centre (JRC) of the European Commission set-up a website about methods to detect melamine.
irmm.jrc.ec.europa.eu/melamine

Members of the European Union are required under Commission Decision 2008/757/EC to ensure that products containing at least 15% of milk product, originating from China, are tested before import into the Community. Products containing more than 2.5 mg/kg melamine must immediately be destroyed.

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:

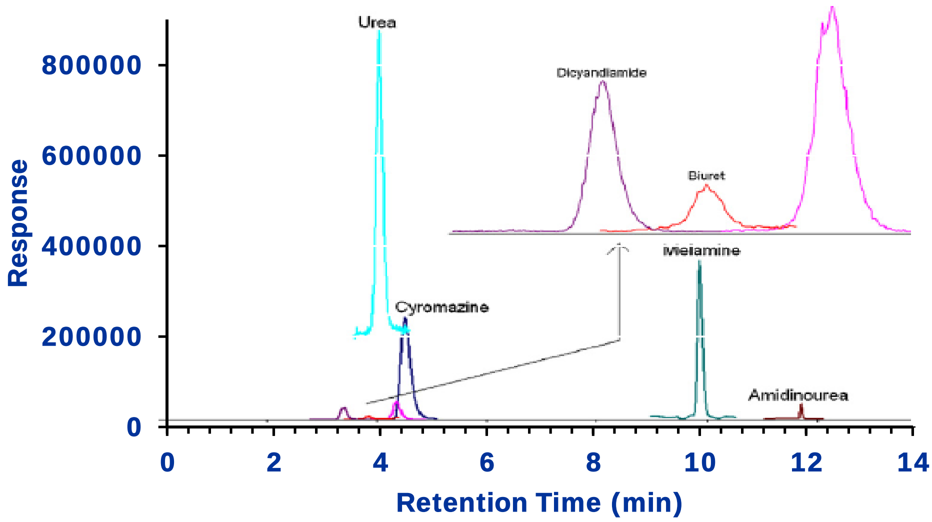
- FDA Laboratory Information Bulletin 4487 (not yet available online)
- Journal of Chromatography A 1220 (2012) 101–107
- FDA Laboratory Information Bulletin 4421

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