A Rapid iMethod™ Test for Analysis of Melamine and Cyanuric Acid in Milk

iMethod™ Test for Analysis of Melamine and Cyanuric Acid in Milk Version 1.0 for Cliquid® Software

The following description outlines the instrument requirements and expected results obtainable from the Phenomenex iMethod™ Test for melamine and cyanuric quantification in milk when using an AB SCIEX API 3200™ or 3200 QTRAP® instrument.

Sample preparation is based upon a simple dilution followed by protein precipitation, centrifugation and then clean up by solid phase extraction. More in depth sample preparation, and instrument parameter information is included as part of the standard operating procedure provided with the method.

Solvents, standards and any supplies required for sample preparation are not. The separation consists of the use of a methanol / acetonitrile / ammonium formate gradient with separation on a Phenomenex Luna 3u HILIC 200 Å Column, 100 x 2 mm Column. An example chromatogram of the separation achieved is shown in figure 1.

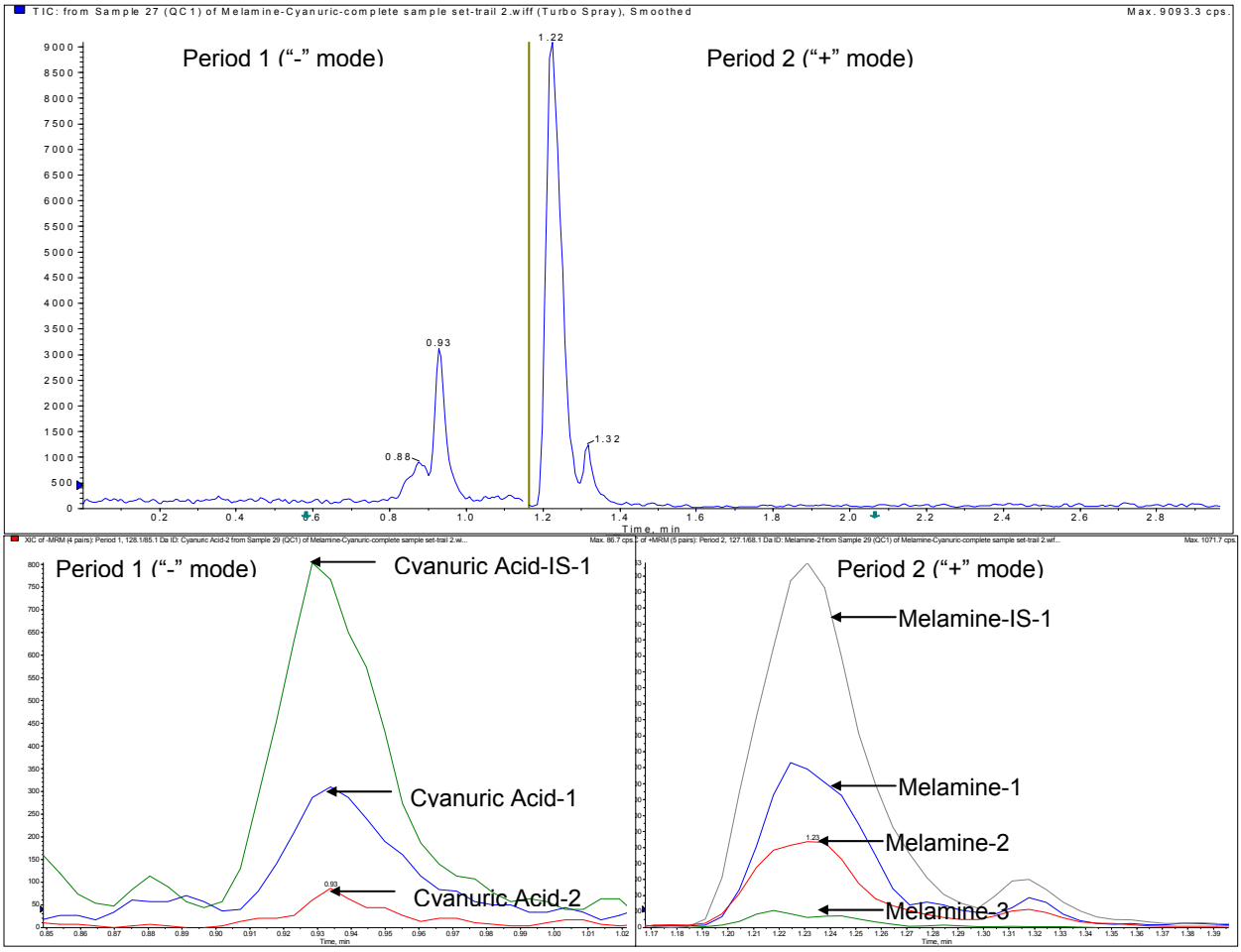


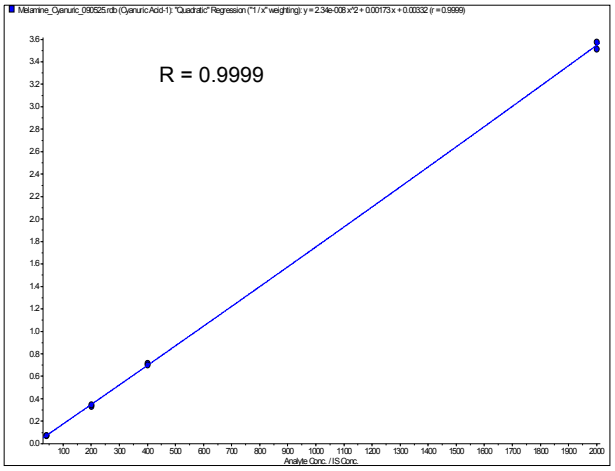
Figure 1: Chromatogram of a 200 ng/ml melamine and cyanuric acid standard run on a 3200 QTRAP® LC/MS/MS System

Results

This method was evaluated using both 4 non-extracted calibration standards with relevant concentration at 40, 200, 400 and 2000 ng/mL and 4 spiked calibration solution with relevant concentration at 40, 200, 400 and 2000 ng/mL. % CV and S/N values for the target analytes were obtained using 2 replicates at 200 ng/mL with 2 injections for each (n=4). With non-extracted calibration standards, accuracy is from 97 to 103% and % CV values are 0.3 to 3.1. With spiked calibration standards, accuracy is from 91 to 109% and % CV values are 3.4 to 19.2. The estimated detection limits for each analyte are more than sufficient to allow the analytical method to be used for either confirmation or quantitation.

The following chromatogram and calibration curves are representative of the performance obtained on the instrument using the method described here, and may not be representative of performance on any other instrument.

Cyanuric Acid



Melamine

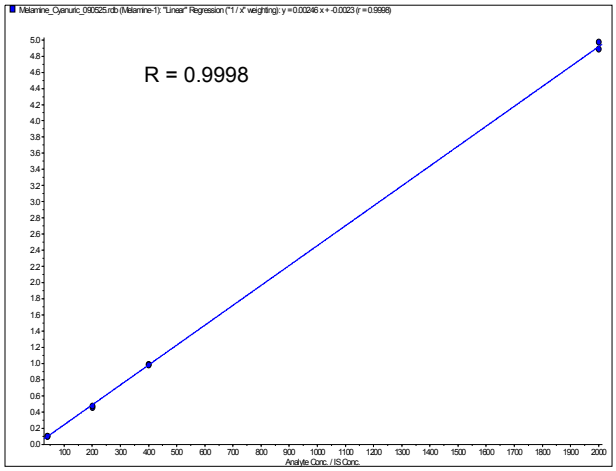


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Figure 2: Representative calibration curves for primary quantitation ions of melamine and cyanuric acid included in the method with a concentration range from 40- 2000 ng/ml. Calibration is performed in the Quadratic mode with 1/x weighting. Cyanuric acid is Quad and Melamine is Linear.

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.

Representative Signal-to-Noise Ratios at 200 ng/ml (Spiked Samples with SPE)

Analyte	S/N*	% CV	Recovery**
Cyanuric Acid	54.2	9.5	126.0
Melamine	57.0	9.3	98.5

* Signal-to-noise (S/N) is the peak height divided by the noise measured at three standard deviations of the noise.
** Recovery was obtained using non-extracted standard calibration curve.

Table 1: Recovery and % CV value of spiked sample with SPE at concentration 200 ng/ml, prepared with SPE on Strata Melamine cartridges, including the % CV estimates from 4 replicate samples, with 2 injections for each sample (n=8).

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™) 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
- Melamine and cyanuric acid (www.sigmaaldrich.com)
- LC/MS-Grade Water, Methanol, Acetonitrile, and Ammonium Formate
- A Phenomenex 3u HILIC 200 Å Column, 100 x 2 mm HPLC column
- A Phenomenex Strata Melamine (50u, 80A) cartridge, 200 mg/3 mL
- Pipettes and standard laboratory glassware

Please note that the Phenomenex HILIC HPLC column is required but not included with this iMethod™ Test. This method can also be run on other HPLC systems, given that they are supported for use by Cliquid® Software and the retention times are updated to reflect the configuration used.

Important Note

The purchase and use of certain of the chemicals listed above 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.

Legal Acknowledgements / Disclaimers

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 us 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.

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