Development of U. S. EPA Method 559 for the Determination of Nonylphenol and 4-tert-Octylphenol in Drinking Water by Solid Phase Extraction and LC/MS/MS

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The 1996 amendments to the SDWA required EPA to establish a Drinking Water Contaminant Candidate List (CCL)

- Must decide to regulate or not regulate a minimum of 5 CCL contaminants every 5 years

- The first CCL was published in 1998 - updated every 5 years

- The Final CCL 4 was published in November 2016 and contains 97 chemicals or chemical groups and 12 microbial contaminants
UCMR Background

◆ Nationwide occurrence data needed on CCL chemicals

◆ EPA’s OGWDW collects occurrence data under the Unregulated Contaminant Monitoring Regulation (UCMR)

◆ EPA methods for drinking water contaminants are needed for UCMR which meet our drinking water method development goals

◆ Conduct monitoring for up to 30 unregulated contaminants in a 5 year UCMR cycle
  ✔ likely to occur in drinking water
  ✔ could be harmful
  ✔ testing methods exist

◆ UCMR 4: January 2017 – December 2021
Method Development Process

1. Optimize Instrumentation (chromatography, mass calibrate, tune, evaluate instrument stability)

2. Determine Best Calibration (linear/quadratic, internal standards)

3. Optimize SPE (select sorbent, surrogates, solvents, solvent volumes, sample volume, evaporation parameters)

4. Determine Interferences (Are DQOs met in various difficult matrices?)

5. Determine Preservatives (select antimicrobial & dechlor)

6. Determine Aqueous & Extract Holding Times and LCMRLs

7. Does Method Meet DQOs?
   - YES: Write Method
   - NO: Revise Technical Approach

8. Multi-Lab Verification

9. Peer Review

10. Available for Use in Future UCMR
Method Development Goals

◆ 70-130% recovery with <30% RSD

◆ Laboratory reagent blank (LRB) no more than 1/3 the minimum reporting level (MRL)

◆ Preservation
  ✓ Dechlorinating agent
  ✓ Antimicrobial

◆ Establish sample and extract holding times – ideally ≥14 days

◆ Lowest concentration minimum reporting limits (LCMRLs) goal – less than health reference level (HRL)

◆ HRL for nonylphenol is 105 µg/L
What is Nonylphenol?

- Nonylphenol is used to make plastics, detergents, paints, pesticides, personal care products
- Many products have “down the drain” applications and are flushed into the water supply

<table>
<thead>
<tr>
<th>Technical, Branched Nonylphenol</th>
<th>Linear Nonylphenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mostly branched C9-alkyl phenols</td>
<td>Linear C9 chain</td>
</tr>
<tr>
<td>CAS# 84852-15-3</td>
<td>CAS# 104-40-5</td>
</tr>
<tr>
<td>Best represents commercially produced NP found in environment</td>
<td>Laboratory generated chemical not found in environment</td>
</tr>
</tbody>
</table>

One example of a 4-branched-NP isomer

Method will report technical, branched NP, CAS #84852-15-3
System Background Contribution

NP is a common laboratory contaminant

Trapping column used to separate LC system contamination away from injected NP peak
Drinking Water Procedure

Preservatives
- Sodium bisulfate = antimicrobial
- Ascorbic acid = dechlor

250 mL sample

Add IS

Oasis HLB, 150 mg
Strata-X, 100 mg

Adjust to 5 mL final volume with acetone

Add IS

Rinse cartridge with reagent water and dry under vacuum

Rinse and elute bottle with 2 x 2 mL acetone

Possible Surrogates
- 4-n-Nonylphenol-\(^{13}\)C\(_6\) (linear)
- 4-n-Nonylphenol-2,3,4,5-\(d_4\), OD (linear)
- 4-n-Octyl-\(d_{17}\)-phenol (linear)
- 4-tert-Octylphenol-\(^{13}\)C\(_6\) (branched)

Internal Standard
- 4-(1,3-Dimethyl-1-ethylpentyl) phenol-\(^{13}\)C\(_6\)

LC/MS/MS

Optimized Conditions
## Evaporation Study

<table>
<thead>
<tr>
<th>Nonylphenol % Recovery</th>
<th>Precision % RSD (reps)</th>
<th>Evaporation Temperature</th>
<th>Minimum Extract Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>86.0%</td>
<td>2.1% (n = 5)</td>
<td>40 °C</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>90.3%</td>
<td>1.3% (n = 5)</td>
<td>30 °C</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>100%</td>
<td>2.7% (n = 4)</td>
<td>30 °C</td>
<td>2 mL</td>
</tr>
<tr>
<td>96.4%</td>
<td>1.6% (n = 4)</td>
<td>No evaporation</td>
<td>No evaporation</td>
</tr>
</tbody>
</table>

- Henry’s Law Constant = 11.02 Pa*m³*mol⁻¹, vapor pressure = 4.55 mPa at 25 °C – potentially semivolatile
- Room temperature N₂ evaporation possible option
- Minimal gain in concentration factor, added complexity, and potential loss of recovery = **No Evaporation**
Mobile Phase Conditions

- Sensitivity increases as mobile phase pH approaches the pKa (10.3)
- Ammonium hydroxide (0.01%) used to increase pH to 10
  - Still 1 - 2 pH units below upper pH limit of C18 LC columns
  - Column damage after two weeks!!

<table>
<thead>
<tr>
<th>Standard Concentration</th>
<th>NP Area</th>
<th>Summary of Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 µg/L</td>
<td>2573</td>
<td>0.01% acetic acid in A/B</td>
</tr>
<tr>
<td>150 µg/L</td>
<td>3621</td>
<td>5 mM ammonium acetate in A/B pH = estimate 6.5</td>
</tr>
<tr>
<td>150 µg/L</td>
<td>18437</td>
<td>no modifiers/neutral pH</td>
</tr>
<tr>
<td>150 µg/L</td>
<td>323825</td>
<td>0.01% ammonium hydroxide in A/B pH = 10.1</td>
</tr>
<tr>
<td>150 µg/L</td>
<td>308900</td>
<td>0.2 mM ammonium fluoride in A pH = 6.0</td>
</tr>
</tbody>
</table>

NH₄F provides good sensitivity at near neutral pH
NP Contamination – Punctured Vial Septa

Four replicate blank injections from the same vial

- Repeat injections from same vial show increase NP area
  - Vial Septa?
  - Laboratory Air?

One injection per vial to eliminate potential NP contamination
<table>
<thead>
<tr>
<th></th>
<th>Oasis HLB ng/L n = 11</th>
<th>Strata-X ng/L n = 6</th>
<th>Brand C ng/L n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>7.64</td>
<td>8.06</td>
<td>15.4</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.50</td>
<td>1.51</td>
<td>1.88</td>
</tr>
<tr>
<td>Minimum</td>
<td>4.42</td>
<td>5.70</td>
<td>12.18</td>
</tr>
<tr>
<td>Maximum</td>
<td>12.45</td>
<td>9.89</td>
<td>17.48</td>
</tr>
</tbody>
</table>

- Brand C cartridge average about 2X higher than other cartridges
- Brand C minimum LRB near or above the maximum of other cartridges

Brand C cartridge discarded as Method 559 option
Mean Recovery and Precision (n=4) of Laboratory Fortified Blanks Comparing Elution Solvent and Volume

Samples fortified at 4 µg/L, Oasis HLB, 150 mg

Nonylphenol 4-n-Octyl-d_{17}-phenol (SUR) 4-n-NP-2,3,5,6-d_{4},OD (SUR) 4-tert-Octylphenol-13C_{6} (SUR)

2 x 2 mL methanol elution 2 x 4 mL methanol elution 2 x 2 mL acetone elution

2 X 2 mL acetone chosen for elution
# Performance of Linear Versus Branched Alkylphenol Surrogates in Drinking Water Matrices

The chart below illustrates the performance of different alkylphenol surrogates in various water matrices. The data shows the mean recovery of surrogates at different concentrations in ground water and surface water samples.

- **Nonylphenol**
  - Ground Water (4µg/L)
  - Ground Water (1µg/L)
  - Surface Water #1 (4µg/L)
  - Surface Water #1 (1µg/L)
  - Surface Water #2 (4µg/L)

- **4-n-Octyl-d17-phenol (SUR)**
  - Linear
  - Oasis HLB, 150 mg

- **4-n-NP-2,3,5,6-d4,OD (SUR)**
  - Linear

- **4-tert-Octylphenol-13C6 (SUR)**
  - Branched

The chart indicates that branched surrogates are chosen for the method, as shown by the highlighted note. This choice is likely due to superior performance or better detection properties in the specific matrices tested.
Mean Recovery and Precision ($n=4$) in Laboratory Fortified Blanks

- Oasis HLB, 100 ng/L
- Oasis HLB, 600 ng/L
- Strata-X, 100 ng/L
- Strata-X, 600 ng/L

Results meet DQOs of 70-130%, <30% RSD
Mean Recovery and Precision (n=4) in Laboratory Fortified Drinking Water Samples

Results meet DQOs of 70-130%, <30% RSD
Aqueous Holding Time Study

Tap water from a surface water source, fortified at 600 ng/L, n=4

- Sodium bisulfate and ascorbic acid preservation

**Oasis HLB, 150 mg**

**SUR not held**
Extract Holding Time Study

Tap water from a surface water source, fortified at 600 ng/L, n=4

Extracts stored in autosampler vials at 4 °C

% Mean Recovery

Day 0  Day 7  Day 14  Day 21  Day 28

4-tert-Octylphenol  Nonylphenol  4-tert-Octylphenol-13C6 (SUR)

SUR not held

Extracts stored in autosampler vials at 4 °C
Drinking Water Method Verification Approach

◆ 4 Laboratories
  ✓ Additional labs for M559 to evaluate LRB background

◆ Labs provided written method

◆ Demonstrate method performance data
  ✓ LRB evaluation
  ✓ accuracy and precision in reagent water
  ✓ accuracy and precision in at least one matrix
  ✓ LCMRL

◆ Provide feedback on written method & any analytical issues encountered

◆ Multi-lab LCMRLs are used to determine MRLs for the UCMR
Highly variable background levels of NP found during verification

- Reagent water likely source

<table>
<thead>
<tr>
<th>LRB</th>
<th>Lab 1</th>
<th>Lab 2</th>
<th>Lab 3</th>
<th>Lab 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>2.8</td>
<td>1.3</td>
<td>31.7</td>
<td>24.3</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.8</td>
<td>0.4</td>
<td>10.1</td>
<td>11.1</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.9</td>
<td>0.9</td>
<td>24.1</td>
<td>12.1</td>
</tr>
<tr>
<td>Maximum</td>
<td>3.8</td>
<td>1.9</td>
<td>56.5</td>
<td>37.3</td>
</tr>
</tbody>
</table>
Results meet DQOs of 70-130%, <30% RSD
### LCMRL Values

<table>
<thead>
<tr>
<th>Analyte</th>
<th>EPA LCMRL</th>
<th>Lab 1 LCMRL</th>
<th>Lab 2 LCMRL</th>
<th>Lab 3 LCMRL</th>
<th>Lab 4 LCMRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-tert-Octylphenol</td>
<td>4.9</td>
<td>6.4</td>
<td>15</td>
<td>4.9</td>
<td>12</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>24</td>
<td>29</td>
<td>113</td>
<td>10</td>
<td>38</td>
</tr>
</tbody>
</table>

**Reference Guidelines**

<table>
<thead>
<tr>
<th>Guideline</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRL</td>
<td>105 µg/L</td>
</tr>
<tr>
<td>Minnesota Department of Health</td>
<td>20 µg/L</td>
</tr>
</tbody>
</table>

- LCMRL value for nonylphenol well below current HRL guideline
Summary

- Rugged, standardized, sensitive method developed for nonylphenol and 4-tert-octylphenol in drinking water
- Investigated best labeled SUR and IS standards for method, chose branched octylphenol for SUR and branched nonylphenol for IS
- Meets DQOs for two types of SPE cartridges, reagent water, and drinking water from ground water and surface water sources
- Aqueous and extract holding times of 28 days
- Multi-lab verification, easily meet HRL limit for nonylphenol
Multi-Laboratory Verification
Acknowledgements

❖ American Water Central Laboratory – Belleville, IL
   ➢ Bill Deckelmann, Katie Kohoutek

❖ Babcock Laboratories, Inc – Riverside, CA
   ➢ David Schiessel, Susann Thomas

❖ Eurofins Eaton Analytical, LLC – Monrovia, CA
   ➢ William Lipps, Ali Haghani

❖ Eurofins Eaton Analytical, LLC – South Bend, IN
   ➢ Yongtao (Bruce) Li, Joshua Whitaker
Questions??