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, 100 x 2.1mm, 3µm
SLAQGDC20-3UM - Selectra Aqueous C18, Guard Cartridge**,** 10 x 2.1mm, 3µm
SLGRDHLDR – Guard Cartridge Holder

April 2015

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, 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.

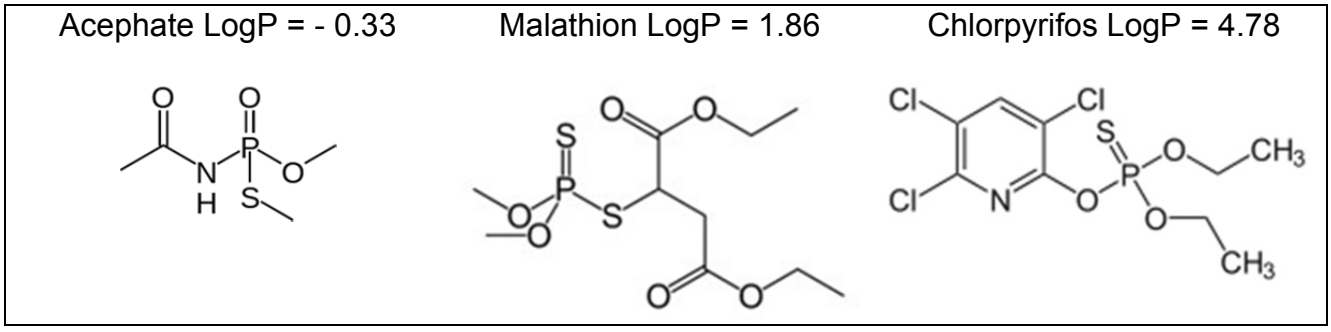

Figure 1. Examples of OP pesticides showing varied structures polarities

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.

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.

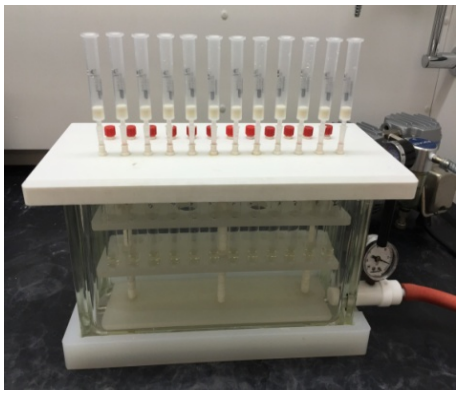

Figure 2.
FASt Setup

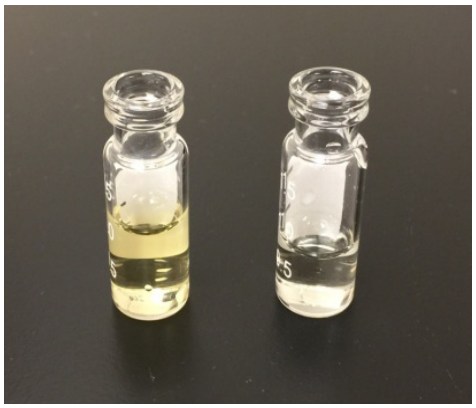

Figure 3.
Urine Sample: Before and after Extraction

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System
Mass Spec: Thermo Scientific TSQ Vantage tandem MS
Polarity: ESI +
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm
Column temperature: 40 °C

Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 10 µL		
Gradient program: **Mobile phase A:** 20 mM ammonium formate in water **Mobile phase B:** 0.1 % formic acid in MeOH		
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

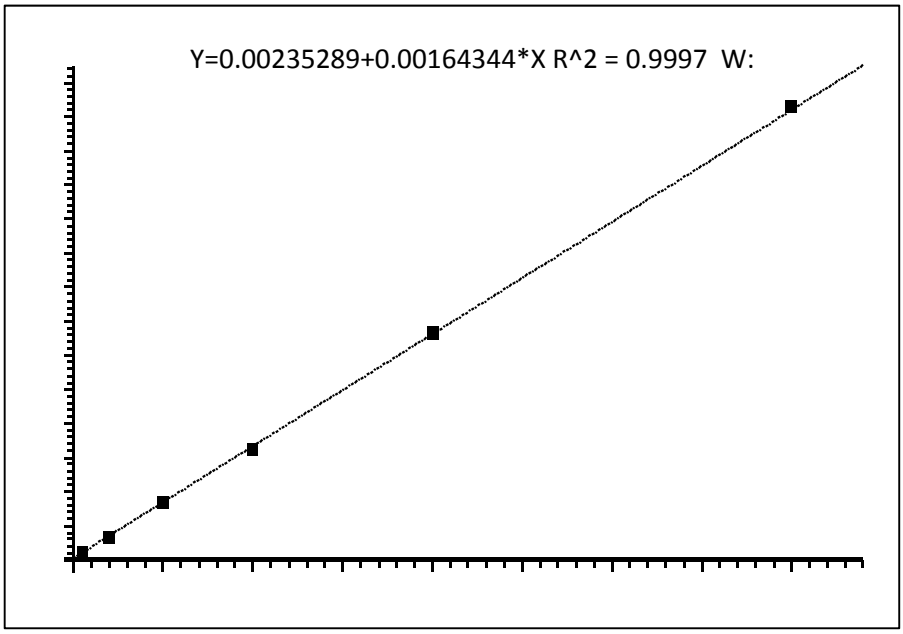

Figure 4. Calibration Curve of Methamidophos

Recovery and RSD Data – Spiked Urine Samples

Compound	Spiked at 10 ng/mL		Spiked at 50 ng/mL		Spiked at 200 ng/mL	
	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**

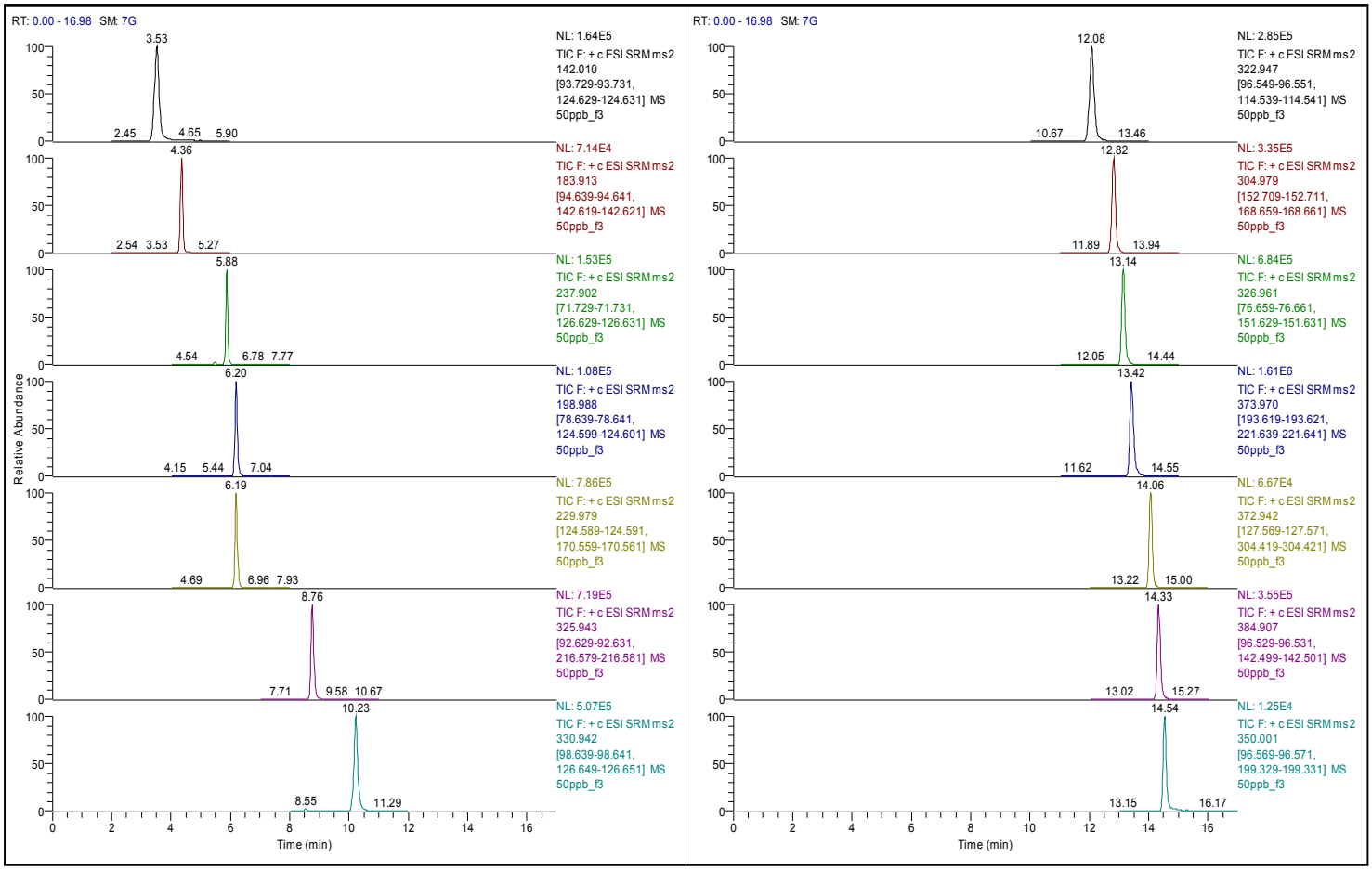

Figure 5. Chromatogram of a 50 ng/mL Solvent Standard

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