

## 中国産植物性たん白に関する経緯等

### 1 米国での経緯

(1) 米国食品医薬品局（FDA）は、5月4日までにペットフードが原因とされるペットの死亡事故について、以下の内容を公表。

- ・ ペットフードを食べた犬や猫が相次いで死亡し、原料として使用した中国産小麦グルテン及びコメ濃縮たん白から、メラミンを検出。
- ・ ペットフード中のたん白質を多く見せかけるため、メラミンが加えられた可能性があり、FDA担当官が、中国政府の担当者から事情を聴取。

(2) FDA及び米国農務省（USDA）は、4月28日及び30日に豚用及びブロイラー用飼料にもメラミンの混入があったとして、以下の内容を公表。

- ・ カリフォルニア州の養豚農家の豚の尿中からメラミンを検出。メラミンが混入し回収されたペットフードが当該農家に販売されていたことが原因。
- ・ インディアナ州にあるブロイラー約30農場と、ブロイラー種鶏8農場で、本年2月にメラミンの混入飼料を使用。
- ・ FDAとUSDAは、当該鶏肉の摂取によりヒトに健康被害が生じる可能性は非常に低いと考えている。
- ・ ただし、USDAは、メラミンの混入飼料を摂取した鶏肉を食用にすることを認めないこととし、FDAとUSDAは、混入飼料を使用した農場がないか、調査を継続。

(3) FDAは、4月27日に「輸入上の注意（Import alert）」において、全ての中国産植物性たん白（小麦グルテン、コメグルテン、コメたん白、コメ濃縮たん白、コーングルテン、コーングルテンミール、コーン副生産物、大豆たん白、大豆グルテン、アミノ酸及びタンパク加水分解物を含むたん白及び緑豆たん白）を対象に輸入時検査を実施することを公表。

### 2 厚生労働省の対応

今回の混入に係る中国の小麦グルテン等の製造業者が一企業に止まらない可能性が出てきたことから、厚生労働省は、中国産植物性たん白の検査を行うこととし、5月2日に医薬食品局安全部監視安全課輸入食品安全対策室長から各検疫所長あての通知（食安輸発第0502001号）を発出。

(別添2)



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## Updated FCC Developmental Melamine Quantitation (HPLC-UV)

April 2, 2007

### Sample Preparation/Extraction:

Wheat gluten: Ground in a Retsch ZM 100 centrifugal rotor mill (ring sieve 0.5 mm).

Moist pet food: Moist chunks and gravy were blended to the consistency of a gritty pudding prior to sampling.

Samples weighed into glass scintillation vials. Extract using 50:50 acetonitrile:water.

Procedure: Add indicated volume of extraction solvent (see note below) <sup>1</sup>. **Cap and vortex thoroughly, get aggressive with this step (critical due to slow dissolution rate of melamine)**. Sonicate 30 minutes. Filter portion of extract through 2-stage GMF-nylon (0.45 m m) filters. Dilute filtered extract 250  $\mu$ l extract + 750  $\mu$ l solvent <sup>2</sup> to maintain solubility of matrix components.

<sup>1</sup> Wheat gluten: Extract in proportion 0.1 g to 10 ml. For melamine contents above 2% w/w, extract in proportion 0.05 g to 15 ml.

<sup>1</sup> Moist pet food: Extract in proportion 2.0 – 2.5 g to 10 ml.

<sup>2</sup> Solvent for final dilution may be water or 0.1 N HCl. Final dilution is necessary for compatibility with ion-pairing chromatography. We have observed some differences in behavior between the wheat gluten and pet food samples with respect to maintaining solubility during final dilution (these are most likely matrix components which fall out). 0.1 N HCl seems to help maintain solubility for final dilution of the wheat glutes.

### HPLC-UV Operating Parameters:

Column: Zorbax Rx C8 (retention is too high on C18 column)

Buffer: 10 mM citric acid, 10 mM sodium octane sulfonate, adjusted to pH 3.0

Mobile phase: 85:15 buffer:acetonitrile

Flow rate: 1.0 ml/min.

Injection volume: 10  $\mu$ l

Column thermostat: 40 °C (column thermostating is necessary for ion-pair separations)

Detection wavelength: 240 nm

Spectral collection: 200 – 400 nm (look for  $\lambda_{\max}$  near 236 nm)

Retention time: 4.2 - 4.3 min.

Run time: 10 min.

### Standard Preparation:

Stock standard was prepared in 76:24 acetonitrile: water. A check stock standard was prepared in 60:40 acetonitrile: water. Dilutions were made in either water or 0.1N HCl giving equivalent calibration curves.

### Figures of Merit:

Linear range: established from 1.0 – 400  $\mu$ g/ml; calibration range 1.0 – 200  $\mu$ g/ml used for most of work

Reproducibility: duplicate preparations agree within 0.1 - 5% relative basis

Spike/recovery (based on spiking solid melamine powder into test matrix prior to extraction): 90 -110%.

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**Interim GC-MS Method for Screening and Confirmation of Melamine and Related Analogs (PRLNW)**  
**(Adapted from Forensic Chemistry Center SOP T015)**

April 25, 2007

**PURPOSE:**

This procedure provides a general guide for the sample preparation and analysis for melamine and related analogs using gas chromatography/mass spectrometry. This procedure is designed to screen a TMS derivative of the extract.

**SCOPE:**

This procedure is applicable to rice protein and wet and dry type pet food. This procedure involves extractions in DEA.

**RESPONSIBILITY:**

It is the responsibility of the analyst to note any modifications to this procedure in the worksheet.

**DEFINITIONS AND ACRONYMS:**

TMS – trimethylsilyl  
DEA – diethylamine

**SAFETY CONSIDERATIONS:**

Accepted safety measures should be employed when working with chemicals and pressurized gases.

**EQUIPMENT:**

Agilent 5973 GC-MS system equipped with a 30m HP-5MS column (or equivalent)  
Reacti-Vap – Evaporating/Heating Module

**REAGENTS:**

DEA: Sigma  
Pyridine: Fisher, Certified A.C.S.  
BSTFA with 1% TMCS: bis(trimethylsilyl)trifluoroacetamide with 1%  
Trimethylchlorosilane (e.g. Sylon BFT, Supelco)

**QC ELEMENTS:**

A blank should be run at the onset of each analysis and then randomly throughout the analysis if there is suspicion of carryover. A fortified sample should be analyzed with each set of samples to demonstrate effective system performance. Bracket the samples with standard injections and use the average response for approximate quantitation.

**PROCEDURE:**

This procedure should be used with the GC operating in the splitless mode.

***Method***

Weigh out ~0.5g rice protein or ~1g of pet food into 50mL centrifuge tube

Add 20mL of 20%DEA in water

Vortex for 1 minute

Sonicate for 30 minutes. For pet foods, chill in an ice bath for 5-10 minutes prior to next step.

Centrifuge 10 minutes at 5000rpm (if possible)

Filter through 0.45µm nylon filter (This step may be a challenge for high protein matrices. Less sample or more 20% DEA may be helpful.)

Take 40µL of filtrate (less if a high level or more if more sensitivity is required)

Evaporate to dryness

Add 600µL pyridine

Add 200µL BSTFA

Vortex

Heat in reactor block at 70°C for 30 minutes

Cool

Filter through 0.45µm nylon filter into injection vial (if necessary – inspect visually)

Filter into injection vial

The approximate retention times of the 3-TMS derivatives are as follows (minutes):

Melamine	~12.4
Ammeline	~11.7
Ammelide	~10.7
Cyanuric Acid	~9.5

Blanks: Use a 3:1 mix of pyridine : BSTFA for blank injections. Since a reagent blank is being run with each batch of samples it may also be used for blank injections for monitoring carryover.

Samples: In general, if sample results are in excess of 100µg/mL (in the injection vial), appropriate dilutions should be made with pyridine/BSTFA to achieve an on column concentration no greater than 50µg/mL.

#### **Sample Fortification:**

This method was successfully applied to detect melamine and related analogs in wet and dry pet food composites and dry rice protein. This method was able to detect melamine levels down to 10 ppm. Sample fortification experiments to determine the limit of detection for melamine in the specific matrix of interest should be performed by the individual laboratories.

#### *Standard Preparation*

- (1) Melamine, CAS 108-78-1, Cat. 240818-5G, Aldrich
- (2) Ammelide, CAS 645-93-2, Cat. A0645, TCI America
- (3) Ammeline, CAS 645-92-1, Cat. A0676, TCI America
- (4) Cyanuric Acid, CAS 108-80-5, Cat. C0459, TCI America

Stock standard solutions are prepared in 20% DEA in water at ~1mg/mL. Some sonication may be required for the ammelide and ammeline standards.

Mixed working standard is prepared by diluting stock standard solutions into a mixture at ~50 µg/mL for each standard. Use this mixed standard solution to prepare the TMS derivatives as previously described and for determination of approximate quantitative results.

#### **Instrument Parameters:**

The following are the general parameters for each of the above mentioned methods:

##### GC Conditions:

Inlet Temperature	280°C
Detector Temperature	290°C
Injection Mode	Splitless
Injection Volume	1µL (use pyridine in rinse vials and watch waste levels)

Carrier Gas Flow He at 1.0mL/minute

##### Oven Program:

100°C (hold 1 minute) to 210°C at 10°C/minute  
210 to 300°C @ 30°C/minute (Hold 10 minutes)

##### MS Conditions:

Autotune	Use regular autotune and add a +306V multiplier bump
Acquisition parameters:	El; scan mode, 60-500 amu
Sampling Rate	set at 2 to keep the scan rate at 3.32 scans/sec (minimize spectral skew)
Threshold	100
Filament Delay	7 minutes
MS Temp	230°C (Source); 150°C (Quad)

Run time: 25 minutes

### Recovery data

Fortification experiments were performed on a rice protein concentrate and a dry pet food sample. The data is summarized below:

Dry Pet Food: (average of three recoveries at 500, 1000 and 2000µg/g)

Melamine	87%
Cyanuric acid	66%
Ammeline	88%
Ammelide	99%

Rice Protein Concentrate (one recovery at ~2000µg/g each component using solid std)

Melamine	87%
Cyanuric acid	120%
Ammeline	87%
Ammelide	105%

### Peak Identification and Results:

Figure 1 is a standard chromatogram showing the cyanuric acid TMS derivative (9.55 minutes), ammelide TMS derivative (10.74 minutes), ammeline TMS derivative (11.68 minutes) and melamine TMS derivative (12.41 minutes). Figures 2 through 5 represent the mass spectra of each of the melamine analogs.

Approximate quantitative results were calculated using the average response from the standard injections that bracket the batch of sample injections.

Figure 1: Standard chromatogram showing the TMS derivatives of target compounds

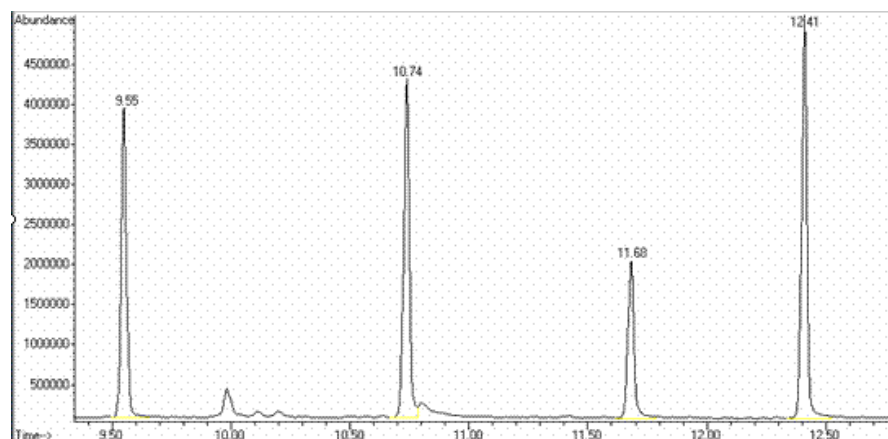


Figure 2: Cyanuric Acid TMS derivative

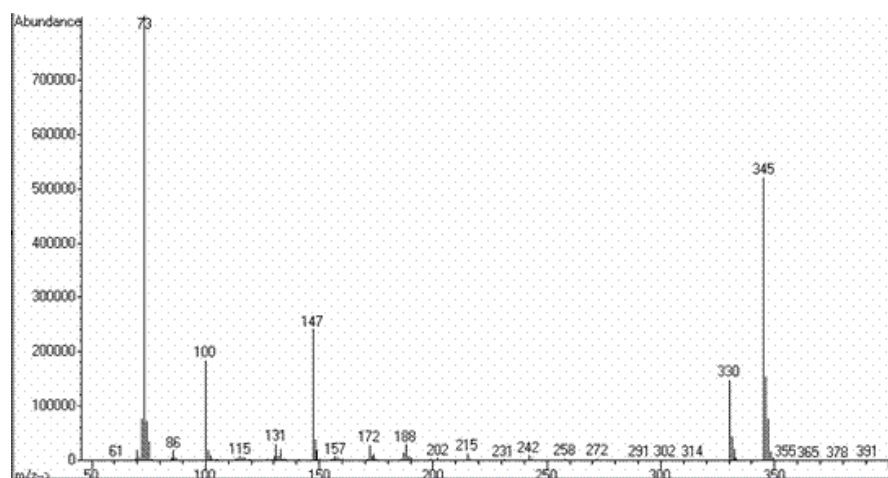


Figure 3: Ammelide TMS derivative

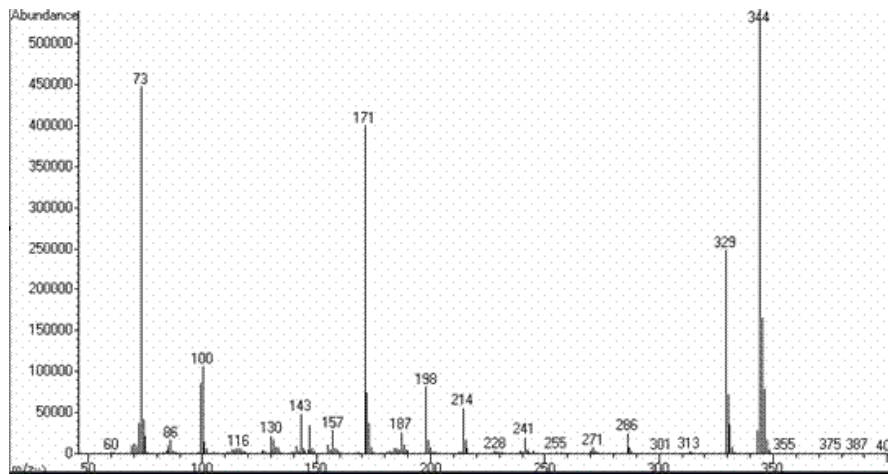


Figure 4: Ammeline TMS derivative

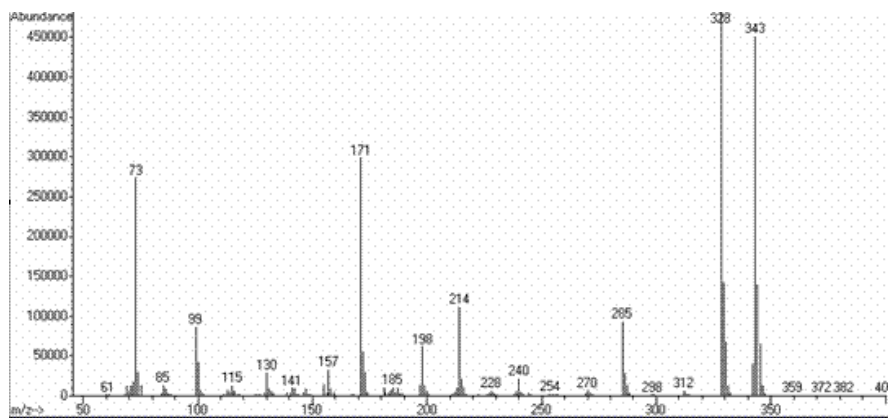
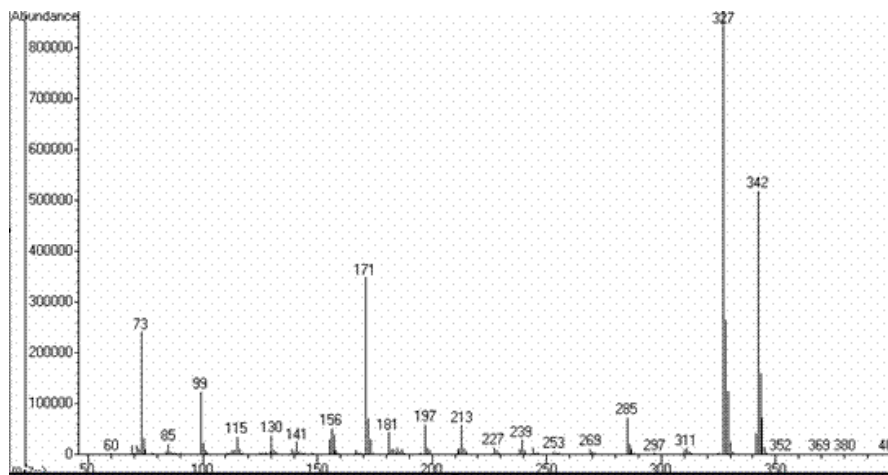


Figure 5: Melamine TMS derivative



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