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

	Content expressed as per cent phenol			
Sample no.	**Opp**	**Ptap**	**Obpcp**	**Notes:**
1	2.50	2.20	4.00	**K salt**
2	5.13	***3.47***	6.84	**Na salt**
3	***0.04***	***0.07***	***0.08***	
4	4.90	2.50	***10.10***	
5	4.02	1.20	4.90	
6	2.35	3.47	3.80	
7	***10.00***	2.00	8.50	

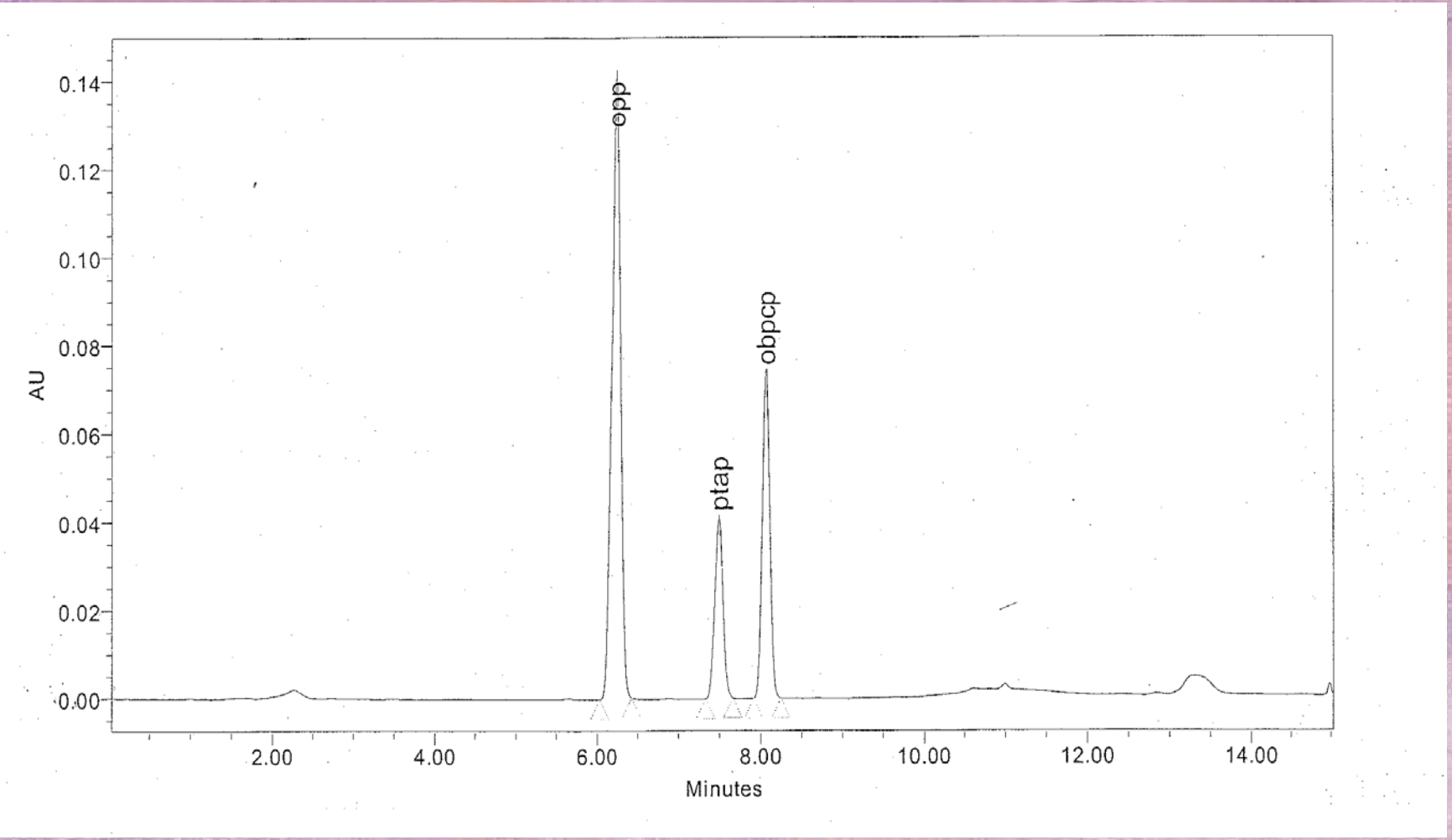
LC Columns Used:

- 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**

Analyte	No. of Labs (outliers)	Mean % [a]	s_r [b]	s_R [c]	RSD_r [d]	RSD_R [e]	r [f]	R [g]	HorRat	Outlier Labs [h]
OPP	13(1)	4.840	0.0719	0.2791	1.49	5.77	0.201	0.781	1.83	2-C
	13(1)	0.047	0.0005	0.0025	0.98	5.43	0.001	0.007	0.86	2-C
	14(0)	2.463	0.0751	0.1551	3.05	6.30	0.210	0.434	1.80	
	13(1)	3.649	0.0698	0.2196	1.91	6.02	0.195	0.615	1.83	16-C
	14(0)	9.953	0.1166	0.6123	1.17	6.15	0.326	1.714	2.17	

[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] $r = 2.8 * s_r$.
[g] $R = 2.8 * s_R$.
[h] C= Cochran outlier; SG = single Grubbs outlier.

Analyte	No. of Labs (outliers)	Mean % [a]	s_r [b]	s_R [c]	RSD_r [d]	RSD_R [e]	r [f]	R [g]	HorRat	Outlier Labs [h]
PTAP	13(1)	2.551	0.0397	0.1730	1.56	6.78	0.111	0.484	1.95	2-C
	12(2)	0.054	0.0009	0.0050	1.12	5.95	0.002	0.014	1.03	2,4-C
	14(0)	3.959	0.0870	0.2103	2.20	5.31	0.244	0.589	1.63	
	14(0)	1.143	0.0389	0.0891	3.40	7.80	0.109	0.249	1.99	18-SG
	13(1)	1.961	0.0467	0.1074	2.38	5.48	0.131	0.301	1.52	18-SG

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[h] C= Cochran outlier; SG = single Grubbs outlier.

Analyte	No. of Labs (outliers)	Mean % [a]	s_r [b]	s_R [c]	RSD_r [d]	RSD_R [e]	r [f]	R [g]	HorRat	Outlier Labs [h]
OBPCP	14(0)	10.150	0.3267	0.5469	3.22	5.39	0.915	1.53	1.91	
	13(1)	0.095	0.0014	0.0053	1.49	5.57	0.004	0.015	0.98	2-C
	13(1)	4.276	0.0598	0.2356	1.40	5.51	0.167	0.660	1.71	19-C
	13(1)	4.855	0.0920	0.2709	1.90	5.58	0.258	0.758	1.77	16-C
	14(0)	8.815	0.1142	0.4845	1.30	5.50	0.320	1.357	1.91	

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Analyte	No. of Labs (outliers)	Mean % [a]	s_r [b]	s_R [c]	RSD_r [d]	RSD_R [e]	r [f]	R [g]	HorRat	Outlier Labs [h]
Results for the salt forms of OPP, PTAP, and OBPCP										
OPP	14(0)	2.614	0.0520	0.1495	1.99	5.72	0.146	0.419	1.65	
	14(0)	6.123	0.0859	0.3835	1.40	6.26	0.241	1.074	2.06	
PTAP	13(1)	2.150	0.0540	0.1182	2.51	5.50	0.151	0.331	1.54	8-SG
	14(0)	3.933	0.0734	0.2508	1.87	6.38	0.206	0.702	1.96	
OBPCP	14(0)	4.471	0.1107	0.3877	2.47	8.67	0.310	1.086	2.72	
	13(1)	8.252	0.1037	0.4942	1.26	5.99	0.290	1.384	2.06	12-C

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[f] r = 2.8*sr.
[g] R = 2.8*sR.
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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**
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Questions?

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Thank You!