Total Solution for Melamine Analysis by Agilent SPE, LC, GC/MS and Triple Quadrupole LC/MS
Agilent Solutions for Analyzing Melamine in Food

**Brodest portfolio of solutions** lets you select the protocol that meets your needs

- **GC/MS method**: rapid screening and confirmation
- **LC method**: routine quantitation
- **Triple Quadrupole LC/MS**: sensitive and selective for screening, confirmation, and quantitation
- **SampliQ SPE**: removes milk matrix interferences

**Agilent innovative technologies** for better lab productivity and faster results

- **Backflush** using innovative Capillary Flow Technology minimizes the requirement for sample preparation and shortens the GC cycle time up to five-fold
- **Fast data review** software for faster analysis time.
- High-sensitivity melamine analysis with **Triple Quadruopole LC/MS**
- **Broad column portfolio** to meet different analysis needs
Why Agilent for Melamine Analysis?

Agilent expertise in food safety:

- Experience with melamine analysis: supported US FDA and Chinese government to develop standard methods (China GB)
- Extensive collaborations with government and private food labs around the world
- Hundreds of publications by Agilent scientists

Reliable analytical systems for continuous lab operation:

- Proven reliability through a large installed base in food laboratories worldwide: private labs, government labs, and industry labs

Agilent support:

- Support to keep systems running at top performance and ensure uninterrupted lab operation
- Training courses to meet customers’ needs
- A large selection of columns for LC- and GC-based applications and SPE sample preparation for melamine analysis
Agilent Offers Solutions to Meet Different Needs

- **SPE Sample Preparation**: SampliQ SCX is used to remove complex sample interferences.
- **GC/MS**: J&W DB5-ms capillary column, TMS derivatization for sample screening and confirmation.
  
  *Optional backflush* based on Agilent’s Capillary Flow Technology can minimize the requirement of sample preparation and shorten the GC cycle time up to five-fold.
- **HPLC**: Reversed-phase Zorbax SB-C8 column in ion-pair mode for routine quantitation
  
  *Optional rapid resolution LC method*: RRHT column to significantly increase speed
  
  *Alternative Ion-Exchange LC method*: Zorbax 300SCX Ion-Exchange column to eliminate the need for ion-pair reagent. Easy to match with China GB LC-QQQ method for confirmation.
- **Triple Quadrupole LC/MS**: Zorbax Rx-Sil column is employed to run in hydrophilic interaction mode (HILIC) to better match with electrospray (ESI) LC/MS, provides extremely simple, sensitive, and selective testing for both melamine and cyanuric acid analysis.
  
  *Alternative Ion-Exchange* Zorbax 300SCX column can be used for the analysis of melamine in dairy products.
GC/MS Methods

GC/MS is used for sample screening and confirmation.

Improved method using Capillary Flow Technology to reduce run time and sample preparation.
Standard GC/MS Method

- Screening, confirmation, and quantitation in various matrices for the presence of melamine and related compounds
- Sample needs to be derivatized before injection into GC/MS
- Analysis time can be long due to sample matrix
GC/MS Sample Preparation Procedure

**Extraction**
- Weigh ~ 0.5 g sample
- Add 20 mL of methanol
- Sonicate for 30 minutes
- Centrifuge for 6 minutes
- Filter using 0.45 µm filter

**TMS-Derivatives**
- Transfer 40 µL filtrate to a GC vial
- Evaporate to dryness (70 °C, nitrogen)
- Add 50 µL pyridine, 50 µL BSTFA
- Incubate at 70 °C for 30 min
**GC/MS Experimental Conditions**

### GC Conditions
- **Inlet Temp EPC,** Split/Splitless @ 250 ºC
- **Injection Volume** 1 µL, Split 3:1
- **Carrier Gas** Helium, Constant Flow Mode, 1.3 mL/min
- **Oven Program** 75 ºC (1 min); 30 ºC /min to 300 ºC (1 min)
- **Post Run** 300 ºC hold 5 min
- **Transfer Line** 280 ºC

### MS Conditions
- **MS** EI, SIM/Scan
- **Solvent Delay** 4.2 min
- **MS Temp** 230 ºC (Source); 150 ºC (Quad)
- **Scan Mode** mass range (40-450 amu)
- **SIM Mode Ion** melamine: 342, 327*, 171, 99; cyanuric acid: 345*, 330, 188

### Ordering information:
- 7890A/5975C with MSD ChemStation E.01.00 or later
- Column: HP-5ms 19091S-433 30 m x 0.25 mm x 0.25 µm
- Or DB-5ms 122-5532 30 m x 0.25 mm x 0.25 µm
Chromatogram of GC/MS Method

Real Milk Powder Sample
Data Analysis: SIM/Scan for Simultaneous Confirmation and Quantitation with Backflush

Abundance

- Cyanuric Acid tri-TMS
- Melamine tri-TMS

Time→ Abundance

- Real Milk Powder Sample
Improved GC/MS Method with Backflush*

➢ Optional backflush to improve lab productivity

➢ Compliant with FDA, China GB method but faster

➢ Agilent reliable and easy-to-use Capillary Flow Technology based backflush can minimize the requirement of sample preparation and shorten the GC cycle time up to five-fold

➢ Requires additional hardware and method setup

*See detail in application note on www.agilent.com/chem/melamine
Backflush GC/MS Configuration

Ordering information:
Same as FDA, China GB GC/MS configuration **PLUS:**
Capillary Flow Technology 3-way splitter: P/N G3183B + Aux EPC: G1530-63309
Experimental Conditions

GC/MS conditions:
Same as standard GC/MS method

Backflush Conditions
Restrictor 0.71 m x 180 µm id
Outlet pressure 2 psi (60 psi for post-run)
Backflush duration 5 min

Easy tool to develop a backflush method using your MSD ChemStation
Using Backflush Can Reduce Run Time 5-fold

Without backflush and baking the column for more than 50 min at 300 °C

Blank run after backflush - successfully cleaned the column

Saving 55 min
Summary for GC/MS Methods

- Rapid screening and confirmation for melamine and related compounds.
- SIM/Scan can be used for simultaneous confirmation and quantitation.
- Backflush helped reduce run time from 75 min to 15 min.
- No need for SPE clean-up, but requires derivatization.
- Agilent’s improved method is fully compliant with FDA, China GB methods.
SPE Sample Preparation

Agilent’s newly introduced SampliQ SCX SPE removes complex sample matrix interferences from milk and related products.
**SPE Method for LC and LC/MS/MS**

**Step 1: Extraction**

- Milk, milk powder, yogurt & ice-cream
- Weigh 2 g into 50-mL tube
- Add 15 mL 5% TCA solution + 5 mL acetonitrile
- Sonicate for 10 min, vertically shake for 10 min, centrifuge at 4000 rpm for 10 min
- Filter the supernatant, bring to 25.0 mL with 5% TCA solution
- (5.0 mL of the above extract + 5.0 mL$^1$ water for SPE clean-up)

**Step 2: SPE Clean-up**

- **SampliQ SCX cartridge Condition:** using 3 mL methanol + 3 mL water
- **Load:** the above extract$^1$
- **Wash:** using 3 mL water + 3 mL methanol at <1 mL/min
  - Dry the cartridge by applying vacuum
- **Elute:** using 6 mL 5% ammonium hydroxide in methanol
- **Collect:** Evaporate to dryness under N$_2$ (50° C), reconstitute in 1.0 mL mobile phase and filter into a LC vial

**Ordering information:** SampliQ P/N: 5982-3236 (3 mL, 60mg)
HPLC Methods*

FDA, China GB standard reversed-phase method is used for routine quantitation

Optional Rapid Resolution LC (RRLC) method can significantly increase sample throughput

Agilent-developed Ion Exchange Chromatography (IEC) method is a better match with China GB LC-QQQ method for melamine analysis in dairy products

*See detail in application note on www.agilent.com/chem/melamine
Reversed-Phase LC (recommended by FDA, China GB)

- Routine quantitation method in various matrices for the presence of melamine
- US FDA reference method with detailed procedure
  http://www.fda.gov/cvm/melamine04022007.htm
- No need for derivatization, but requires SPE cleanup
Reversed-phase LC Conditions

Column: Zorbax SB-C8, 4.6 mm x 250 mm x 5 µm
Buffer: 10 mM citric acid, 10 mM sodium octane sulfonate, adjusted to pH 3.0
Mobile phase: 92:8 buffer: acetonitrile
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Column thermostat: 30°C
Detection wavelength: 240 nm
Run time: 20 min

Ordering information:
LC system: Any Agilent LC (1200 HPLC, 1200SL RRLC or 1120 Compact LC)
Column: Zorbax SB-C8 4.6 mm x 250 mm x 5 µm (P/N: 880975-906)
Reversed-phase LC: yogurt Sample

20 µg/mL Melamine

Contaminated yogurt sample after clean-up by SampliQ SCX SPE cartridge (PN: 5982-3267)
Optional Rapid Resolution LC (RRLC) Method

- Rapid routine quantitation in various matrices for the presence of melamine
- Total analysis time less than 6 min
- No need for derivatization, but requires SPE cleanup
RRLC Conditions

Column: Zorbax SB-C8 RRHT, 4.6 mm x 50 mm x 1.8 µm
Buffer: 10 mM citric acid, 10 mM sodium octane sulfonate, adjusted to pH 3.0
Mobile phase: 92:8 buffer: acetonitrile
Flow rate: 1.5 mL/min
Injection volume: 8 µL
Column thermostat: 30 °C
Detection wavelength: 240 nm
Run time: 6 min

Ordering information:
LC system: Agilent 1200 RRLC system
Column: Zorbax SB-C8 RRHT, 4.6 mm x 50 mm x 1.8 µm (P/N:827975-906)
Melamine, DAD1 A
Area = 17.7609855*Amt - 3.0706106


Correlation: 0.99996

Amount[wt%]
RRLC Result: Yogurt Sample

-same sample, more than 3 times faster

0.8 µg/mL Melamine

Contaminated yogurt sample after clean-up by SampliQ SCX SPE cartridge (PN: 5982-3267)
RRLC Result: Liquid Milk

Liquid milk, 0.3 mg/kg

Melamine

0.8 µg/mL Standard

Liquid milk spiked with 8 µg/mL melamine

Melamine
Alternative LC Method – Ion Exchange Chromatography (IEC)

- Fast, simple way for routine quantitation of melamine in milk and related products
- Compatible with China GB LC-QQQ method for confirmation
- Not an FDA or China GB standard LC method but a simple and faster method for the analysis of melamine in milk
- Less interference from milk matrix components
IEC Conditions

Column: ZORBAX 300SCX 4.6 x 150 mm, 5 µm
Buffer: 50 mM ammonium formate solution, adjust pH to 3.0 with formic acid
Mobile phase: 15:85 buffer: acetonitrile
Flow rate: 1.0 mL/min.
Injection volume: 10 µL
Column thermostat: 30 °C
Detection wavelength: 240 nm
Run time: 5.5 min

Ordering information:
LC system: Any Agilent LC (1200 HPLC, 1200SL RRLC, or 1120 Compact LC)
Column: ZORBAX 300SCX 4.6 mm x 150 mm x 5 µm (P/N 883952-704)
IEC Method Result

Melamine:
LOD 0.05 ug/mL, S/N=3.9
IEC – Milk Powder Sample

Note: no SPE clean-up was used for sample
HPLC Methods Summary

- LC methods are suitable for routine quantitation.
- SPE sample cleanup to eliminate matrix interferences.
- FDA, China GB Reversed-phase LC method is easy to set up with the most commonly used HPLC mode and column, but requires the use of an environmentally unfriendly ion-pairing reagent as buffer and longer retention time.
- Optional RRLC significantly improved the speed of analysis.
- Agilent-developed IEC method eliminates the need for the ion-pair reagent; it is simple, fast, more forgiven to matrix interferences and a better match with LC/MS/MS.
Triple Quadrupole LC/MS Methods*

Highly sensitive and selective method for screening, quantitation and confirmation

HILIC method is referenced by FDA research method for the analysis of melamine & cyanuric acid

Ion-exchange is referenced by China GB method for the analysis of melamine in dairy products

*See detail in application note on www.agilent.com/chem/melamine
Triple Quadrupole LC/MS with HILIC Chromatography

- Simple, sensitive, and selective for screening, confirmation, and quantitation
- No need for derivatization but requires SPE cleanup
- Simultaneously analyze melamine and cyanuric acid
## HPLC Parameters for Triple Quadrupole LC/MS

**HILIC Method**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HPLC system</strong></td>
<td>Agilent 1200 RRLC</td>
</tr>
<tr>
<td><strong>Column</strong></td>
<td>Agilent Zorbax-Rx Sil, 2.1 x 150 mm, 5 µm P/N 883700-901</td>
</tr>
<tr>
<td><strong>Injection Volume</strong></td>
<td>10 µL</td>
</tr>
<tr>
<td><strong>Temp</strong></td>
<td>40 °C</td>
</tr>
<tr>
<td><strong>Flow rate</strong></td>
<td>0.2 mL/min</td>
</tr>
<tr>
<td><strong>Mobile phase</strong></td>
<td>A - 5 mM Ammonium acetate@Water</td>
</tr>
<tr>
<td></td>
<td>B - 5 mM Ammonium acetate@ACN</td>
</tr>
<tr>
<td><strong>Isocratic</strong></td>
<td>95% B</td>
</tr>
</tbody>
</table>
# MS Parameters for Triple Quadrupole LC/MS

## HILIC Method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS system</td>
<td>Agilent LC/MS/MS QQQ</td>
</tr>
<tr>
<td>Ion source</td>
<td>ESI</td>
</tr>
<tr>
<td>Polarity</td>
<td>Positive and Negative</td>
</tr>
<tr>
<td>Nebulizer gas</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>Ion spray voltage</td>
<td>4000V</td>
</tr>
<tr>
<td>Source temperature</td>
<td>350 °C</td>
</tr>
<tr>
<td>Resolution</td>
<td>Q1 (unit) Q3 (unit)</td>
</tr>
<tr>
<td>Scan mode</td>
<td>Multiple Reaction Monitoring (MRM)</td>
</tr>
<tr>
<td>Segment</td>
<td>Segment 1= 0~4 min negative for cyanuric acid</td>
</tr>
<tr>
<td></td>
<td>Segment 2= 4~6 min positive for melamine</td>
</tr>
<tr>
<td>Delta EMV</td>
<td>600 V</td>
</tr>
</tbody>
</table>
## MRM Conditions

<table>
<thead>
<tr>
<th>Time</th>
<th>Compound</th>
<th>Precursor</th>
<th>Product</th>
<th>Dwell (ms)</th>
<th>Fragmentor (V)</th>
<th>Collision Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.9</td>
<td>Melamine</td>
<td>127</td>
<td>85</td>
<td>200</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>127</td>
<td>68</td>
<td>200</td>
<td>100</td>
<td>35</td>
</tr>
</tbody>
</table>
Triple Quadrupole LC/MS HILIC Method Result - Spiked 50 ppb

- **Corn Meal**:
  - +/- TIC MRM (** → **) corn ~50ppb water-1uL.d
  - Abundance vs. Acquisition Time (min)
  - Peak at 2.737

- **Wheat Gluten**:
  - +/- TIC MRM (** → **) Gluten ~50ppb water-1uL.d
  - Abundance vs. Acquisition Time (min)
  - Peak at 2.722

- **Rice Protein**:
  - +/- TIC MRM (** → **) rice ~50ppb water-1uL.d
  - Abundance vs. Acquisition Time (min)
  - Peak at 2.735

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Triple Quadrupole LC/MS with HILIC Chromatography - High Selectivity and Sensitivity

Noise (PeakToPeak) = 3.0; SNR (10.354 min) = 16.0

Melamine 50 ppt
S/N=16.0 (pk-pk)
Triple Quadrupole LC/MS — Ion-Exchange LC-MS

- Simple, sensitive, and selective for screening, confirmation, and quantitation of melamine in milk products
- No need for derivatization but requires SPE clean up
## Triple Quadrupole LC/MS Ion-Exchange LC Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC system</td>
<td>1200 LC system with binary pump</td>
</tr>
<tr>
<td>Column</td>
<td>Agilent Zorbax 300SCX, 2.1×150 mm, 5 µm</td>
</tr>
<tr>
<td></td>
<td>P/N: 883952-704</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.2 mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>40 °C</td>
</tr>
<tr>
<td>Mobile phase A</td>
<td>10 mM NH₄Ac/acetic acid pH adjusted to 3.0;</td>
</tr>
<tr>
<td>Mobile phase B</td>
<td>ACN</td>
</tr>
<tr>
<td></td>
<td>A:B=20:80</td>
</tr>
<tr>
<td>Run time</td>
<td>10 min</td>
</tr>
</tbody>
</table>
**Triple Quadrupole LC/MS Ion-Exchange MS Conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS system</td>
<td>Agilent 6410A LC/MS/MS</td>
</tr>
<tr>
<td>Ion source</td>
<td>ESI</td>
</tr>
<tr>
<td>Polarity</td>
<td>Positive</td>
</tr>
<tr>
<td>Nebulizer gas</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>Ion spray voltage</td>
<td>4000V</td>
</tr>
<tr>
<td>Dry gas temperature</td>
<td>350 °C</td>
</tr>
<tr>
<td>Dry gas flow rate</td>
<td>9 L/min</td>
</tr>
<tr>
<td>Nebulizer pressure</td>
<td>40 psi</td>
</tr>
<tr>
<td>Resolution</td>
<td>Q1 (unit) Q3 (unit)</td>
</tr>
<tr>
<td>Scan mode</td>
<td>Multiple Reaction Monitoring (MRM)</td>
</tr>
</tbody>
</table>
Triple Quadrupole LC/MS Ion-Exchange Result – Milk Powder

Melamine assay: 18.3 ppb
Triple Quadrupole LC/MS Ion-Exchange Result – Calibration Curve

LOQ: limit of quantitation (S/N>10) peak to peak 0.5 ppb
LOD: limit of detection (S/N>3) peak to peak 0.2 ppb
Triple Quadrupole LC/MS Summary

- Simple, sensitive, and selective for screening, confirmation, and quantitation.
- No need for derivatization but requires SPE cleanup.
- Zorbax Rx-Sil normal-phase column is employed to run in hydrophilic interaction mode (HILIC) to match with electrospray (ESI) LC/MS and simultaneously analyze melamine and cyanuric acid.
- Ion-exchange Zorbax 300SCX column-based method is a simple and fast equivalent to China GB method.
Total Solution for Melamine Analysis

- **SPE Sample Preparation:** to remove complex sample interferences.
- **GC/MS:** for sample screening and confirmation.
  
  *Optional backflush* to shorten the GC cycle time up to five-fold.
- **HPLC:** for routine quantitation
  
  *Optional RRLC method:* to significantly increase speed
  
  * Ion-Exchange LC method:* Fast, simple, robust and compatible with LC-QQQ
- **Triple Quadrupole LC/MS:** HILIC to better match with electrospray (ESI) LC/MS for both melamine and cyanuric acid analysis.
  
  *Ion-Exchange – MS* for the analysis of melamine in dairy products.

For More Information: