Application Note

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μSPEed | Application Note 2018

Combining μSPEed and ePrep for River Water Trace Pesticide Analysis without Surrogate Standards

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INTRODUCTION

The simplicity, accuracy and precision of Eprep's μSPEed cartridges combined with ePrep Sample Preparation Workstation (figure 1) can make the sample preparation processes easier, faster and reproducible.

Typically, a surrogate standard is add to the sample at the beginning of the preparation procedure to account for analyte loss or degradation. This is particularly important for analytes with limited insolubility, stability and unknown interaction with solvents used during the sample preparation process.

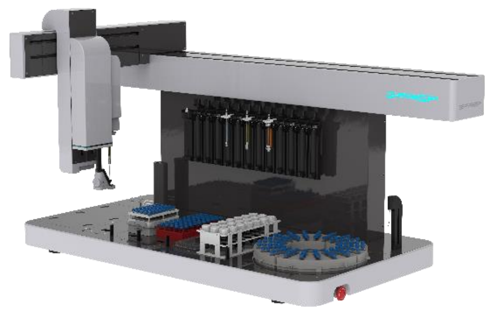

Figure 1 EPREP Sample Preparation Workstation

In this work, we proposed a new method to avoid samples losses, particularly for new and emerging compounds where surrogates and deuterated compounds are not available.

If the entire standards and sample are taken through identical sample preparation procedures, including SPE, recoveries can be accurately determined for sample analysis eliminating need for surrogate. However, to do this Solid Phase Extraction (SPE) techniques must be robust, accurate and reproducible. μSPEed cartridges (figure 2) are capable of this level of performance, due to their high extraction efficiency.

This application note describes a μSPE method for the accurate determination of organochloride pesticides in river water without the need for expensive and difficult to source deuterated surrogate standards. Spiked Pure Water, River Water and ppt level standards and samples are analysed.

AIM

The aim of this work, is to develop a sample preparation workflow for the analysis of trace organochloride pesticides in sedimentary river water without using a surrogate standard. The method includes several steps such as preparation of standards for instrument calibration, μSPE calibration standards, blanks, spiked blanks and sedimentary river water to ensure and validate the accuracy and reproducibility of the method.

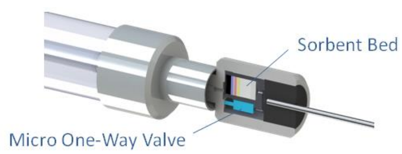

Figure 2 μSPEed Cartridge

PROCEDURE

Automated Liquid Handling

Using a fully automated liquid handling method on the EPREP sample preparation workstation a 6 point calibration curve at 0, 10, 20, 30, 40 and 50 ppb was prepared along with check standards and spiked/unspiked sedimentary river water samples for analysis. All the dispense heights were set at 25mm to avoid any droplet formation. Aspiration speed at 2000 uL/min and dispense speed at 100 uL/min.

µSPEed Extraction Workflow

Aqueous samples were loaded onto µSPEed cartridges, C18RPS-3µm/120Å (EPREP), washed with ultrapure water and then eluted using 100µl of MeOH. Conditioning and equilibration steps carried out at 200 µL/min flow rate, all other steps at 60 µL/min. Operational workflow is described in Figure 3..

Recoveries were determined by loading 10 cartridges with 25ppb water samples and eluted.

The same SPE treatment was used for Spiked and Unspiked water samples.

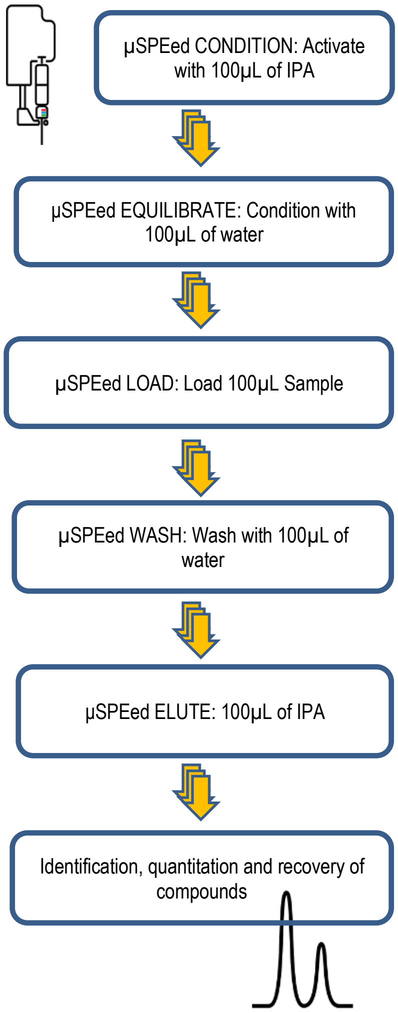

Figure 3 SPE workflow

Chromatography

Instrument: **Thermo Scientific Trace 1300 GC**
Column: DB-5MS UI Length: 25m Diameter: 0.25 mm Film: 0.25uM
Split flow: 50 ml/min
Splitless time: 1.00 min
Purgeflow: 5 ml/min
Temperature profile: 25°C/min rise to 9min, 9°C/min until 25min, hold for 5min

Detection:

Detector: **Thermo ISQ QD Single Quadrapole**
MS transfer line temperature: 270
Ion source temperature: 280
Scan range: 50 - 500
Injection volume: 1uL

RESULTS

Instrument Calibration

A 50ppb standard solution containing twenty different pesticides was used for instrument calibration. Each Pesticide compound was identified from 3 major ions and compared to library reference spectra. Quantification was based on abundance of the most common ion. Figure 4 shows a representative chromatogram with identified compounds and an example separation.

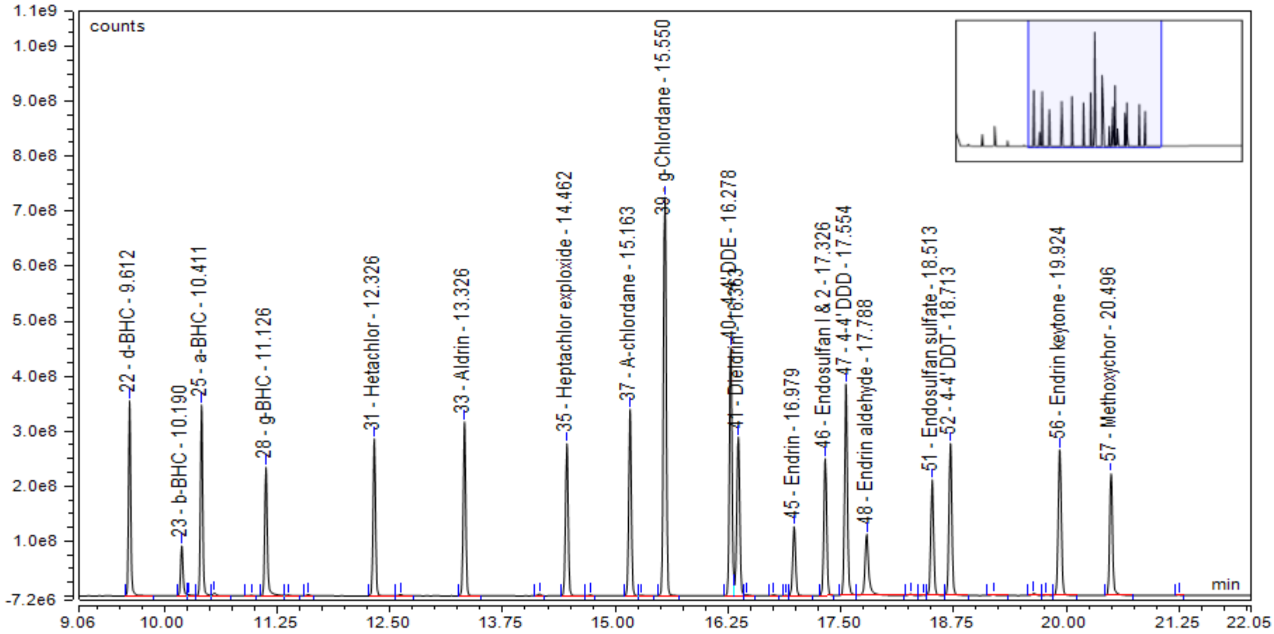

Figure 4 Identification of standardpesticides from a 50ppb standard by GC-MS

Instrument Precision was determined through 7 consecutives injections of the check standard and accuracy was determined through the calibration curve linearity. Results are summarized in Table 1 and example Calibration curves of 2 compounds (Heptaclor exposide and b-BHC, respectively) are shown in Figure 5,

Table 1 Reproducibility for the 20 target compounds analysed by 7 injections into GC-MS

Compound	d-BHC	b-BHC	a-BHC	g-BHC	Hetachlor	Aldrin	Heptachlor epoxide	A-chlordane	g-Chlordane	4-4' DDE	Dieldrin	Endrin	Endosulfan I & 2	4-4' DDD	Endrin aldehyde	Endosulfan sulfate	4-4' DDT	Endrin keytone	Methoxychor
R^2	0.996	0.998	0.995	0.993	0.990	0.994	0.994	0.992	0.998	0.988	0.995	0.979	0.988	0.982	0.962	0.978	0.981	0.989	0.962
%RSD	3.1	3.7	4.9	7.4	2.6	3.5	2.9	3.3	2.5	2.7	2.9	5.0	3.0	2.8	2.3	3.4	3.2	2.9	4.2

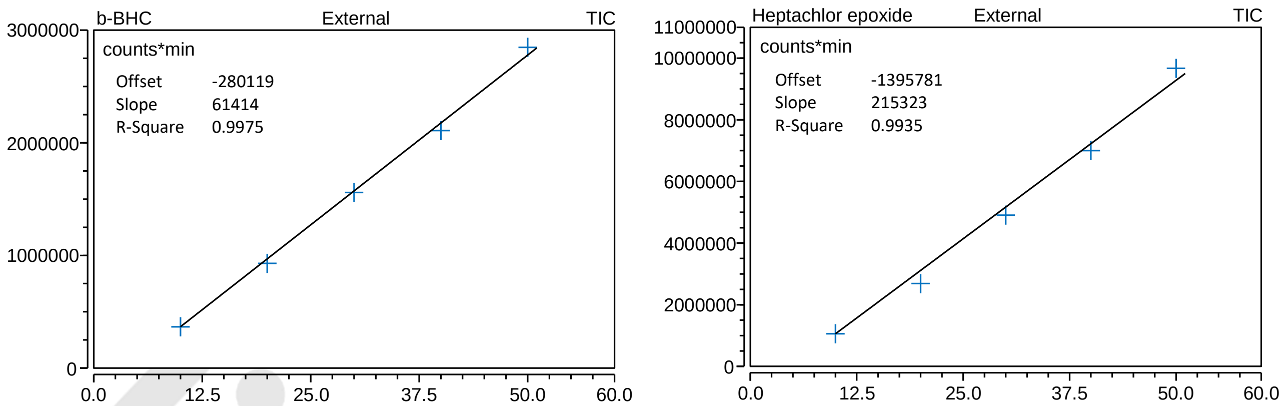

Figure 5 Calibration curves of 2 pesticides standards (a)Heptachlor epoxide and (b) b-BHC Analysed by GC-MS

Validation:

Validation of the SPE process was carried out, by comparison of 2 calibration curves, obtained from the external calibration method and after the SPE process. Figure 2 shows the results obtained from the calibration curve carried out through SPE process. These results demonstrate that the SPE process is still accurate and reproducible for quantification purposes.

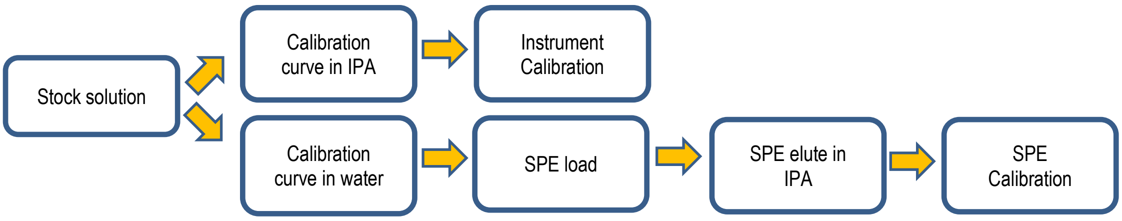

Table 2 Calibration and recovery results carried through SPE process

	d-BHC	b-BHC	a-BHC	g-BHC	Hetachlor	Aldrin	Heptachlor exploxide	A-chlordane	g-Chlordane	4-4' DDE	Dieldrin	Endrin	Endosulfan I & 2	4-4' DDD	Endrin aldehyde	Endosulfan sulfate	4-4' DDT	Endrin keytone	Methoxychor
SPE R^2	0.948	0.930	0.943	0.937	0.940	0.945	0.946	0.933	0.936	0.946	0.950	0.928	0.932	0.941	0.901	0.907	0.948	0.929	0.908
SPE %RSD	7.6	6.9	7.9	6.3	6.2	3.8	5.2	7.2	6.5	8.3	4.3	4.7	9.4	8.0	7.6	8.8	8.5	4.4	11.5

Method recovery was investigated by determining a spiked standard in an ultrapure water sample (50ppb for each pesticide); the recovery range was from 86 to 103% demonstrating that this SPE method provides good selectivity and suitability for the analysis of pesticides in water samples.

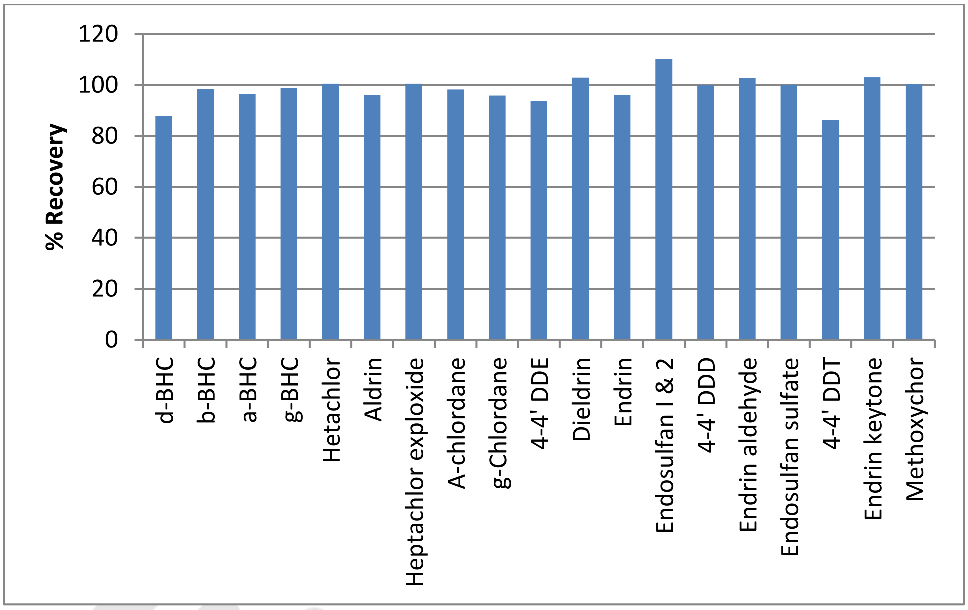

Figure 6: Average recoveries from 4 standards diluted in ultrapure water each loaded on µSPEed cartridges and analysed by GC-MS

Identification of pesticide compounds in water samples at significantly lower levels was also attempted. A 10 times concentration factor was used to detect samples spiked with 1ppb of pesticides and another sample was spiked with 100ppt of pesticides and concentrated to 100 times its original concentration. Figures 7 and 8, show the chromatographic separation of the 19 target compounds at 1ppb and 100ppt level in 2 water samples, respectively. The implementation of multiple load and elute passes may increase the effectiveness of the concentration and may allow for reliable detection at the ppt range.

Table 3 Recovery of pesticides from concentrated samples diluted to 1ppb and 100ppt in water samples and analysed by GC-MS

	% Recovery of 10x concentration	% Recovery of 100x concentration
d-BHC	113	94
b-BHC	130	111
a-BHC	119	102
g-BHC	105	91
Hetachlor	58	41
Aldrin	49	34
Heptachlor epoxide	64	44
A-chlordane	53	34
g-Chlordane	53	36
4-4' DDE	58	51
Dieldrin	53	32
Endrin	62	48
Endosulfan I & 2	69	53
4-4' DDD	54	42
Endrin aldehyde	81	64
Endosulfan sulfate	91	76
4-4' DDT	57	
Endrin keytone	86	72
Methoxychor	66	58

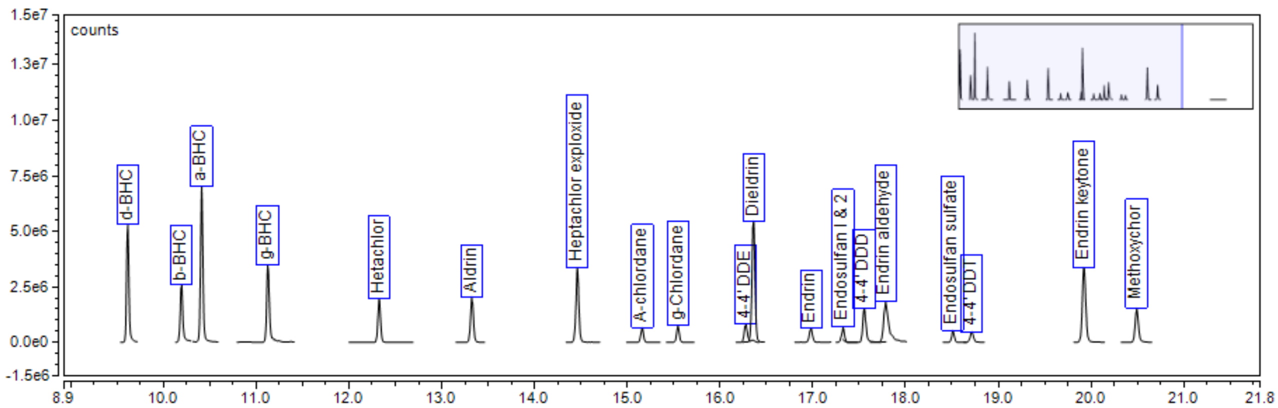

Figure 7 Chromatogram of a 1ppb water sample concentrated 10 x to 10ppb analysed by GC-MS

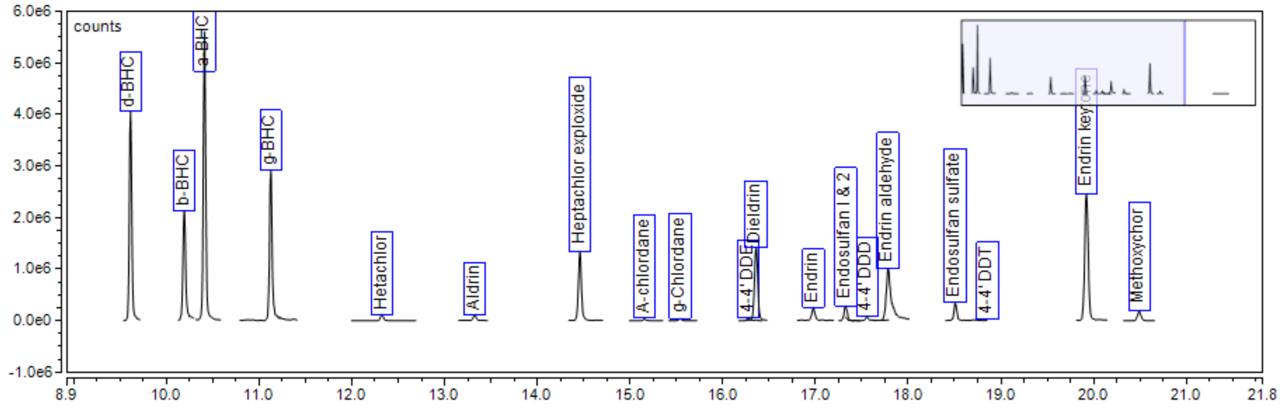

Figure 8 Chromatogram of a 100ppt water sample concentrated 100 x to 10ppb analysed GC-MS

Application of the developed methodology to a sedimentary river water sample

_Sedimentary river water sample (figure 10) was used unfiltered and untreated when passed through µSPEed C18 Cartridges for pesticide trapping. Figure 11 shows sedimentary deposit left in the µSPEed cartridge following load and elution of pesticides; demonstrating that the SPE method can be used without any filtering before trapping and clean up.

This method was tested against dirty sedimentary river water samples. These samples had no further clean up procedure other than the SPE method. The resulting chromatogram in Figure 9 show no interference form humus/sedimentary components on GC/MS analysis of the µSPEed elution of pesticides.

Recoveries of the 20 spiked compounds (see Table 3) are lower in sedimentary river water than the ones found in a clean water sample, due to suspected adsorption of the sedimentary components in the µSPE cartridges.

Table 4 Average recoveries from spiked sedimentary river water samples

	% Recovery
d-BHC	72
b-BHC	71
a-BHC	97
g-BHC	72
Hetachlor	69
Aldrin	75
Heptachlor epoxide	68
A-chlordane	71
g-Chlordane	66
4-4' DDE	72
Dieldrin	70
Endrin	74
Endosulfan I & 2	73
4-4' DDD	69
Endrin aldehyde	77
Endosulfan sulfate	75
4-4' DDT	76
Endrin keytone	77
Methoxychor	74

Figure 10, Sedimentary River Water

Figure 11, µSPEed cartridge with sediment

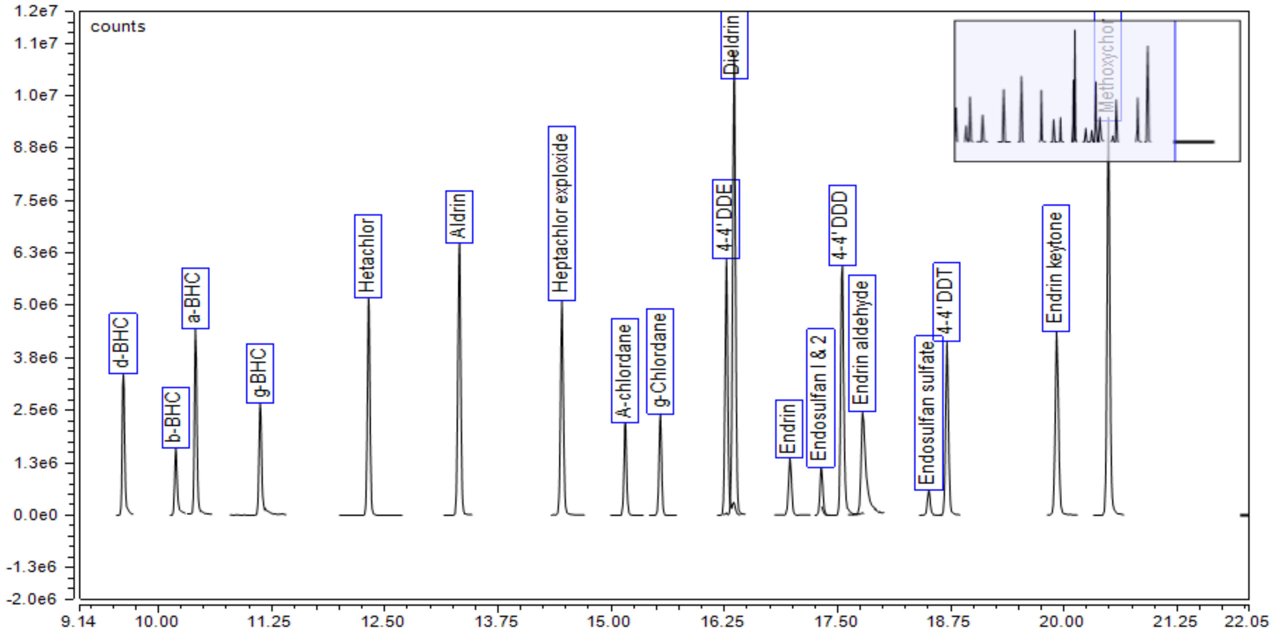

Figure 9 Chromatogram of a Spiked sedimentary river water sample EIC

CONCLUSION

SPE remains an essenital part of any chemists lab toolkit for concentrating and clean up of samples. µSPEed cartidges have the ability to reduce the amount of sample required, concentrating a 100mL sample into just 50uL while eliminating the need for large and complex elution steps with incredibly small effective elution volumes. The high effiency of the µSPEed catridges combined with the small particle size (3µm) of the sorbent bed allows almost a complete eltimination of any other sample preparation techniques like filtering or centrifuging, with the entire sample simply being loaded onto the cartidge.

Applying automated solutions like the ePrep sample prepartion workstation can also help eliminate the complex and laborious sample preparation processes that remain common in the modern lab. The eprep workstation has shown its capability of generating accurate and linear calibration curves while also providing a simple method for time-saving automation

With the emergence of new pollutants each year its often difficult to find certified references and duterated standards to use as an internalstandard. By introducing a calibration curve spiked to the matrix of interest and then following through with the entire sample prep procedure you can effectivly calibrate your detection to account for any affects that the matrix may introduce. This app note provides a simple way to use this method, combined with an accurate and fast automated system and highly effiecnt SPE extraction methods to quickly, accuratly and rapidly analyse pesticide samples even in the most dificulte of water matrixes.

EPREP WORKFLOW

Instrument Calibration Standards, 50, 100, 150, 200, 250ppb {1.5mL Vial} Pesticide Std [x1] { Add Reagent: 300uL of IPA (for needle dipping)

a) Serial Dispense: Start = 50, Increment =50 (50, 100, 150, 200 and 250uL) of 200ppb Pesticide Std [Asp: Auto@75uL/sec, Disp: 25mm@60uL/sec]
b) Make up to Volume: 1000uL IPA [Asp: Auto@75uL/sec, Disp: 25mm@60uL/sec]

uSPEd Calibration Standards, 50, 100, 150, 200, 250ppb {1.5mL Vial} Pesticide Std [x2] {Task Group #1-3}

a) Add Reagent: 300uL of Water (for needle dipping)
b) Serial Dispense: Start = 50, Increment =50 (50, 100, 150, 200 and 250uL) of 200ppb Pesticide Std [Asp: Auto@75uL/sec, Disp: 25mm@60uL/sec]
c) Make up to Volume: 1000uL Water [Asp: Auto@75uL/sec, Disp: 25mm@60uL/sec]

Spike Pure Water [x2] {Task Group #4}

a) Reagent: 300uL of Pure Water (for needle dipping
b) Add Reagent: 125uL of 200ppb Pesticide Std [Asp: Auto@75uL/sec, Disp: 25mm@60uL/sec]
c) Make up to Volume: 1000uL Pure Water [Asp: Auto@75uL/sec, Disp: 25mm@60uL/sec]

Spiked River Water [x3] {Task Group #7}

a. Reagent: 300uL of River Water (for needle dipping)
b. Add Reagent: 125uL of 200ppb Pesticide Std [Asp: Auto@75uL/sec, Disp: 25mm@60uL/sec]
c. Make up to Volume: 1000uL River Water [Asp: Auto@75uL/sec, Disp: 25mm@60uL/sec]

µSPEed (Note: No Needle Dipping) {Task Group 9-15}

a. µSPEed Activate: 2 x 200uL IPA
b. µSPEed Condition: 1 x 100uL Pure Water
c. µSPEed Load: 500uL Standards, Sample (Waste, 25mm, Asp=75@60uL/sec, Disp: 16.7@60uL/sec)
d. µSPEed Wash: 100uL water
e. µSPEed Elute: 100uL IPA (25mm, Asp=75@60uL/sec, Disp: 16.7@60uL/sec)

ACKNOWLEDGEMENTS

Matthew Diplock, Prof Philip Doble[1],
[1]*University of Technology, Sydney - School of Mathematical and Physical Sciences, Faculty of Science.*

REFERENCE

USEPA, EPA. "METHOD 3500C ORGANIC EXTRACTION AND SAMPLE PREPARATION." *Environmental Protection Agency, USA* (2007).

USEPA, EPA. "EPA Method 3535A (SW-846): Solid-Phase Extraction" *Environmental Protection Agency, USA* (2007).

USEPA, EPA. "Method 8081B. Organochlorine pesticides by gas chromatography." *Environmental Protection Agency, USA* (2007).

µSPEed Ordering Information

Part Number	Code	Description
Silica Sorbents		
01-10110	µSPEed, C18RPS-3µm/120Å (Pkt 10)	3µm/ 120Å ODS spherical silica packing with high acidic resistance suitable for general organic compound applications.
01-10115	µSPEed, Silica-3µm/120Å (Pkt 10)	3µm/120Å spherical bare silica packing. High purity silica for normal and hilic applications
Speciality – Silica Sorbents		
01-10118	µSPEed, PFAS-3µm/120Å (Pkt 10)	3µm/ 120Å PFAS spherical silica packing. PFAS applications
01-10185	µSPEed, Cxyl-3µm/120Å (Pkt 10)	3µm/ Carboxyl spherical inert silica packing. Customisable chemistry applications
PS-PVB Sorbents		
01-10150	µSPEed, PS/DVB -3µm/ 300Å (Pkt 10)	3µm/ 300Å spherical, crosslinked polystyrene divinyl benzene
01-10151	µSPEed, PS/DVB RP-3µm/ 300Å (Pkt 10)	3µm/ 300Å Phenyl (RP) spherical, crosslinked polystyrene divinyl benzene
01-10155	µSPEed, PS/DVB SAX-3µm/ NP (Pkt 10)	3µm/Non Porous SAX spherical, crosslinked polystyrene divinyl benzene
01-10156	µSPEed, PS/DVB SCX-3µm/ NP (Pkt 10)	3µm/Non Porous SCX spherical, crosslinked polystyrene divinyl benzene