Application Note: 424

Analysis of Melamine and Cyanuric Acid in Food Matrices by LC-MS/MS

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Key Words

- TSQ Quantum Ultra™
- Cyanuric Acid
- Food Safety
- LC-MS/MS

Introduction

In March 2007, several North American manufacturers of pet food voluntarily issued nationwide recall notices for some of their products that were reportedly associated with renal failure in pets[1]. The raw material wheat gluten, used to manufacture the pet food, was imported from China and was identified as the source of contamination[2].

Although initial reports suggested that contamination was confined to pet food, further investigations revealed that melamine-tainted fodder may have been used to feed animals intended for human consumption.[3,4,5] In particular, it was discovered that melamine-contaminated ingredients had been used to prepare feed for chickens, swine, and catfish.[3,4] Consequently, the U.S. Food and Drug Administration (FDA)[3] and the U.S. Department of Agriculture (USDA)[4] have developed methods for the analysis of melamine residues in animal tissue. Both methods use tandem mass spectrometric detection and employ disposable strong cation exchange solid phase extraction (SPE) cartridges to prepare samples for liquid chromatographic analysis.

Experimental

Chemicals and reagents

Unless stated otherwise, all organic solvents used in this work were HPLC grade quality and were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Melamine, cyanuric acid, and 30% (w/w) aqueous ammonia were purchased from Sigma (St. Louis, MO, USA). The internal standards $^{13}C_3$-melamine and -cyanuric acid were prepared using $^{13}C_3$-cyanuric chloride, which was also obtained from Sigma. 18 MΩ water was obtained from a Milli-Q™ (Millipore Corporation, Billerica, MA, US) purification system.

Calibration Standards

Individual solutions (1000 µg/mL) of cyanuric acid and melamine were prepared by dissolving the crystalline compounds in 50% (v/v) aqueous methanol. Aliquots (1 mL) of these solutions were combined and then diluted with 1:3 water-acetonitrile, respectively, to obtain a 10 µg/mL stock solution, from which eight working standards in the range of 1-1000 ng/mL were prepared by serial dilutions with acetonitrile. Calibration standards were prepared by adding 50 µL of the stock solution of the internal standards to 1 mL of each of the eight working standards.

Sample Preparation

Solid samples were homogenized using an Ultra-Turrax® (IKA®-Werke GmbH & Co. KG, Staufen, Germany) homogenizer. After extraction into aqueous 1:1 Water:MeOH, and addition of the internal standards, the samples were prepared by offline ion exchange chromatography using SPE cartridges.

Liquid Chromatography

Aliquots (10-25 µL) of the above extracts were chromatographed on a BioBasic™AX (Thermo Fisher Scientific, Bellefonte, PA) analytical column (2.1×150 mm, 5 µm), which was kept at 30 °C in an oven. The initial mobile phase was composed of acetonitrile-isopropanol-50 mM aqueous ammonium acetate in the ratio of 85:10:5, respectively, and was pumped through the column at a flow of 400 µL per minute.

After 5 min, the mobile phase composition and flow were immediately changed to 9:1 water-acetonitrile, and 500 µL per minute, respectively. These conditions were maintained for 5 min before returning the mobile phase to the initial composition. After 5 min of equilibration, the flow through the column was returned to 400 µL per minute. The column effluent was diverted to waste for the first 1.5 minutes and then switched to the detector for the remaining run time.

MS Conditions – Melamine

MS: Thermo Scientific TSQ Quantum Ultra
Source: Heated Electrospray (H-ESI)
Ionization: Positive ESI
Sheath Gas: 65 units
Auxiliary Gas: 35 units at 250 °C
Ion Transfer Tube Temp: 350 °C
Scan Time: 200 ms/transition
Q1/Q3 Resolution: 0.7 FWHM

SRM Transitions:

Melamine:	*Melamine $^{13}C_3$ (Internal Standard):*
m/z 127→68 @ 32 eV	*m/z* 130→70 @ 32 eV
m/z 127→85 @ 18 eV	*m/z* 130→87 @ 18 eV

QED-MS/MS Conditions:
Collision Energy: 30 eV
Reverse Energy Ramp (RER): 50 eV

MS Conditions – Cyanuric Acid

MS: TSQ Quantum Ultra
Source: Heated Electrospray (H-ESI)
Ionization: Negative ESI
Sheath Gas: 75 units
Auxiliary Gas: 10 units at 250 °C
Ion Transfer Tube Temp: 350 °C
Scan Time: 200 ms/transition
Q1/Q3 Resolution: 0.7 FWHM

SRM Transitions:

Cyanuric Acid:	*Cyanuric Acid $^{13}C_3$ (Internal Standard):*
m/z 128→42 @ 17 eV	*m/z* 131→43 @ 17 eV
m/z 128→85 @ 11 eV	*m/z* 131→87 @ 11 eV

Results

A chromatogram showing a standard mixture of both melamine and cyanuric acid, along with their associated internal standards, is shown in Figure 1. Calibration curves ranging from 1-1000 ppb are shown in Figure 2 and Figure 3 for melamine and cyanuric acid, respectively. The calibrations are linear over the entire range, and a close-up of the lower portion of the calibration curve (1-100 ppb) is shown in the same figure.

Melamine and cyanuric acid were spiked into a matrix of catfish and processed as described in the method section above. A chromatogram of this sample, spiked at 10 ppb for melamine and 50 ppb for cyanuric acid, is shown in Figure 4. Very low noise is observed, emphasizing the effectiveness of the cleanup procedure for a complicated matrix.

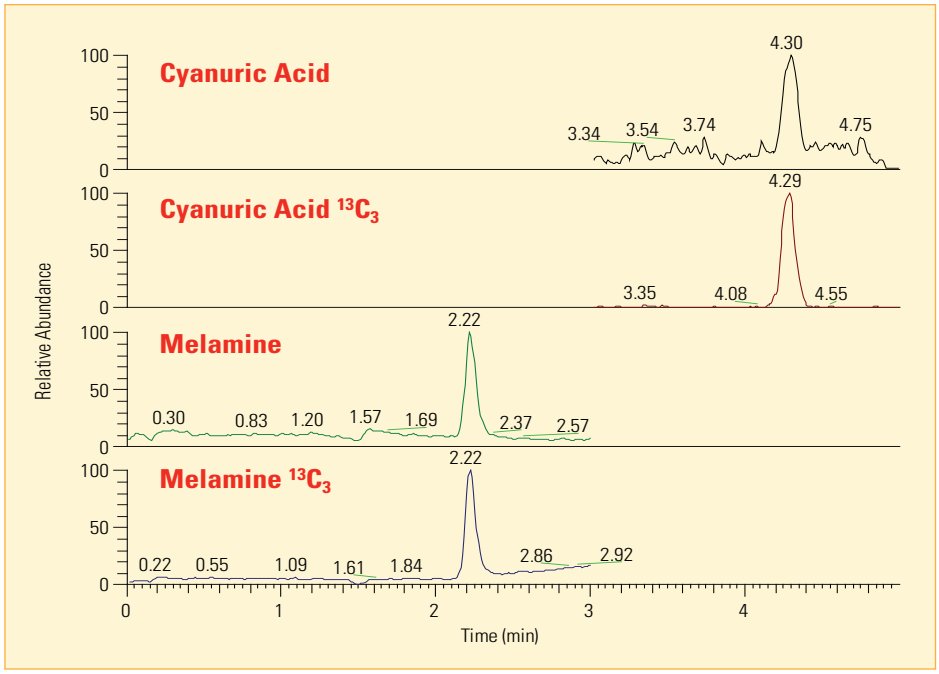

Figure 1. Melamine, cyanuric acid, and their internal standards at a concentration of 1 ppb. From top to bottom, cyanuric acid, cyanuric acid $^{13}C_3$, melamine, and melamine $^{13}C_3$.

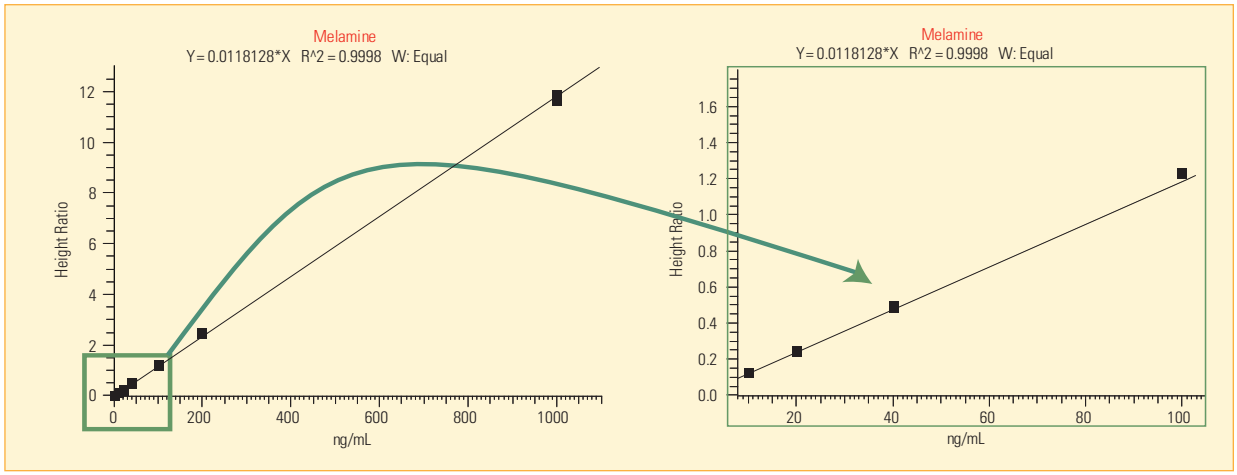

Figure 2. Calibration curve for melamine from 1-1000 ng/mL. The left figure shows the entire calibration range, while the right figure shows the expanded range from 10-100 ng/mL.

Additionally, full spectra data was collected using the standard Quantitation-Enhanced Data-Dependent MS/MS (QED-MS/MS) scan function. QED-MS/MS works by monitoring SRM data, and when the response of a particular SRM reaches a threshold level, the full scan MS/MS is activated. The resulting full scan spectra for melamine at 100 ppb and its internal standard are shown in Figure 5. The full scan data allows for further confirmation of results by eliminating "false positives" and also provides the opportunity to perform a library search. When a full scan QED-MS/MS spectra collected from a catfish sample spiked at 10 ppb was searched against the library, the library search returned melamine as the most likely hit. The results of the library search are shown in Figure 6. The spectrum of the sample and the spectrum that is stored in the library are visible in the lower left quadrant of the figure. The top spectrum is the catfish sample, while the lower spectrum is the reference spectrum. There is good agreement between the two spectra, even though the reference spectrum was generated using standards without matrix.

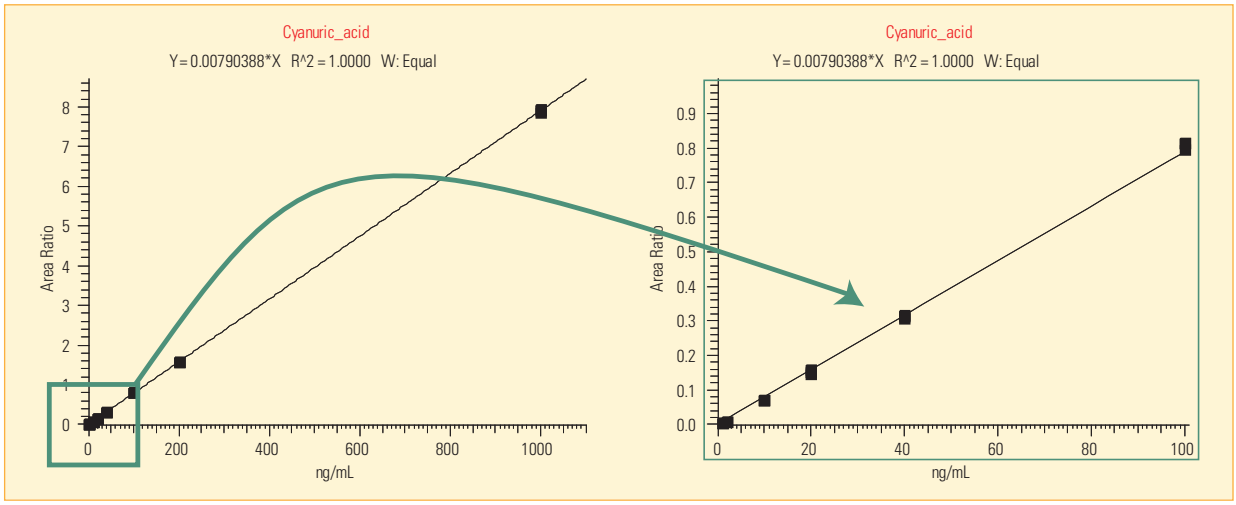

Figure 3: Calibration curve for cyanuric acid from 1-1000 ng/mL. The left figure shows the entire calibration range, while the right figure shows the expanded range from 1-100 ng/mL.

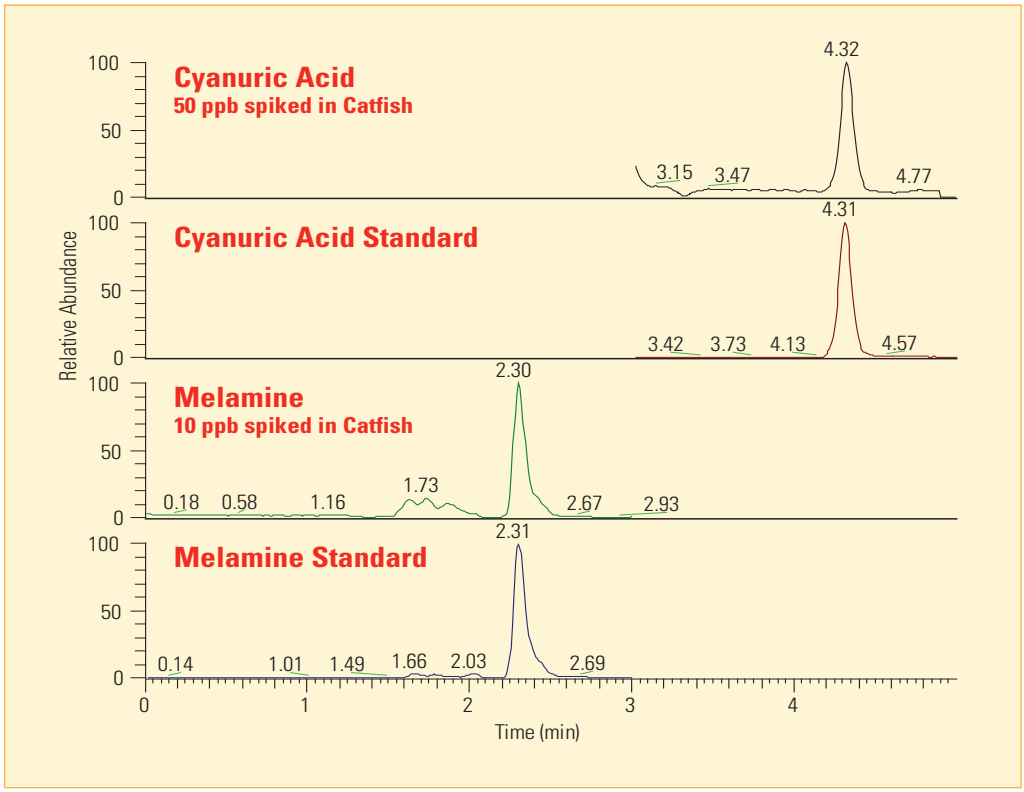

Figure 4: Chromatogram of cyanuric acid and melamine spiked into catfish matrix, at a level of 50 ppb for cyanuric acid, and 10 ppb for melamine

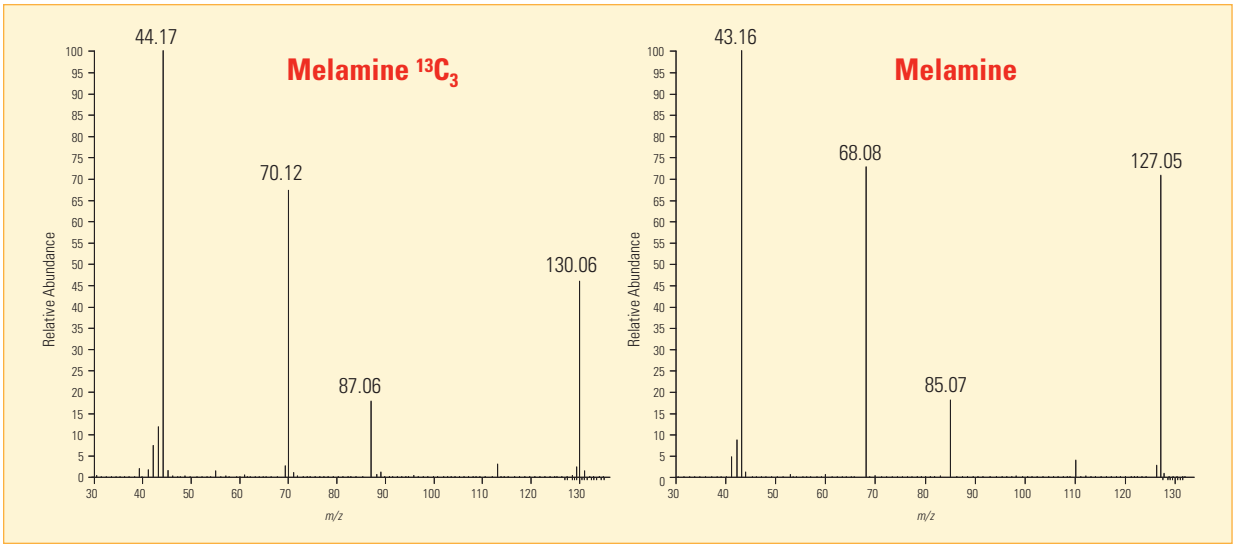

Figure 5: QED-MS/MS spectra for melamine $^{13}C_3$ (left) and melamine (right). Unique, rich, library-searchable spectra are collected in the same chromatographic run, allowing both quantitative and confirmatory full scan data in the same file.

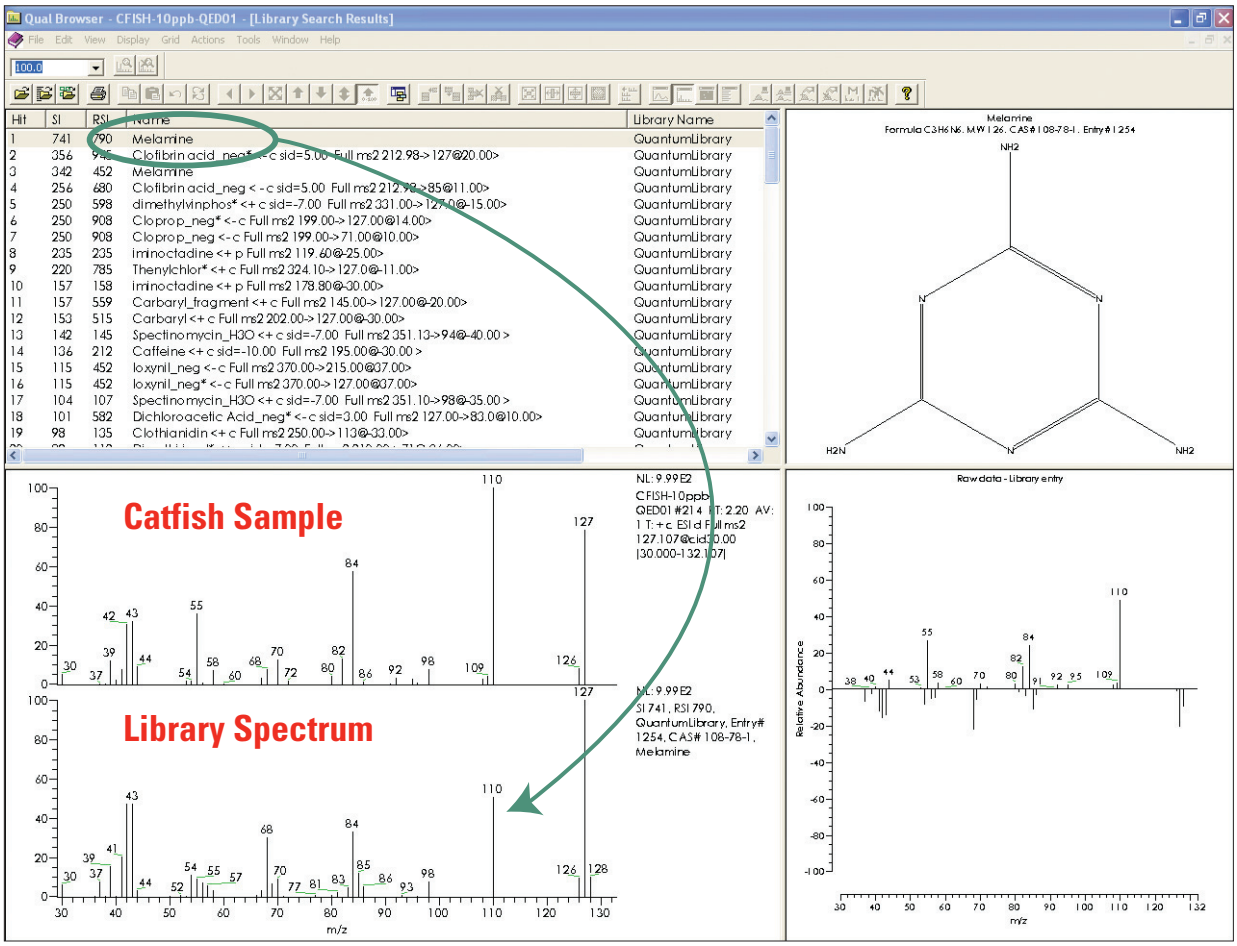

Figure 6: Library search results for melamine spiked at 10 ppb into a catfish matrix. Melamine is the top hit in the search list.

Conclusion

A simple, sensitive, and specific method for the detection and quantitation of melamine and cyanuric acid in food matrices has been demonstrated. The method is robust and allows for the analysis of a large number of samples, without degradation in column performance. Additionally, full scan spectra for Q3 are collected in the same chromatographic run using the QED-MS/MS scan function, permitting a library search of the results to eliminate any false positives.

References:

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