IC-MS Analysis of Haloacetic Acids

Jonathan Beck, PhD, Marketing Specialist for ICMS in Environmental and Food Safety

Terri Christison, Marketing Specialist
Ion Chromatography Sample Preparation

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Disinfection Byproducts in Drinking Water

- Disinfection treatment is essential to eliminate waterborne disease-causing microorganisms
- Ozonation – bromate
- Chlorination (chlorine or chloramine)
  - Chlorite, chlorate
  - Trihalomethanes (THM) and haloacetic acids (HAAs)
- Highly regulated due to associated health issues
  - Chlorite: nervous system, affects fetal development, anemia
  - Bromate: carcinogenic
  - Chlorate: produce gastritis, blood diseases, and acute renal failure.
  - THM & HAAs: chronic exposure could increase risk of cancer
- Regulated under Safe Drinking Water Act
- EPA promulgated to the states
Occurrence of Disinfectant Treatment Byproducts

Haloacetic acids are formed when chlorine or other disinfectants react with naturally occurring organic and inorganic matter in water.

- Trihalomethane: 20.1%
- Cyanogen Chloride: 1.0%
- Haloacetonitrile: 2.0%
- Chloral Hydrate: 1.5%
- Haloacetic Acids: 13.0%
- Unknown Halogenated Organics: 62.4%
# Haloacetic acids (HAA5 and HAA9)

<table>
<thead>
<tr>
<th>Acid</th>
<th>HAA</th>
<th>Formula</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monochloroacetic Acid</td>
<td>MCAA*</td>
<td>ClCH&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td>2.86</td>
<td>187.8</td>
</tr>
<tr>
<td>Dichloroacetic Acid</td>
<td>DCAA *</td>
<td>Cl&lt;sub&gt;2&lt;/sub&gt;CHCO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td>1.25</td>
<td>194</td>
</tr>
<tr>
<td>Trichloroacetic Acid</td>
<td>TCAA *</td>
<td>Cl&lt;sub&gt;3&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td>0.63</td>
<td>197.5</td>
</tr>
<tr>
<td>Monobromoacetic Acid</td>
<td>MBAA *</td>
<td>BrCH&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td>2.87</td>
<td>208</td>
</tr>
<tr>
<td>Dibromoacetic Acid</td>
<td>DBAA *</td>
<td>Br&lt;sub&gt;2&lt;/sub&gt;CHCO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td>1.47</td>
<td>195</td>
</tr>
<tr>
<td>Tribromoacetic Acid</td>
<td>TBAA**</td>
<td>Br&lt;sub&gt;3&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td>0.66</td>
<td>245</td>
</tr>
<tr>
<td>Bromochloroacetic Acid</td>
<td>BCAA**</td>
<td>BrClCHCO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td>1.39</td>
<td>193.5</td>
</tr>
<tr>
<td>Chlorodibromoacetic Acid</td>
<td>CDBAA**</td>
<td>Br&lt;sub&gt;2&lt;/sub&gt;ClCCO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td>1.09</td>
<td>NA</td>
</tr>
<tr>
<td>Bromodichloroacetic Acid</td>
<td>BDCAA**</td>
<td>Cl&lt;sub&gt;2&lt;/sub&gt;ClCCO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td>1.09</td>
<td>NA</td>
</tr>
</tbody>
</table>

*HAA5; **HAA9
Disinfectant Byproducts (DBPs) Regulation

- Total Trihalomethanes (TTHMs) in 1970s
- 1998 U.S. EPA Stage 1 Disinfectants/Disinfection Byproducts (D/DBP) Rule:
  - Seven new regulations, including HAA5 and bromate
  - Monitoring of HAA5 at all plants that disinfect with chlorine
  - Report total MCAA, MBAA, DCAA, DBAA, and TCAA
  - Maximum Contamination Level (MCL) = 0.060 mg/L annual average
  - MCL Goal (MCLG): DCAA should not be present; TCAA < 0.030 mg/L

- 2006 U.S. EPA Stage 2 D/DBP Rule: Reduced MCLG
  - Total HAA5 MCL < 0.060 mg/L
  - MCAA < 0.07 mg/L; TCAA < 0.02 mg/L
  - DCAA should not be present
## Summary of EPA Methods for HAAs (& Bromate, Dalapron)

<table>
<thead>
<tr>
<th>Technique</th>
<th>EPA Method</th>
<th>Thermo Scientific™ Dionex™ IonPac™ Columns</th>
<th>MDL (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Liquid/Liquid Extraction</td>
<td>552.2</td>
<td>GC-ECD</td>
<td>Mono: 0.13–0.20</td>
</tr>
<tr>
<td>2) Derivitization</td>
<td>552.3</td>
<td></td>
<td>Di: 0.02–0.08</td>
</tr>
<tr>
<td>3) GC-ECD</td>
<td></td>
<td></td>
<td>Tri: 0.03–0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kertronic AG24 pre-column + Kertronic AS24A separation column (2 mm i.d.)</td>
<td>Mono: 0.06–0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Di: 0.02–0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tri: 0.04–0.09</td>
</tr>
<tr>
<td>IC-MS, IC-MS/MS</td>
<td>557</td>
<td>Kertronic AG24 pre-column + Kertronic AS24A separation column (2 mm i.d.)</td>
<td>Mono: 0.17–0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Di: 0.06–0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kertronic AG26 pre-column + Kertronic AS26B separation column (4 mm i.d.)</td>
<td>Mono: 0.08–0.27</td>
</tr>
<tr>
<td>2D-IC Suppressed Cond.</td>
<td>Pending</td>
<td>Kertronic AG26 pre-column + Kertronic AS26B separation column (4 mm i.d.)</td>
<td>Mono: 0.08–0.27</td>
</tr>
<tr>
<td>(direct)</td>
<td>302.0,</td>
<td>Kertronic AG26 pre-column + Kertronic AS26B separation column (4 mm i.d.)</td>
<td>Mono: 0.08–0.27</td>
</tr>
<tr>
<td></td>
<td>314</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
U.S. EPA Method 557

Suppressed ion chromatography with MS or MS-MS detection

- Direct injection method with matrix diversion
- Eliminates liquid-liquid extraction and labor intensive derivatization
- Eliminates co-elution issues because MS is a selective detector
- MS/MS provides molecular information assuring confirmation of analyte
- Fully automated
- Recovery > 90%
A Complete Family of Ion Chromatography Systems

- Thermo Scientific Dionex ICS-1100 Basic Integrated Ion Chromatography System
- Thermo Scientific Dionex ICS-1600 Standard Integrated Ion Chromatography System
- Thermo Scientific™ Dionex™ ICS-2100 Reagent-Free™ Ion Chromatography (RFIC™) System
- Thermo Scientific™ Dionex™ ICS-4000 Capillary HPIC™ Ion Chromatography System
- Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ Ion Chromatography System

RFIC

HPIC
Ion Chromatography: Anion-Exchange Mechanism

Advantages of IC

- Direct method
  - No chemical derivitization
- Excellent selectivity
- High sensitivity
  - Low chemical noise
- Ideal for separations of small polar compounds
- Low cost
  - No solvent needed

IC provides needed sensitivity and selectivity
Hydroxide Eluent Generation for Anion Analysis

\[
\text{KOH} + \text{H}_2 \rightarrow \text{KOH}
\]

\[
2\text{H}_2\text{O} + 2\text{e}^- \rightarrow 2\text{OH}^- + \text{H}_2
\]

\[
\text{H}_2\text{O} \rightarrow 2\text{H}^+ + \frac{1}{2}\text{O}_2 + 2\text{e}^-
\]

\[
(\text{H}_2\text{O} \rightarrow 2\text{H}^+ + \frac{1}{2}\text{O}_2 + 2\text{e}^-)
\]

\[
\text{K}^+ \text{Electrolyte Reservoir}
\]

\[
\text{Pt cathode} \rightarrow 2\text{OH}^- + \text{H}_2
\]

\[
\text{EluGen KOH Cartridge}
\]

\[
\text{Pump}
\]

\[
\text{KOH Generation Chamber}
\]

\[
\text{Cation-exchange connector}
\]

\[
\text{Vent}
\]

\[
[KOH] \alpha \frac{\text{Current}}{\text{Flow rate}}
\]
Advantages of Suppressed Conductivity

Eluent (KOH)

Injection Valve

Sample F\(^-\), Cl\(^-\), SO\(_4^{2-}\)

Ion-Exchange Separation Column

KF, KCl, K\(_2\)SO\(_4\)
in KOH

Anion Electrolytically Regenerating Suppressor

HF, HCl, H\(_2\)SO\(_4\)
in H\(_2\)O

Without Suppression

\(\text{µS} \quad \text{µS} \quad \text{µS}\)

Counter ions

Time

With Suppression

\(\text{µS} \quad \text{µS} \quad \text{µS}\)

Time
Dionex ICS-5000+ HPIC IC System

Dionex ICS-5000+ HPIC

Highly Versatile Modular Design

- Dual Reagent-Free IC system
- Improved performance in sensitivity, noise reduction, stable, and ease of use
- Increased temperature control for HAA applications
- Supports smaller particle separation columns and all column formats
- Supports multiple detection techniques
IC Conditions

<table>
<thead>
<tr>
<th>Retention Time (min)</th>
<th>[KOH] mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>7.0</td>
</tr>
<tr>
<td>15.1</td>
<td>7.0</td>
</tr>
<tr>
<td>30.8</td>
<td>18.0</td>
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<tr>
<td>31</td>
<td>60</td>
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<tr>
<td>46</td>
<td>60</td>
</tr>
<tr>
<td>47</td>
<td>7.0</td>
</tr>
<tr>
<td>58</td>
<td>7.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column</th>
<th>Dionex IonPac AG24 (2×50mm), IonPac AS24 (2×250mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppressor</td>
<td>Dionex AERS 500, 2 mm</td>
</tr>
<tr>
<td>Column Temperature</td>
<td>15 °C</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>100 µL</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.3 mL/min KOH gradient, electrolytically generated</td>
</tr>
</tbody>
</table>
TSQ Quantiva and TSQ Endura Overview

**TSQ Endura**

Extreme Quantitative Value

- Best-in-class performance
- Unprecedented usability
- Exceptional robustness

**TSQ Quantiva**

Extreme Quantitative Performance

- Attogram sensitivity
- Unprecedented usability
- Exceptional robustness
**Experimental Details**

- Dionex ICS 5000+ HPIC coupled to a Thermo Scientific™ TSQ Endura™ MS
- Assay – Halo Acetic Acids
  - EPA method 557
  - Regulated compounds, disinfection byproducts
  - Calibration curve ranged from 0.25-20ppb
  - Who is interested in HAAs? “Anyone who drinks water!” – Richard Jack
- EPA regulates 5 HAAs*, but there are currently 9 total that are of interest. This analysis contains all 9.
  - MCAA Monochloro AA*
  - DCAA Dichloro AA*
  - TCAA Tricloro AA*
  - MBAA Monobromo AA*
  - DBAA Dibromo AA*
  - TBAA Tribromo AA
  - BCAA Bromochloro AA
  - DBCAA Dibromochloro AA
  - DCBAA Dichlorobromo AA

* HAAs: Halo Acetic Acids
# Mass Spectrometer Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Ion Source Polarity</td>
<td>Negative Ion Mode</td>
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<tr>
<td>Spray Voltage</td>
<td>3200 V</td>
</tr>
<tr>
<td>Vaporizer Gas Pressure</td>
<td>45 units N₂</td>
</tr>
<tr>
<td>Auxiliary Gas Pressure</td>
<td>10 units N₂</td>
</tr>
<tr>
<td>Capillary Temperature</td>
<td>200 C</td>
</tr>
<tr>
<td>Vaporizer Temperature</td>
<td>200 C</td>
</tr>
<tr>
<td>Collision Gas Pressure</td>
<td>1.5 mTorr Argon</td>
</tr>
<tr>
<td>Ion Cycle Time</td>
<td>0.5 seconds</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Q1 (m/z)</th>
<th>Q3 (m/z)</th>
<th>RF lens (V)</th>
<th>CE (V)</th>
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</thead>
<tbody>
<tr>
<td>MCAA</td>
<td>92.9</td>
<td>35.0</td>
<td>67</td>
<td>10</td>
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<tr>
<td>MBAA</td>
<td>136.9</td>
<td>79.0</td>
<td>60</td>
<td>13</td>
</tr>
<tr>
<td>DCAA</td>
<td>126.9</td>
<td>82.9</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>DBAA</td>
<td>216.8</td>
<td>172.8</td>
<td>72</td>
<td>12</td>
</tr>
<tr>
<td>BCAA</td>
<td>172.9</td>
<td>128.9</td>
<td>70</td>
<td>11</td>
</tr>
<tr>
<td>TCAA</td>
<td>160.9</td>
<td>116.9</td>
<td>45</td>
<td>8</td>
</tr>
<tr>
<td>BDCAA</td>
<td>162.9</td>
<td>81.0</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>DBCAA</td>
<td>206.9</td>
<td>81.0</td>
<td>90</td>
<td>16</td>
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<tr>
<td>TBAA</td>
<td>252.8</td>
<td>81.0</td>
<td>70</td>
<td>17</td>
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<tr>
<td>Dalapon</td>
<td>140.9</td>
<td>96.8</td>
<td>56</td>
<td>7</td>
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<tr>
<td>Bromate</td>
<td>126.9</td>
<td>110.9</td>
<td>90</td>
<td>22</td>
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<tr>
<td>MCAA-ISTD</td>
<td>94.0</td>
<td>35.0</td>
<td>67</td>
<td>10</td>
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<tr>
<td>MBAA-ISTD</td>
<td>138.0</td>
<td>79.0</td>
<td>60</td>
<td>13</td>
</tr>
<tr>
<td>DCAA-ISTD</td>
<td>128.0</td>
<td>84.0</td>
<td>70</td>
<td>10</td>
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<tr>
<td>TCAA-ISTD</td>
<td>162.0</td>
<td>118</td>
<td>45</td>
<td>8</td>
</tr>
</tbody>
</table>
IC-MS Flow Diagram

High-Pressure Non-Metallic Pump

Eluent Generator (OH⁻ or H⁺)

CR-TC

Sample Inject (Autosampler)

Waste

Separation Column AS 24

Electrolytic Eluent Suppressor

Conductivity Detector

Makeup Pump (ACN or IPA)

Data Management

0.31 µS

TSQ Endura MS
1 ppb HAA standard, mixture of 9 HAAs

- **MCAA**
- **MBAA**
- **DCAA**
- **BCAA**
- **DBAA**
- **TCAA**
- **BDCAA**
- **BDCAA**
- **DBCBA**
- **TBAA**
LSSM of HAA, Dalapon and Bromate 20ppb spike

Bromate
Dalapon
DCAA
BCAA
DBAA
TCAA
DCBAA
DBCAA
TBAA
Overlaid Chromatograms with Divert Windows

1. MCAA
2. MBAA
3. Bromate
4. Dalapon
5. DCAA
6. BCAA

7. DBAA
8. TCAA
9. DCBAA
10. DBCAA
11. TBAA
Divert to waste, eliminates salts from matrix

\[ \text{Cl}^- \quad \text{CO}_3^{2-} \quad \text{SO}_4^{2-} \quad \text{NO}_3^- \]
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Calculated MDL (ppb)</th>
<th>EPA Method 557 MDL (ppb)</th>
<th>EPA Method 552.2 MDL (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCAA</td>
<td>0.105</td>
<td>0.20</td>
<td>0.273</td>
</tr>
<tr>
<td>MBAA</td>
<td>0.104</td>
<td>0.064</td>
<td>0.204</td>
</tr>
<tr>
<td>DCAA</td>
<td>0.044</td>
<td>0.055</td>
<td>0.242</td>
</tr>
<tr>
<td>DBAA</td>
<td>0.021</td>
<td>0.015</td>
<td>0.066</td>
</tr>
<tr>
<td>BCAA</td>
<td>0.059</td>
<td>0.11</td>
<td>0.251</td>
</tr>
<tr>
<td>TCAA</td>
<td>0.033</td>
<td>0.090</td>
<td>0.079</td>
</tr>
<tr>
<td>BDCAA</td>
<td>0.141</td>
<td>0.050</td>
<td>0.091</td>
</tr>
<tr>
<td>DBCAA</td>
<td>0.214</td>
<td>0.041</td>
<td>0.468</td>
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<tr>
<td>TBAA</td>
<td>0.159</td>
<td>0.067</td>
<td>0.820</td>
</tr>
<tr>
<td>Dalapon</td>
<td>0.050</td>
<td>0.038</td>
<td>N/A</td>
</tr>
<tr>
<td>Bromate</td>
<td>0.059</td>
<td>0.020</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Conclusions

• Demonstrated the analysis of 9 HAAs, Bromate and Dalapon using IC coupled to MS/MS
• Detection limits exceed the requirements of the EPA method
• No derivitization steps required prior to analysis
• Low chemical noise with suppressor to increase sensitivity, eliminate ion suppression, and enable compatibility with MS
• Ion Chromatography offers excellent separations and selectivity for HAAs
• Dionex ICS-5000+ offers temperature control which is critical for this method
• No sample prep, besides addition of internal standards for analysis
• MS/MS offers specificity and sensitivity over single quadrupole methods
• TSQ Endura offers excellent performance at an attractive price point
Future Application Plans for ICMS

- Polar Pesticides in Drinking Water and Food
  - AMPA, Glyphosate, Phosphonic acid, Perchlorate, Fosetyl-Al, Maleic hydrazide, HEPA, Ethephon, MPPA, Glufosinate, N-acetyl-glufosinate

- Perchlorate in Drinking Water

- Additional Disinfection byproducts including HAAs of Iodine
Targeted Quantitation: EPA 543 on TSQ Quantiva MS and EQuan MAX Plus
Current Title: 
*Determination of Selected Organic Chemicals in Drinking Water by On-Line Solid Phase Extraction-Liquid Chromatography / Tandem Mass Spectrometry (On-line SPE-LC/MS/MS)*

- First EPA promulgated method using Online SPE (Thermo Scientific™ EQuan MAX Plus system)
- Online SPE Analog of EPA 540
- Currently in Draft Form Status, scheduled release Sept 2014
- Thermo has been testing the method in conjunction with the US EPA Office of R&D, National Exposure Research Laboratory – **Jody Shoemaker – EPA Cincinnati, OH.**

- Analytes:
  - 3-Hydroxycarbofuran
  - Bensulide
  - Fenamiphos
  - Fenamiphos sulfone
  - Fenamiphos sulfoxide
  - Tebuconazole
  - Tebufenozide
EPA 543 Method

- EQuan MAX Plus system with TSQ Quantiva MS
- Load Column – Waters Oasis HLB* (Alternative Thermo Scientific column being tested)
- LC Column – aQ 100 x 2.1mm 2.6 mm
- 2mL injection volume
- Drinking water matrix

*Required by the EPA method.
Environmental Analysis (Water)

EQuan MAX Plus
For targeted quantitation (TSQ)
or
Targeted/non-targeted screening and quantitation using High Resolution Accurate Mass (Orbitrap platform)
EQuan MAX Plus: What is it?

• Turnkey method for assaying environmental water samples (pesticides, antibiotics, etc.) at low ppt levels
  • On-line sample clean-up and preconcentration
    • 2 Columns: Loading and Analytical
    • 2 pumps
  • High injection volumes
    • 1mL-20mL
  • Standard injection volumes
    • 1-100 uL
EQuan MAX Plus: Targeted Quantitation

- Couple EQuan MAX Plus with any TSQ Quantum from Thermo Scientific for the most sensitive and selective experiments.

- **Fast Positive Negative Switching**
  - 25ms

- **TraceFinder Software**
  - Built in SRM parameters
  - Built in EQuan Methods

- TSQ Quantum Access MAX
- TSQ Endura
- TSQ Quantiva
EQuan MAX Plus: Non-targeted screening and Quantitation

- Couple EQuan MAX Plus with the Exactive Orbitrap instruments (Exactive Plus or Q-Exactive Plus).
- High Resolution Accurate Mass (HRAM)
- All ions are collected in every experiment.
- Re-interrogate your data at a later time
- Quantitation and screening methods are easy to set up since there are no compound dependant parameters to optimize.
# SPE - standard enrichment procedure

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Sample preparation:</strong> 1L sample filtration internal Std.</td>
</tr>
<tr>
<td>2</td>
<td><strong>Conditioning:</strong> MeOH / H₂O</td>
</tr>
<tr>
<td>3</td>
<td><strong>Extraction:</strong> 60mg Oasis</td>
</tr>
<tr>
<td>4</td>
<td><strong>Washing:</strong> H₂O/MeOH (95/5)</td>
</tr>
<tr>
<td>5</td>
<td><strong>Drying:</strong> air</td>
</tr>
<tr>
<td>6</td>
<td><strong>Elution:</strong> MeOH</td>
</tr>
<tr>
<td>7</td>
<td><strong>Detection:</strong> LC-MSMS</td>
</tr>
</tbody>
</table>

40 samples: ~ 5h  
~ 2d
Calibration Curve for 3-Hydroxycarbofuran

Calibration Range = 0.5-200 ng/L
### EPA 543 Detection Limits and Chromatogram

<table>
<thead>
<tr>
<th>Compound</th>
<th>LCMRL (ng/L)</th>
<th>Detection Limit (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxycarbofuran</td>
<td>&lt; 0.5</td>
<td>0.31</td>
</tr>
<tr>
<td>Bensulide</td>
<td>2.1</td>
<td>0.71</td>
</tr>
<tr>
<td>Fenamiphos</td>
<td>&lt;0.2</td>
<td>0.061</td>
</tr>
<tr>
<td>Fenamiphos sulfone</td>
<td>&lt;0.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Fenamiphos sulfoxide</td>
<td>&lt;0.5</td>
<td>0.10</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>0.46</td>
<td>0.41</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>14</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Mid Level Calibrator, 6 or 15 ng/L**

![Chromatogram Image]
Thank you!