

A New, Fast and Sensitive LC-MS/MS Method for the Accurate Quantitation and Identification of Melamine and Cyanuric Acid in Pet Food Samples

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Overview

Recent issues with the determination of Melamine and Cyanuric Acid in wheat gluten imported from China and the subsequent animal deaths and recall of millions of pet food products have highlighted the need for both food manufacturers and regulatory agencies to utilize fast and accurate analytical techniques to proactively ensure product safety.¹

A fast and sensitive LC-MS/MS method was developed for the analysis of Melamine and Cyanuric Acid utilizing a simple extraction, with a run time of 10 minutes, and with limits of quantitation of Melamine and Cyanuric Acid below 1 µg/kg. In addition the method provides an extra degree of confidence through the use of Multiple Reaction Monitoring (MRM) ratios for compound identification.

Introduction

While GC-MS methods have been developed for the analysis of Melamine and Cyanuric Acid in wheat, rice and other gluten products, these methods require extensive sample clean-up with hazardous solvents and derivatization.² Additionally reported limits of detection are only in the mg/kg range.

In comparison to GC-MS the developed LC-MS/MS method has the following benefits:

- Reduced sample preparation and run time
- Superior quantitative results with less starting material
- Results with a higher degree of confidence
- The ability to analyze a wide range of contaminants



Experimental

Chemicals

Melamine (108-78-1) and Cyanuric Acid (108-80-5) standards were obtained from Sigma-Aldrich.

Extraction

Liquid-Liquid-Extraction (LLE) of pet food samples was performed using the following procedure:

- Add 5 mL of acetonitrile to 5 g of homogenized sample
- Mix thoroughly and let stand for 15-30 minutes
- Add 5 mL of saturated NaCl, mix and let stand for another 15-30 minutes
- Separate by centrifugation and inject the top layer

Further dilution of the extract with water/acetonitrile (50/50) might be necessary if the sample is heavily contaminated.

Liquid Chromatography

A Shimadzu Prominence LC system containing a CBM-20A system controller, two LC-20AD pumps, a semi-micro gradient mixer SUS-20A, and a SIL-20AC autosampler was used. Separation was performed on a GL Science Inertsil HILIC 5 μ m (150x3 mm) column at a temperature of 40°C with a mobile phase of (A) acetonitrile + 10 mM ammonium acetate and (B) water + 10 mM ammonium acetate at a flow of 0.5 mL/min. The gradient using normal phase conditions is listed in Table 1. An injection volume of 5 μ L was used.

Table 1. LC conditions for the analysis of Melamine and Cyanuric Acid on an Inertsil HILIC 5 μ m column

Time (min)	Flow (mL/min)	A (%)	B (%)
0.1	0.5	97	3
5.0	0.5	20	80
5.5	0.5	3	97
5.6	0.5	97	3
10.0	0.5	97	3

Mass Spectrometry

An AB SCIEX API 3200™ LC/MS/MS System equipped with Turbo V™ source and Electrospray Ionization (ESI) probe was used to detect both targeted analytes in Multiple Reaction Monitoring (MRM) mode. Precursor and product ions with corresponding Declustering Potentials (DP) and Collision Energies (CE) of the detection of Cyanuric Acid in negative polarity and Melamine in positive polarity are given in Table 2.

Table 2. MS/MS parameters of the detection of Melamine and Cyanuric Acid using an API 3200™ LC/MS/MS system

Compound	Q1 (amu)	Q3 (amu)	DP (V)	CE (V)
Cyanuric Acid	128	42	-30	-30
	128	85	-30	-13
Melamine	127	85	44	26
	127	68	44	48
	127	60	44	27

Results and Discussion

Melamine and Cyanuric Acid were separated using a normal phase gradient on a Hydrophilic Interaction Chromatography (HILIC) column. Periods were adjusted to allow detection in negative and positive polarity with highest sensitivity. An example chromatogram is given in Figure 1.

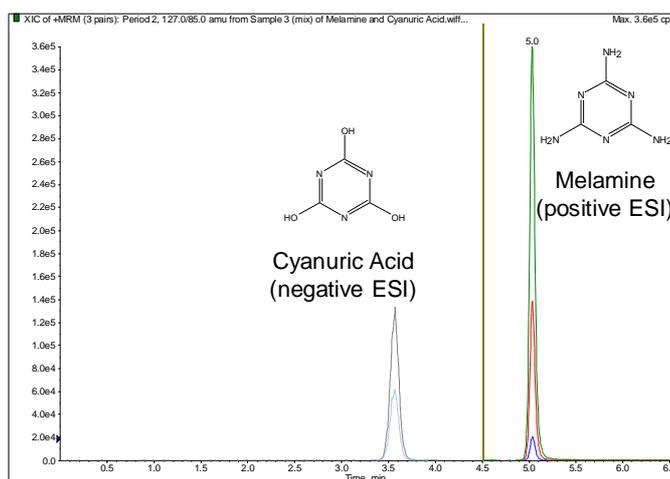


Figure 1. LC separation of Melamine and Cyanuric Acid with MS/MS detection in negative and positive Electro Spray Ionization (ESI)

Linearity and reproducibility of the developed method was studied by 5 replicate injections at each concentration level. The example calibration line of Melamine given in Figure 2 highlights the linear range of 3 orders of magnitude and the reproducibility and accuracy of this method. Limits of detection were found below 1 μ g/kg for Melamine and Cyanuric Acid.

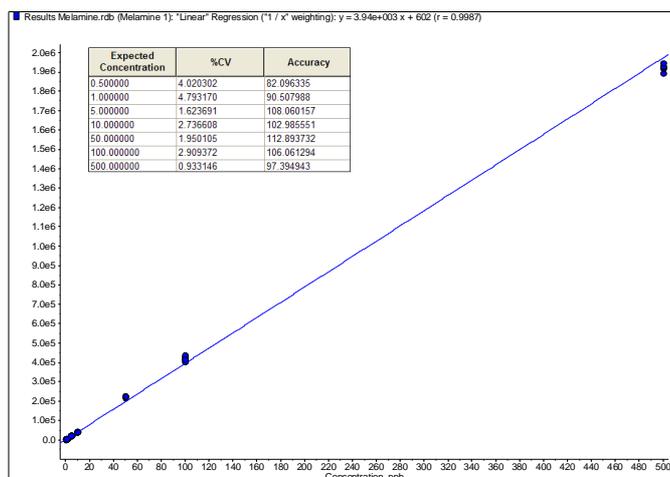


Figure 2. Calibration line of Melamine (MRM: 127/85) over a range of 0.5 to 500 ppb with coefficients of variation (%CV) and accuracy for each concentration (5 replicate injections)

Figure 3 shows a chromatogram with Signal-to-Noise ratios and detected MRM transitions used for identification.

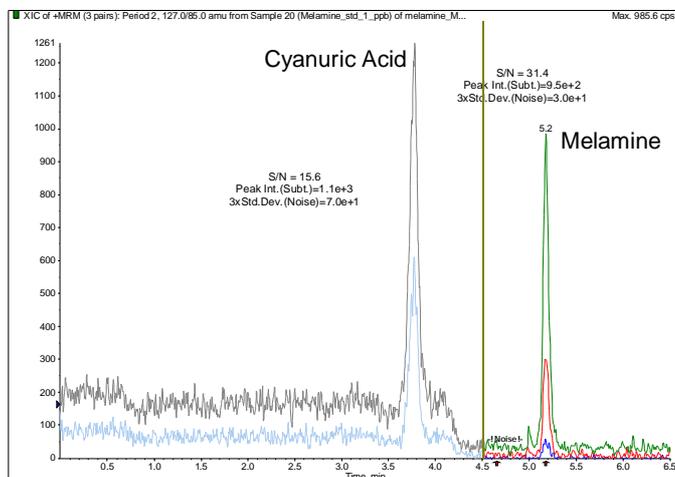


Figure 3. Signal-to-Noise and confirmatory MRM transition at a concentration of 1 ppb of Melamine and Cyanuric Acid

Several pet food samples were extracted and analyzed for both targeted analytes. Examples of a blank and a contaminated cat food sample are given in Figure 4.

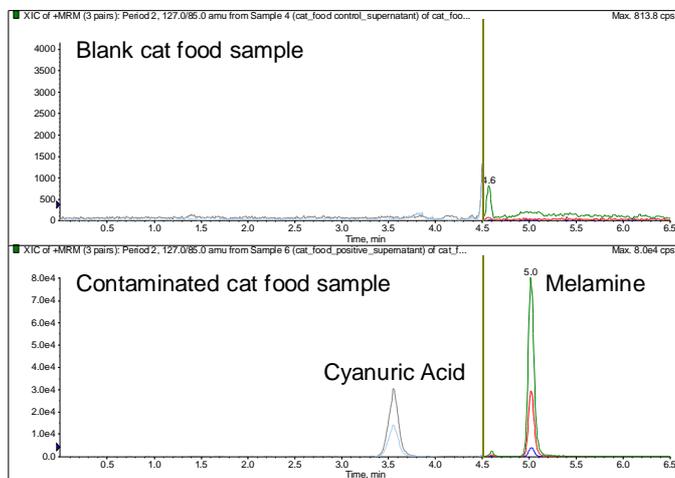


Figure 4. LC-MS/MS chromatograms of a blank and contaminated cat food sample

Cliquid® Software and iMethod™ Tests

The presented method is available as an iMethod™ Test and can be used stand-alone or integrated into Cliquid™ Software.

Cliquid™ Software provides a simple four step work flow, to perform the analysis and to automatically generate both

quantitative and confirmatory reports according to regulatory guidelines. Figure 5 shows a report with MRM ratios to identify the presence of Melamine and Cyanuric Acid in contaminated cat food samples.

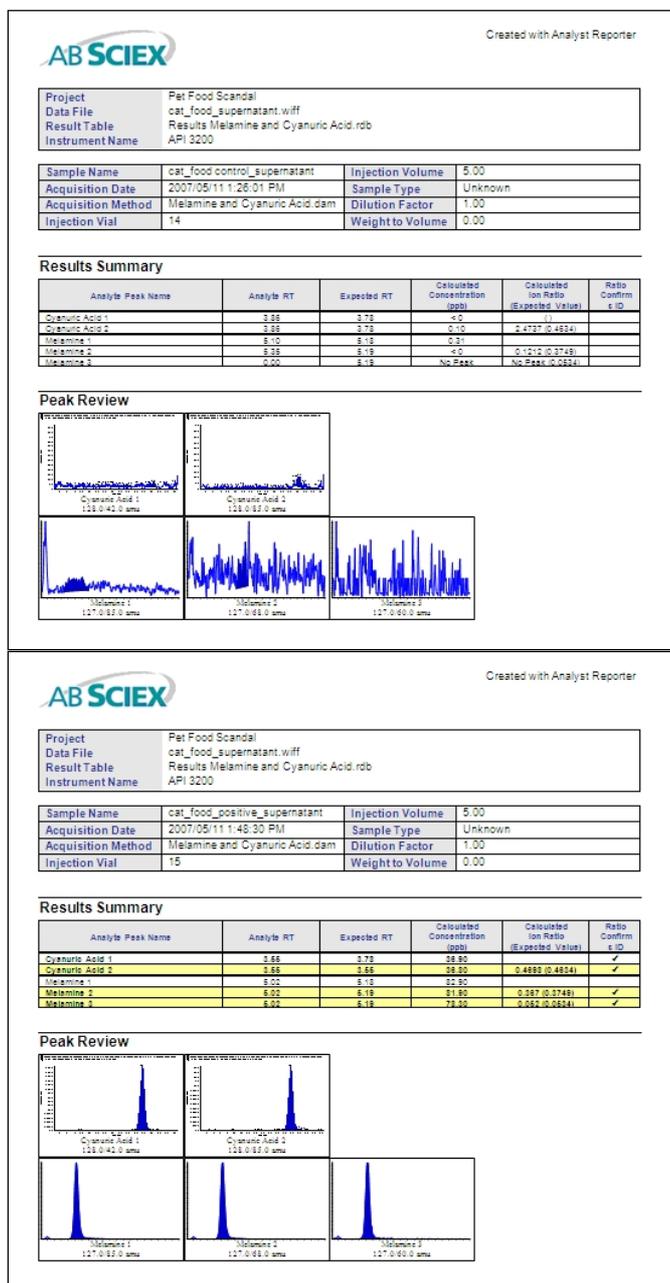


Figure 5. A report generated automatically by Cliquid™ Software with confirmatory results of Melamine and Cyanuric Acid findings in cat food samples

Summary

A fast and sensitive LC-MS/MS method was developed for the quantitation and identification of Melamine and Cyanuric Acid in pet food samples.

A simple extraction followed by LC separation on a HILIC column and MS/MS detection in negative and positive Electrospray Ionization using an API 3200™ LC/MS/MS System enables the quantitation of Melamine and Cyanuric Acid below 1 µg/kg. The method provides an extra degree of confidence through the use of Multiple Reaction Monitoring (MRM) ratios for compound identification.

Future investigation will include the optimization of recovery by adjusting pH during sample preparation and the use of isotopically labeled internal standards to correct for possible matrix effects during ionization.

Acknowledgements

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References

- ¹ http://en.wikipedia.org/wiki/2007_pet_food_crisis
- ² <http://www.fda.gov/cvm/MelaminePresence.htm>

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