Determination of Organophosphate Pesticides in Urine Using a 'Filter And Shoot' (FASt®) Extraction and LC-MS/MS

UCT Part Numbers

CSFAS203
Clean Screen FASt®
200 mg, 3 mL

SLAQ100ID21-3UM
Selectra® Aqueous C18 Column
100 x 2.1mm, 3 µm

SLAQGDC20-3UM
Selectra® Aqueous C18 Guard
Column 10 x 2.1 mm, 3 µm

SLGRDHLDR-HPOPT
Guard Cartridge Holder

Summary:

Organophosphate pesticides (OP) are a diverse group of compounds. Derived from phosphoric acid they exhibit varied physicochemical properties. They are used extensively as nerve poisons to kill target pests (usually insects). However, their toxicity extends to mammals and they can adversely affect the human nervous system, even at low exposure levels. For example, in 2013, 23 Indian students were killed from cooking oil contaminated with monocrotophos. OP pesticides are unstable and breakdown relatively quickly through hydrolysis and exit the human body via urine; thus monitoring OP pesticides and their metabolites in urine can indicate any recent exposures.

Extracting OP pesticides can be a challenge due to their varied physicochemical properties. Liquid/Liquid (L/L), solid phase extraction (SPE), supported liquid extraction (SLE), and QuEChERS work for mid to non-polar compounds, but not for polar compounds due to insufficient analyte partitioning between the aqueous and organic phases or retention on typical reverse phase sorbents.

In this application a simple, fast sample preparation approach for LC/MS analysis of 13 OP pesticides in urine samples was conducted. This method efficiently retains the unwanted matrix components and particulates to the sorbent and frits while allowing the analytes of interest to pass through the sorbent bed, and collected for direct LC-MS/MS analysis.

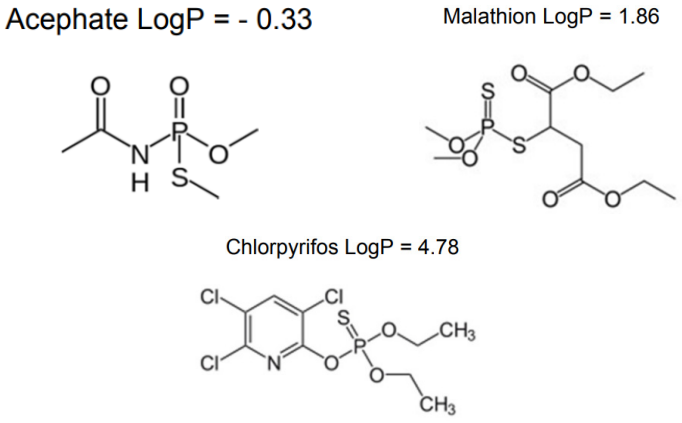

Figure 1. Examples of OP pesticides showing varied structures polarities

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Sample Preparation:

1) Hydrolyze urine sample with beta-glucuronidase if there are any glucuronide or sulfate- conjugated metabolites.
2) Mix 0.5 mL* urine sample with 0.5 mL acetonitrile containing internal standard(s), vortex for 1 min.
3) Apply the mixed sample to FASt column (or well plate), apply a low vacuum and collect the filtrate.
4) Mix 200 µL filtrate with 800 µL reagent water**, vortex and analyze by LC-MS/MS.

* Less sample volume can be used for 96-well plate application.
** Water dilution was needed for better retention of a couple polar OP pesticides, which is not necessary if only mid to non-polar compounds are analyzed.

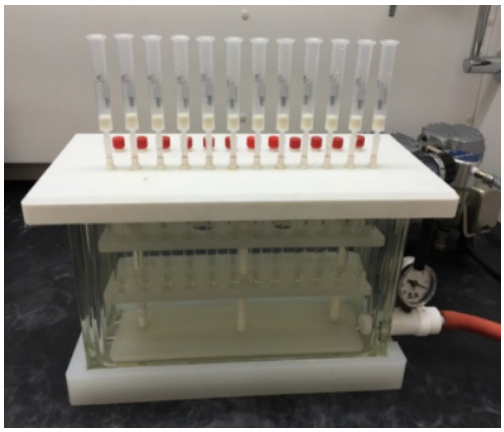

Figure 2. FASt Setup

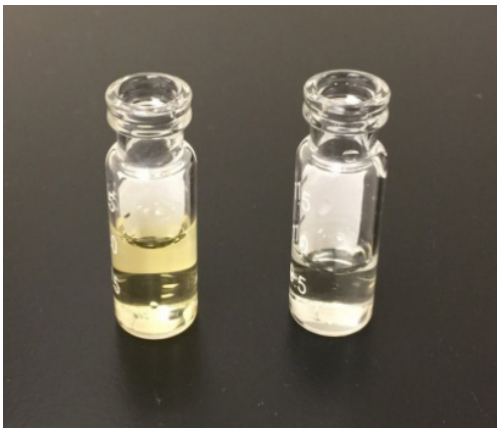

Figure 3. Urine Sample: Before and after extraction

LC-MS/MS Method	
HPLC	Thermo Scientific Dionex UltiMate 3000® LC System
Mass Spec	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI+
Column	Selectra® Aqueous C18 100 x 2.1 mm, 3 µm (**PN: SLAQ100ID21-3UM**)
Guard column	Selectra® Aqueous C18 Guard Column 10 x 2.0 mm, 3 µm (**PN: SLAQGDC20-3UM**)
Column Temperature	40 °C
Column Flow Rate	0.300 mL/min
Auto-sampler Temperature	10 °C
Injection Volume	10 µL
Mobile Phases	**Mobile phase A:** 20 mM ammonium formate in water **Mobile phase B:** 0.1 % formic acid in MeOH

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Gradient Program		
Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
0.5	100	0
3	50	50
4.5	50	50
6	35	65
9	35	65
13	5	95
15	5	95
15.1	100	0
19	100	0

Divert mobile phase to waste from 0 - 2 and 16 - 19 min to prevent ion source contamination.

Results:

Retention Times, SRM Transitions & Linearity					
Compound	RT (min)	Parent ion	Product ion 1	Product ion 2	Linearity (R^2)
Methamidophos	3.53	142.0	93.7	124.6	0.9997
Acephate	4.36	183.9	142.6	94.6	0.9990
Dicrotophos	5.88	237.9	126.6	71.7	0.9994
o,o,o-triethylphosphorothioate	6.20	199.0	124.6	78.6	0.9991
Dimethoate	6.19	230.0	124.6	170.6	0.9990
Famphur	8.76	325.9	216.6	92.6	0.9980
Malathion	10.23	330.9	126.7	98.6	0.9963
Sulfotep	12.08	322.9	96.6	114.5	0.9977
Diazinon	12.82	305.0	168.7	152.7	0.9995
TPP (IS)	13.14	327.0	151.6	76.7	NA
Pyrazophos	13.42	374.0	221.6	193.6	0.9945
Profenofos	14.06	372.9	127.6	304.4	0.9980
Ethion	14.33	384.9	142.5	96.5	0.9974
Chlorpyrifos	14.54	350.0	96.6	199.3	0.9988

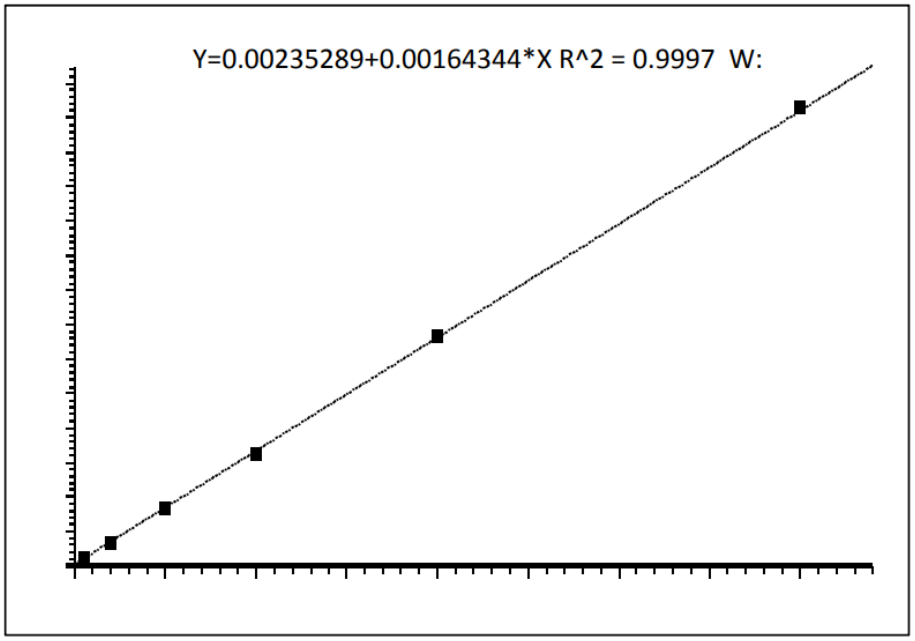

Figure 4. Calibration Curve of Methamidophos

Recovery and RSD Data – Spiked Urine Samples						
	Spiked at 10 ng/mL		Spiked at 50 ng/mL		Spiked at 200 ng/mL	
Compound Name	**Recovery %**	**RSD% (n=6)**	**Recovery %**	**RSD% (n=6)**	**Recovery %**	**RSD% (n=6)**
Methamidophos	**96.3**	2.4	**110.6**	2.8	**101.1**	14.2
Acephate	**92.5**	7.8	**92.1**	7.5	**87.8**	9.6
Dicrotophos	**96.2**	5.9	**103.5**	2.0	**94.3**	5.7
o,o,o-triethylphosphorothioate	**102.0**	11.4	**112.6**	3.4	**101.0**	3.7
Dimethoate	**103.2**	4.7	**109.7**	4.3	**104.4**	2.5
Famphur	**106.5**	9.5	**112.3**	2.6	**106.5**	3.4
Malathion	**104.9**	7.3	**110.4**	1.7	**105.6**	3.1
Sulfotep	**87.3**	8.1	**93.4**	3.7	**92.1**	6.4
Diazinon	**94.8**	5.8	**103.7**	1.1	**104.1**	2.3
Pyrazophos	**104.6**	8.7	**114.8**	0.9	**101.6**	3.4
Profenofos	**90.7**	6.2	**100.8**	4.3	**101.1**	6.8
Ethion	**84.9**	6.6	**101.2**	2.4	**98.7**	8.3
Chlorpyrifos	**91.4**	5.6	**99.5**	7.5	**104.7**	5.5
Overall mean	**96.6**	**6.9**	**105.0**	**3.4**	**100.2**	**5.8**

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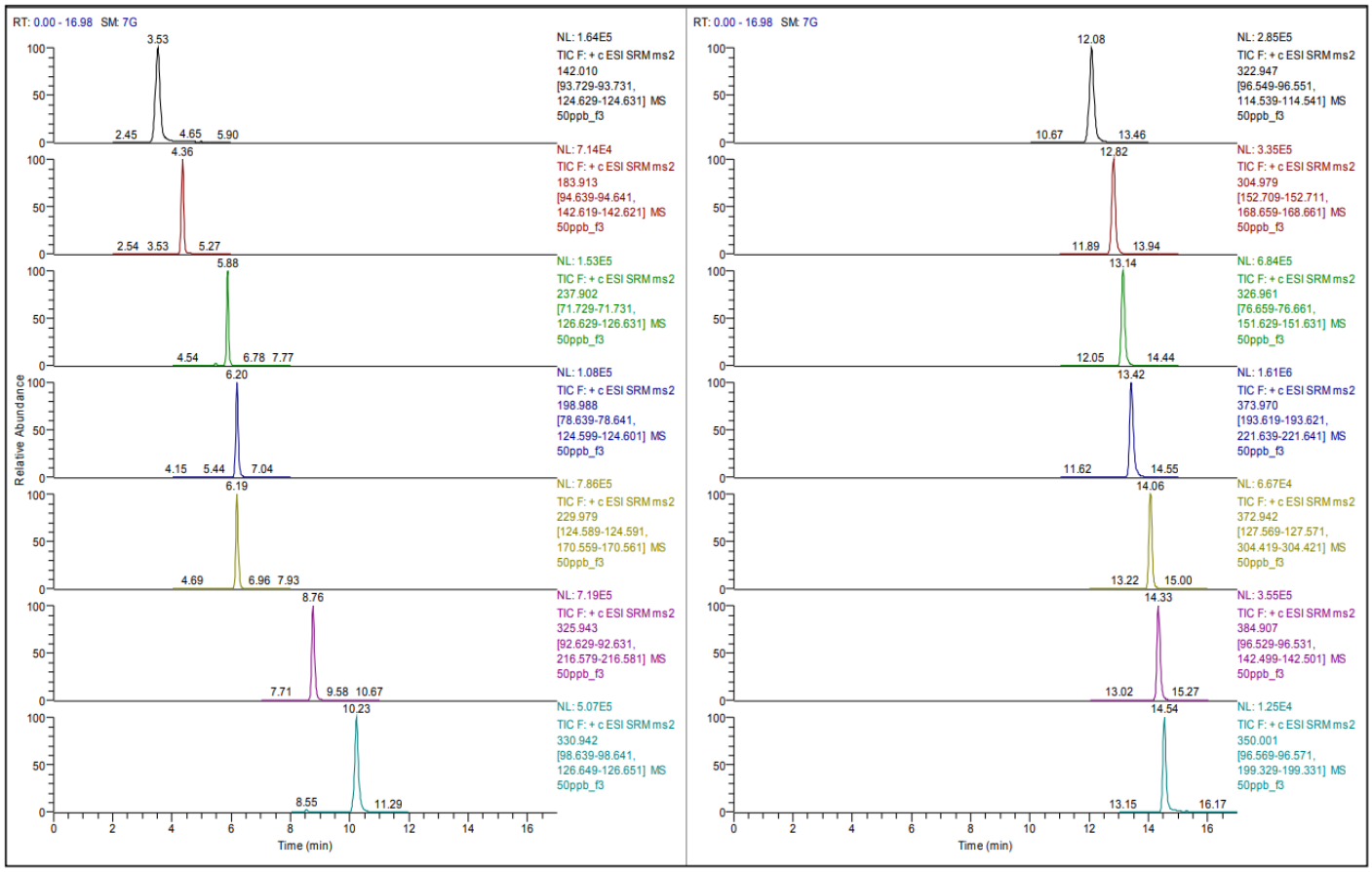

Figure 5. Chromatogram of a 50 ng/mL solvent standard

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UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com