

A Rapid iMethod™ Test for the Analysis of Melamine and Cyanuric Acid in Pet Food

iMethod™ Test Melamine and Cyanuric Acid Version 2.0 for Cliquant® Software

Recent issues with the presence of Melamine in wheat gluten and milk based ingredients from China have highlighted the need for both food manufacturers and regulatory agencies to utilize fast and accurate analytical techniques like LC/MS/MS to proactively ensure product safety.

The following fast and sensitive method was developed for the analysis of melamine and cyanuric acid in food using a simple liquid-liquid extraction with a run time of less than 10 minutes and limits of quantitation below 1 µg/kg. Two MRMs are used per compound with ion ratios calculated to provide an extra degree of confirmation.

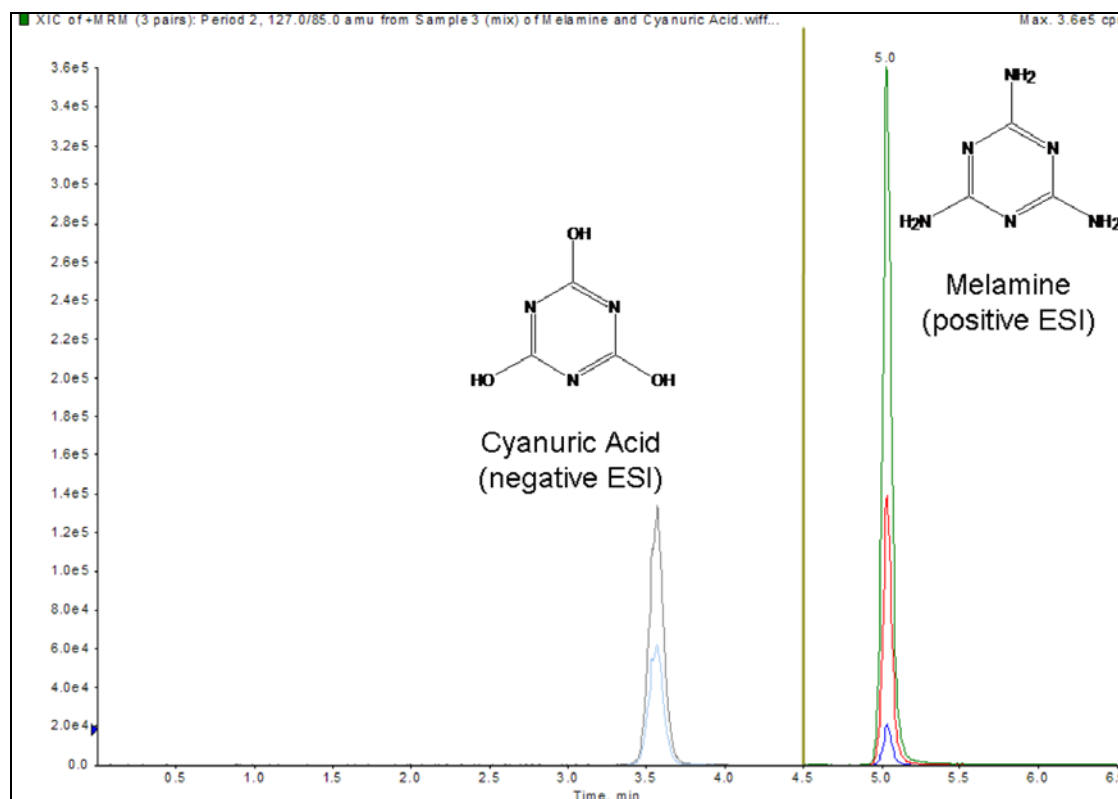


Figure 1: LC separation of melamine and cyanuric acid utilizing Electrospray Ionization with positive/negative switching.

Calibration

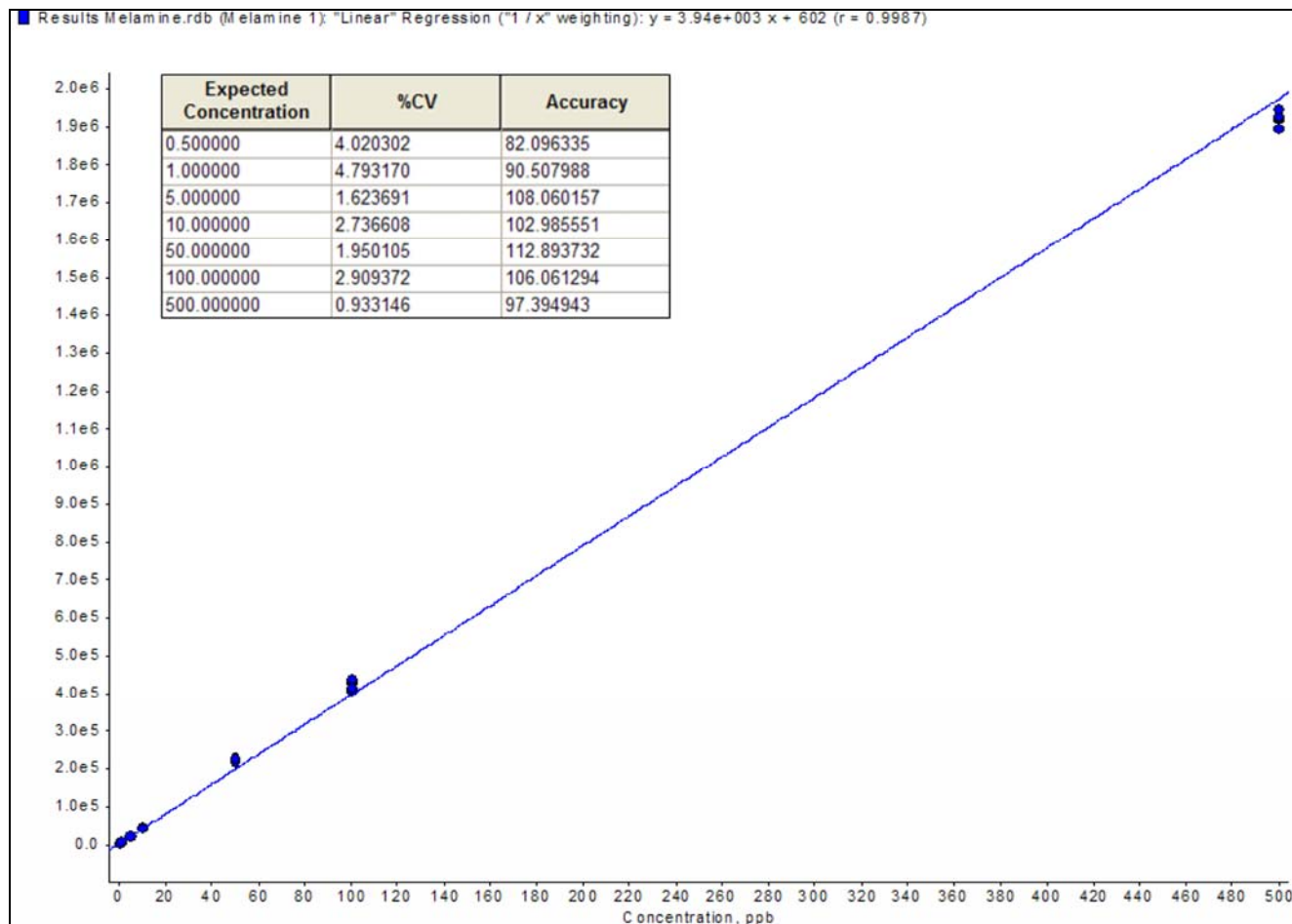


Figure 2. Calibration curve for melamine (MRM: 127 / 85) over a range of 0.5 to 500 ppb

Please note that the results presented above were obtained using a single instrument and single set of standards and samples. Prior to production use, the method should be fully validated with real samples, and the results here may not be typical for all instruments. Variations in LC column properties, chemicals, environment, instrument performance and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

Results

The developed extraction procedure was validated by spiking two types of pet food, biscuit and wet food, from different manufacturers at high and low concentration levels. These spiked samples were analyzed in triplicate to evaluate recovery and reproducibility. Recoveries were 116% for the spiked samples containing melamine and at concentrations of 20 and 200 mg/kg and 68% for cyanuric Acid.

Linearity and reproducibility was studied by performing 5 replicate injections at each concentration level. The example calibration curve given in Figure 2 highlights the linear dynamic range of 3 orders of magnitude achieved with a detection limit of less than 1 ppb for both melamine and cyanuric acid.



System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 Series (3200 QTRAP[®] or API 3200[™]) or 4000 Series (4000 QTRAP[®] or API 4000[™]) LC/MS/MS System
- A Shimadzu Prominence 20A LC System with reservoir tray and bottles, system controller CBM-20A, 100 µL mixer, 2 isocratic pumps LC-20AD, 3 channel degasser, autosampler SIL-20AC, column oven CTO-20AC or Agilent 1100/1200 LC system with binary pump G1312A (without static mixer), well plate auto sampler, and thermostated column oven.
- Melamine and cyanuric acid standards (www.sigmaaldrich.com)
- LC/MS grade water, ammonium acetate and acetonitrile
- A 250 mL conical flask
- 15 ml centrifuge tubes plus a centrifuge able to run at 5000 rpm
- A GL Science Inertsil HILIC 5 µm (150 x 3 mm) column
- Pipettes and standard laboratory glassware

Ordering Information

Product Name	Part Number
<i>iMethod™ Test for Melamine and Cyanuric Acid Version 2.0 for Cliquid® Software</i>	Free download at: http://www.absciex.com

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Analysis of Melamine and Cyanuric Acid, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site training are available to assist in the deployment of the iMethod™ Test and are recommended for inexperienced users. Please consult your local sales representative for more details.

Important Note

The iMethod™ Test described above has been designed by AB SCIEX to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It is up to the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst[®] Software may be required to do so.

The purchase and use of certain of the chemicals listed below may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements. AB SCIEX is not responsible for user's compliance with any statute or regulation, or for any permit or approval required for user to implement any iMethod procedure.

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