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 Cliquid® 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](attachment:image.png)

**Figure 1:** LC separation of melamine and cyanuric acid utilizing Electrospray Ionization with positive/negative switching.
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 autosampler, 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 um (150 x 3 mm) column
- Pipettes and standard laboratory glassware

Ordering Information

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<thead>
<tr>
<th>Product Name</th>
<th>Part Number</th>
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<tbody>
<tr>
<td>iMethod™ Test for Melamine and Cyanuric Acid V2.0 for Cliquid® Software</td>
<td>Free download at: <a href="http://www.absciex.com">http://www.absciex.com</a></td>
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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 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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