Use of Automated Solid Phase Extraction to Quantify Organochlorine Pesticides in Wastewater in Compliance with EPA Method 608.3

Biotage

Michael Ebitson[1], Andrew Taylor[1]
[1]Biotage, 16 Northwestern Dr, Salem, NH, USA
Andrew.taylor@biotage.com

Introduction

Many of the pesticides that were used decades ago have now been banned, due to their known impact on the health of animals and humans. To ensure our continued safety, it's important to be able to screen for pesticides in our food and water. The U.S. EPA has published several methods to outline the extraction and quantification of pesticides in water. Some of the methods cover a wide range of contaminants and include pesticide compounds – Methods 525.2, 525.3, 625.1, to name a few. Other methods are a bit more specific to pesticides – Method 549.2 for extracting paraquat and diquat compounds, and Method 608.3 for extracting organochlorine pesticides, for example. The extraction of pesticides can be challenging and processing samples by liquid-liquid extraction can result in poor accuracy and reproducibility. These challenges were taken into account for Method 608 and the latest revision (608.3) was published to formally allow the use of disk-based solid phase extraction (SPE) over liquid-liquid extraction (LLE). In addition to reducing solvent usage and streamlining the extraction process, SPE provides an opportunity to elute your target analytes directly into hexane.

This work will present data to validate the use of an automated solid phase extraction system to extract 21 pesticide compounds from wastewater samples. Target analytes were retained using C18 solid phase extraction media, and the extracts were dried, evaporated and concentrated for quantification by GC-ECD. The automated workflow increased sample throughput, while reducing solvent usage and maintaining compliance with EPA Method 608.3.

EPA Method 608.3

Overview:

- Method for extracting and quantifying organochlorine pesticides and PCBs in wastewater samples
- Uses GC/HSD (halogen specific detector) such as an electron capture detector (ECD) for quantification

Challenges:

- Waste water samples can form challenging emulsions when extracted by separatory funnel
- SPE of wastewater samples requires proper pre-filtration to ensure that the entire sample can be processed through the disk

Solid Phase Extraction Steps

Overview:

- Condition disk with DCM to ensure cleanliness of disk
- Activate C18 media in disk using MeOH, rinse with water
- Load sample, slowly if possible to maximize interaction time with media
- Air dry to remove residual water from the disk
- Elute with acetone – a transition solvent that is miscible with both water and DCM
- Elute with chlorinated solvent, such as DCM (like dissolves like)
- Dry, then concentrate extract to approximately 5 mL, solvent exchange into hexane since DCM will interfere with a halogen specific detector
- Analyze sample extract on a GC/HSD

Sample Preparation Workflow

Figure 1: Biotage® Horizon 5000 automated solid phase extraction system and TurboVap® II concentrator

Target Analytes

Compound	Compound
Alpha-BHC	4,4'-DDE
Gamma-BHC	Dieldrin
Beta-BHC	Endrin
Heptachlor	4,4'-DDD
Delta-BHC	Endosulfan II
Aldrin	4,4'-DDT
Heptachlor epoxide	Endrin Aldehyde
Endosulfan I	Endosulfan Sulfate

Table 1: Compounds analyzed from Table 1 of EPA method 608.3. This list includes all of the Priority Pollutant pesticides.

Disk Holder Setup

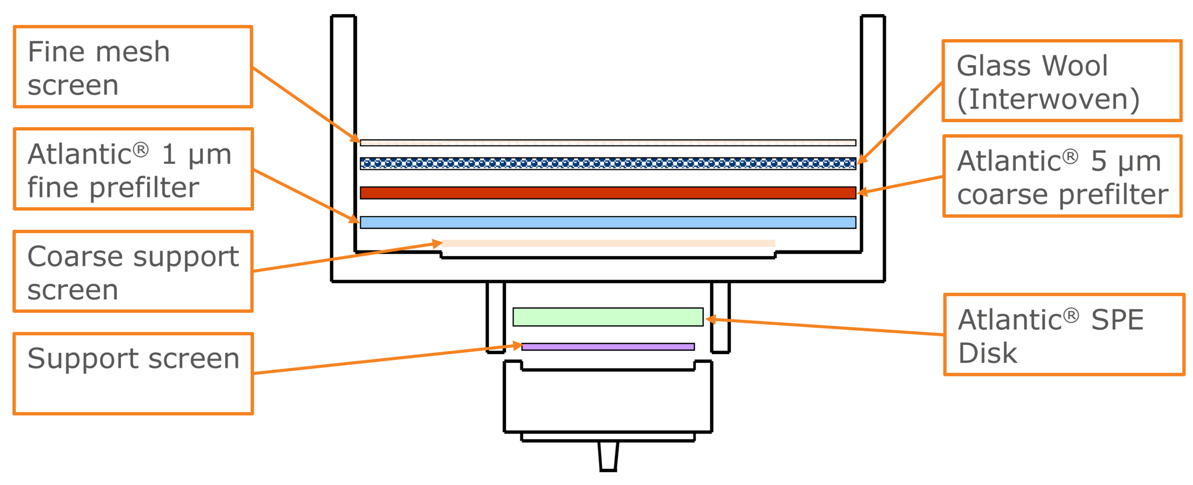

Figure 2: Configuration of the fast flow disk holder for a very challenging sample matrix

Applicable pre-filter materials for samples with high levels of particulates:

- Atlantic® 1 µm fine pre-filter
- Atlantic® 5 µm coarse pre-filter
- Glass wool
- Fine mesh screen (for large visible particulates)
- Filter aid (such as diatomaceous earth)

Display system (Figure 1) equipped with:

- Fast flow disk holders
- 8270/625.1 One-Pass kit (not necessary for 608.3)
- Cap adapters that fit to various bottle neck sizes

Extraction Method

Step	Solvent	Volume (mL)	Purge Time (s)	Pump Rate (#)	Sat. Time (s)	Soak Time (s)	Drain Time (s)
Condition SPE Disk	DCM	40	60	6	3	60	120
Condition SPE Disk	MeOH	40	60	3	3	60	10
Condition SPE Disk	MeOH	20	30	3	3	30	10
Condition SPE Disk	DI Water	20	60	3	3	15	10

Step	Sample Flow Rate (#)	Done Loading Sample Delay (s)
Load Sample	4	60

Step	Dry Time (s)	Pump Rate (#)	N_2 Blanket
Air Dry Disk	600	6	Off

Step	Solvent	Volume (mL)	Purge Time (s)	Pump Rate (#)	Sat. Time (s)	Soak Time (s)	Drain Time (s)
Elute Sample Container	Acetone	40	20	3	3	60	90
Elute Sample Container	DCM	40	15	3	3	60	90
Elute Sample Container	DCM	40	15	3	3	60	90
Elute Sample Container	DCM	40	15	6	3	60	120

Table 2: Biotage® Horizon 5000 method for EPA Method 608.3 when equipped with a fast flow disk holder

QC Performance

- A method blank and four replicate spikes were extracted and analyzed in order to complete the initial demonstration of capability. The concentration of the analytes of interest varied. The concentration of each analyte is listed in **Table 3**
- Three separate MDL studies were performed; one at 0.005 ppb, one at 0.01ppb and one at 0.02ppb. Each study consisted of seven replicates of each concentration listed above. The standard deviation as well as the appropriate Student's t value were used to calculate the MDL. The data for the 0.01 ppb study is listed in **Table 4**.

Benefits of Solid Phase Extraction (SPE) for 608.3

- Reduced solvent volume from 180 mL for SFLLE to 40 mL for SPE
- SPE dramatically reduces the chance of forming an emulsion
- Completely automated SPE reduces the workload for a technician, freeing them up to perform other extractions or analyses
- Reduced solvent volume means less DCM emissions during a time of ever-growing regulations around DCM usage

Initial Demonstration of Capability (IDC)

Compound	Avg % Rec (4 replicates)	Method Acceptance Criteria (%)	Spike Conc (µg/L)	RSD (%)
alpha-BHC	77.9	49-130	25	1.4
gamma-BHC	81.0	43-130	25	1.1
beta-BHC	85.5	39-130	25	0.5
Heptachlor	76.6	43-130	25	0.6
delta-BHC	67.8	51-130	25	0.4
Aldrin	73.3	54-130	25	1.1
Heptachlor epoxide	82.0	57-132	25	0.2
Endosulfan I	103.8	57-141	25	1.0
4,4'-DDE	90.2	54-130	50	0.8
Dieldrin	93.4	58-130	50	1.1
Endrin	98.8	51-130	50	1.4
4,4'-DDD	98.9	48-130	50	0.6
Endosulfan II	96.5	22-171	50	0.6
4,4'-DDT	103.0	46-137	50	1.3
Endrin aldehyde	89.9	N/A	50	1.2
Endosulfan sulfate	92.5	38-132	50	0.3
Decachlorobiphenyl (surrogate)	87.7	N/A	100	0.9

Table 3: IDC study performed at various concentrations

Method Detection Limit (MDL)

Compound	Avg % Rec (4 replicates)	Method Acceptance Criteria (%)	RSD (%)	Std Dev (s)	MDL (µg/L)
alpha-BHC	74.3	49-130	7.2	0.001	0.002
gamma-BHC	74.3	43-130	7.2	0.001	0.002
beta-BHC	48.6	39-130	22.0	0.001	0.003
Heptachlor	100.0	43-130	---	0.000	0.001*
delta-BHC	54.3	51-130	9.8	0.001	0.002
Aldrin	70.0	54-130	---	0.000	0.001*
Heptachlor epoxide	80.0	57-132	---	0.000	0.001*
Endosulfan I	95.7	57-141	5.6	0.001	0.002
4,4'-DDE	90.0	54-130	---	0.000	0.001*
Dieldrin	90.0	58-130	---	0.000	0.001*
Endrin	95.7	51-130	5.6	0.001	0.002
4,4'-DDD	80.0	48-130	---	0.000	0.001*
Endosulfan II	70.0	22-171	---	0.000	0.001*
4,4'-DDT	120.0	46-137	---	0.000	0.001*
Endrin aldehyde	64.3	N/A	8.3	0.001	0.002
Endosulfan sulfate	107.1	38-132	20.0	0.002	0.007

Table 4: MDL study performed at 0.01µg/L
**Not enough significant figures to report value, rounded up to nearest decimal*

Conclusions

- The completely automated Biotage® Horizon 5000 solid phase extraction system showed phenomenal accuracy and precision in both the initial demonstration of capability and the method detection limit study per EPA Method 608.3
- Given the proper pre-filter technique, even the most challenging wastewater samples can be processed using disk based solid phase extraction
- IDC studies were performed at varying concentrations (25 and 50 ppb) yielding recovery values well above the method acceptance criteria

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