Mixed Phenols and Phenates by LC- UV AOAC International Collaborative Study

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US EPA Pesticide Registration Guidelines:

• The EPA requires a suitable analytical method for all registered pesticide active ingredients in technical materials and if requested, end use products.

• Enforcement Analytical methods are not required to have a Single Laboratory Validation (SLV) for EPA registration.
US EPA Enforcement Concerns:

- Disagreements over method used have occurred between registrant and FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) laboratory
- Government laboratories need accurate, reliable and rugged methods for their enforcement work.
- The chlorinated phenols, used in many hospital disinfectants had not been collaboratively studied.
Preliminary work:

• We considered two methods for potential collaborative study, a GC method and an LC method.

• A pilot study was conducted for both methods using the same 8 commercial disinfectant samples analyzed by 6 laboratories.

• All laboratories returned data using the LC method, but only 2 laboratories returned data on the GC method.
• From the results and comments from the pilot study, a single laboratory validation for the LC method was conducted by Tom Phillips, Maryland Department of Agriculture to determine the accuracy and repeatability (within lab).

• The SLV was published in the Journal of the AOAC International (T.Phillips, A. Burns, 2010)
Study Design:

- We wanted a minimum of ten laboratories from government, industry and academia to collaboratively study the proposed method.
- We wanted a minimum of five materials encompassing range of % actives and variety of inert ingredients.
Mixed Phenols/Phenates Collaborative Study Design

- Originally had 19 laboratories sign up for the study
- 1 test sample to ensure analyst familiarity with the method and check out the LC system
- 7 Samples selected reflect as much variability as possible:
  - Low levels
  - High levels
  - Salts (phenates)
  - Other active ingredients
Phenols/Phenates Examined:

- **Opp**: Ortho phenyl phenol / phenate
- **Obpcp**: ortho benzyl parachloro-phenol/phenate
- **Ptap**: para tertiary amyl phenol / phenate

These are the top three registered phenols and present as single or multi-active ingredient in 89 different currently registered products.
Participating Laboratories:

- Iowa Department of Agriculture and Land Stewardship
- Robert Wesleyan College
- North Carolina Department of Agriculture and Consumer Services
- Georgia Department of Agriculture
- Steris Corporation
- Lonza Corporation
• Clorox Corporation
• Florida Department of Agriculture/Environmental Services
• US Environmental Protection Agency
• Dow AgroSciences
• Silliker Laboratories
• Kansas Department of Agriculture
• Maryland Department of Agriculture
## Sample Table

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Opp</th>
<th>Ptap</th>
<th>Obpcp</th>
<th>Notes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.50</td>
<td>2.20</td>
<td>4.00</td>
<td>K salt</td>
</tr>
<tr>
<td>2</td>
<td>5.13</td>
<td>3.47</td>
<td>6.84</td>
<td>Na salt</td>
</tr>
<tr>
<td>3</td>
<td>0.04</td>
<td>0.07</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.90</td>
<td>2.50</td>
<td>10.10</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.02</td>
<td>1.20</td>
<td>4.90</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.35</td>
<td>3.47</td>
<td>3.80</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.00</td>
<td>2.00</td>
<td>8.50</td>
<td></td>
</tr>
</tbody>
</table>
LC Columns Used:

- Phenomenex Luna C18(2)
- Waters µBondapak ODS (300x3.9 mm)
- Waters Novapak ODS (200x 4.0 mm)
- Column Engineering Inertsil
- Keystone Scientific Betasil ODS
- Agilent Zorbax Eclipse XDB-ODS
- Phenomenex Kinetix ODS (100x 4.6 mm)
- Whatman Partisphere ODS (250x 4.6 mm)
Method Summary:

- Samples are extracted with acidified methanol
- LC is run with a gradient mobile phase of acidified water/ acetonitrile
- Detector is UV at 285 nm
Typical chromatogram
Samples were tested as blind duplicates

- Data was presented by analyte:
- % concentration found
- Stats for outliers- Cochran and Single Grubbs run
- Predicted relative standard deviation
- Relative standard deviation found
- HorRat was determined for each analyte of interest
<table>
<thead>
<tr>
<th>Analyte</th>
<th>No. of Labs (outliers)</th>
<th>Mean % $^a$</th>
<th>$s_r$ $^b$</th>
<th>$s_R$ $^c$</th>
<th>RSD$_r$ $^d$</th>
<th>RSD$_R$ $^e$</th>
<th>r $^f$</th>
<th>R $^g$</th>
<th>HorRat</th>
<th>Outlier Labs $^h$</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPP</td>
<td>13(1)</td>
<td>4.840</td>
<td>0.0719</td>
<td>0.2791</td>
<td>1.49</td>
<td>5.77</td>
<td>0.201</td>
<td>0.781</td>
<td>1.83</td>
<td>2-C</td>
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<tr>
<td></td>
<td>13(1)</td>
<td>0.047</td>
<td>0.0005</td>
<td>0.0025</td>
<td>0.98</td>
<td>5.43</td>
<td>0.001</td>
<td>0.007</td>
<td>0.86</td>
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<td>14(0)</td>
<td>2.463</td>
<td>0.0751</td>
<td>0.1551</td>
<td>3.05</td>
<td>6.30</td>
<td>0.210</td>
<td>0.434</td>
<td>1.80</td>
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<tr>
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<td>13(1)</td>
<td>3.649</td>
<td>0.0698</td>
<td>0.2196</td>
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<td>6.02</td>
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<tr>
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<td>14(0)</td>
<td>9.953</td>
<td>0.1166</td>
<td>0.6123</td>
<td>1.17</td>
<td>6.15</td>
<td>0.326</td>
<td>1.714</td>
<td>2.17</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Weight percent average of the blind duplicate pair

$^b$ $s_r$ = Standard deviation for repeatability (within laboratory).

$^c$ $s_R$ = Standard deviation for reproducibility (among laboratories).

$^d$ RSD$_r$ = Relative standard deviation for repeatability.

$^e$ RSD$_R$ = Relative standard deviation for reproducibility.

$^f$ $r = 2.8 \times s_r$.

$^g$ $R = 2.8 \times s_R$.

$^h$ C = Cochran outlier; SG = single Grubbs outlier.
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<th>(S_R) (^c)</th>
<th>RSD(_r) (^d)</th>
<th>RSD(_R) (^e)</th>
<th>(r) (^f)</th>
<th>(R) (^g)</th>
<th>HorRat</th>
<th>Outlier Labs (^h)</th>
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</thead>
<tbody>
<tr>
<td>PTAP</td>
<td>13(1)</td>
<td>2.551</td>
<td>0.0397</td>
<td>0.1730</td>
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<td>6.78</td>
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<td>12(2)</td>
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<td>0.0009</td>
<td>0.0050</td>
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<tr>
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<td>14(0)</td>
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<td>13(1)</td>
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<td>0.1074</td>
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<td>5.48</td>
<td>0.131</td>
<td>0.301</td>
<td>1.52</td>
<td>18-SG</td>
</tr>
</tbody>
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<th>(R (^g))</th>
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<tbody>
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<td>OBPCP</td>
<td>14(0)</td>
<td>10.15</td>
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<td>13(1)</td>
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<td>5.50</td>
<td>0.320</td>
<td>1.357</td>
<td>1.91</td>
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</tbody>
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<th>s_R c</th>
<th>RSD_r d</th>
<th>RSD_R e</th>
<th>r f</th>
<th>R g</th>
<th>HorRat</th>
<th>Outlier Labs h</th>
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<tbody>
<tr>
<td>OPP</td>
<td>14(0)</td>
<td>2.614</td>
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<td>0.1495</td>
<td>1.99</td>
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<td>14(0)</td>
<td>6.123</td>
<td>0.0859</td>
<td>0.3835</td>
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<td>6.26</td>
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<td>1.074</td>
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<tr>
<td>PTAP</td>
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<td>2.150</td>
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<td>0.1182</td>
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<td>14(0)</td>
<td>3.933</td>
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<td>0.206</td>
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<tr>
<td>OBPCP</td>
<td>14(0)</td>
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<td>0.1107</td>
<td>0.3877</td>
<td>2.47</td>
<td>8.67</td>
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<td>12-C</td>
</tr>
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Results for the salt forms of OPP, PTAP, and OBPCP

a Weight percent average of the blind duplicate pair
b s_r = Standard deviation for repeatability (within laboratory).
c s_R = Standard deviation for reproducibility (among laboratories).
d RSD_r = Relative standard deviation for repeatability.
e RSD_R = Relative standard deviation for reproducibility.
f r = 2.8*sr.
g R = 2.8*sR.
h C= Cochran outlier; SG = single Grubbs outlier.
Results

• Results were acceptable. There were 26 outliers in 588 data points generated (4.42%)
• Performance of the method compared favorably to the SLV as well.
• AOAC International granted First Action in 2011 (AOAC 2011.26)
Acknowledgements:

- Tom Phillips for conducting the Single Laboratory Validation and being a co-study director
- US EPA for funding this collaborative study
- Thuy Nguyen for allowing us the time to pursue this collaborative study
- Members and friends of AOAC Committee A for their support and input.
Questions?

• Contact information
• Adrian Burns telephone: 410-305-2927
  email: burns.adrian@epa.gov
• Diane Rains telephone: 410-305-2908
  email: rains.diane@epa.gov
Thank You!