EPA Analytical Methods for Cyanotoxins

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William A. Adams, Ph.D.

Office of Ground Water and Drinking Water Standards and Risk Management Division
Technical Support Center
Cincinnati, OH
Overview

- Method development
- EPA methods used for cyanotoxin analysis
- Comparing techniques
General Method Development

Target analyte selection

Storage Stability Study
- Tracks target analyte concentrations in preserved tap water for 5 weeks

Instrument Optimization
- Based on scientific literature and preliminary experiments
- Instrument: Analytical column, eluent, temperature programs, flow, injection volume, assays
- Detectors: Target analyte MS tuning, detector settings, probes

System Background – Laboratory Reagent Blank (LRB)

Precision and Accuracy Measurements
- Accuracy: Low: 50–150% Mid/High: 70–130%
- Precision: Low: ≤30% Mid/High: ≤20%
- Analyzed in three matrixes

LCMRL Calculation – Lowest Concentration Minimum Reporting Level
- The lowest true concentration for which the future recovery is predicted to fall between 50% to 150% with 99% confidence

Multi-Laboratory Demonstration
- At least two outside laboratories

Submitted for EPA clearance
# Microcystins DW Methods Overview

<table>
<thead>
<tr>
<th>Summary Options</th>
<th>ELISA-Field (Tube/Strips)</th>
<th>ELISA-Lab</th>
<th>LC-MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scope</td>
<td>“Total Microcystins and Nodularins”</td>
<td>“Total Microcystins and Nodularins” (EPA Method 546)</td>
<td>6 Specific Microcystin Congeners and Nodularin-R (EPA Method 544)</td>
</tr>
<tr>
<td>Approx. Limit of Quantification (LOQ)</td>
<td>~0.5 – 1 ug/L</td>
<td>~ 0.3 µg/L</td>
<td>~ 0.02 µg/L</td>
</tr>
<tr>
<td>Time to Result</td>
<td>10 – 60 minutes</td>
<td>1 – 4 hours</td>
<td>&lt; one day</td>
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# Cylindrospermopsin and Anatoxin-a DW Methods Overview

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<th>Summary Options</th>
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<tr>
<td>Scope</td>
<td>Cylindrospermopsin and Anatoxin-a</td>
<td>Cylindrospermopsin and Anatoxin-a</td>
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<tr>
<td>Approx. Limit of Quantification (LOQ)</td>
<td>~ 0.3 and 1.0 µg/L</td>
<td>~ 0.06 and 0.02 µg/L</td>
</tr>
<tr>
<td>Time to Result</td>
<td>1 – 4 hours</td>
<td>&lt; one day</td>
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</table>
LC-MS/MS

- EPA finished water methods
  - EPA Method 544 – six selected microcystins and nodularin-R
  - EPA Method 545 – cylindrospermopsin and anatoxin-a
LC-MS/MS

• EPA ambient water methods
  • Single Laboratory Validated Method for Determination of Cylindrospermopsin and Anatoxin-a in Ambient Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) (Nov 2017, EPA 600-R-17-130)
  • Single Laboratory Validated Method Determination of Microcystins and Nodularin in Ambient Freshwaters by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) (Nov 2017, EPA 600-R-17-344)
    • thirteen selected microcystins and nodularin-R
## LC-MS/MS EPA Method 544
*(Selected Microcystins and Nodularin-R)*

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<th>Parameter</th>
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<tbody>
<tr>
<td>Reporting Limit</td>
<td>0.0029–0.022 µg/L (LCMRL)</td>
<td>Sample Preparation</td>
<td>Cell lysing, SPE, concentration</td>
</tr>
<tr>
<td>Sample Collection</td>
<td>500 mL in glass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preservation</td>
<td>Refrigerated samples, frozen extracts, Trizma buffer, ascorbic acid dechlorination, 2-chloroacetamide microbial inhibition, EDTA, 28-day extract and sample hold time</td>
<td>Quality Control</td>
<td>LRB, precision and accuracy demonstrations, MRL confirmation, QCS, calibration checks, surrogate standard, laboratory fortified blank, laboratory fortified sample matrix and duplicate, field duplicate</td>
</tr>
</tbody>
</table>

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\(^1\)EPA Method 544: *Determination of microcystins and nodularin in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS)*; EPA Document No. 600-R-14-474; U.S. Environmental Protection Agency, ORD/NERL: Cincinnati, OH, 2015.
### LC-MS/MS EPA Method 545
(Cylindrospermopsin and Anatoxin-a)\(^1\)

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<tr>
<td>Reporting Limit</td>
<td>0.063 and 0.018 µg/L (LCMRL)</td>
<td>Sample Preparation</td>
<td>Cell lysing, filtration</td>
</tr>
<tr>
<td>Sample Collection</td>
<td>At least 10 mL in glass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preservation</td>
<td>Refrigerated, ascorbic acid dechlorination, sodium bisulfate microbial inhibition, 28-day hold time</td>
<td>Quality Control</td>
<td>LRB, precision and accuracy demonstrations, MRL confirmation, QCS, calibration checks, internal standards, laboratory fortified sample matrix and duplicate, field duplicate</td>
</tr>
</tbody>
</table>

\(^1\)EPA Method 545: Determination of cylindrospermopsin and anatoxin-a in drinking water by liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS); EPA Document No. 815-R-15-009; U.S. Environmental Protection Agency, OW/OGWDW/SRMD/TSC: Cincinnati, OH, 2015.
LC-MS/MS Chromatograms

EPA Method 544

- Nodularin
- MC-RR
- MC-YR
- MC-LA
- MC-LR
- MC-LY
- MC-LF
- C$_2$D$_5$-MC-LR (SUR)

EPA Method 545

- L-phenylalanine-$d_5$
- Uracil-$d_4$
- CYN
- ANA
Enzyme-Linked Immunosorbent Assay (ELISA)

• ELISA is commonly used to detect cyanotoxins
  • Separate assays are used to detect individual or groups of cyanotoxins
• Adda-ELISA results quantify “total microcystins and nodularins”
  • Based on the Adda portion of the molecules
• Calibration curve based on four-parameter logistic function (sigmoidal curve)
Adda-ELISA EPA Method 546  
(Total Microcystins and Nodularins)\(^1\)

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<tr>
<td>Reporting Limit</td>
<td>0.26 µg/L (MC-LR, LCMRL)</td>
<td>Sample Preparation</td>
<td>Cell lysing, filtration</td>
</tr>
<tr>
<td>Sample Collection</td>
<td>&lt;100 mL in glass or PTEG</td>
<td></td>
<td>LRB, precision and accuracy demonstrations, MRL confirmation, QCS, calibration verification, laboratory fortified sample matrix and duplicate</td>
</tr>
<tr>
<td>Preservation</td>
<td>Refrigerated then frozen, sodium thiosulfate dechlorination, 14-day hold time</td>
<td>Quality Control</td>
<td></td>
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</table>

ELISA Calibration Curve

- 80% response
- 60% response
- 50% response
- 40% response
- 20% response

Abs vs. conc MC (ng/mL)
# Microcystin Analytical Comparisons

<table>
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<tr>
<th>Analysis</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
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</table>
| EPA Method 544 -or- Other Microcystin LC-MS/MS Analyses | • Sensitive  
• Speciates microcystins                                  | • Standards not available for all microcystin congeners (limited target analyte list)  
• Instrument limitations considering number of congeners    |
| EPA Method 546 ADDA-ELISA                           | • Cost effective  
• Provides “total” concentration (single number)  
• Faster turnaround for results                         | • Does not speciate microcystins  
• Non-typical calibration  
• Technique is important                             |
Method 544 and Method 546 Results

• Method results may differ from each other
  • M544 was developed and validated to detect only six microcystin congeners and nodularin-R
  • M546 was developed and validated to detect the Adda portion of microcystins and nodularins with varying degrees of assay recognition (cross-reactivities) using MC-LR as the calibration standard

• It is important to understand what is being measured by each technique for proper application
Conclusions

• EPA cyanotoxin methods underwent rigorous method development processes

• Several methods are available for the analysis of various cyanotoxins

• EPA Methods meet typical DW method validation and performance acceptance criteria

• Results are dependent on the analysis being used
Questions?

ada.ms.william@epa.gov

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