Reduced Sample Volume Extractions for US EPA Method 608.3

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Introduction

Pesticides, including insecticides, fungicides, and herbicides, are used extensively to increase agricultural yield. It is important to monitor organochlorine pesticides and polychlorinated biphenyls (PCBs) not just in drinking water (US EPA Method 508), but also in wastewater (US EPA Method 608.3). These wastewater samples can be heavily laden with particulates and sludge, making it difficult to extract an entire liter. This work provides a solution for extracting 100 mL of 608.3 wastewater samples utilizing the Biotage® Horizon 5000, DryDisk® and the TurboVap® II.

Procedure

1. Obtain the 100 mL samples of DI water that will be extracted. Acidify to a pH <2 with HCl. For Matrix Spike (MS) and Matrix Spike Duplicate (MSD) test samples only, 2 mg of oil and grease (hexadecane and stearic acid) was added to the acidified DI water.
2. Fortify samples with surrogates decachlorobiphenyl (DCB) and tetrachloro-m-xylene (TCMX). Fortify laboratory control spike (LCS), MS, and MSD samples with target analytes.
3. Run Biotage® Horizon 5000 extraction method (Table 1).
4. Pour each extract into the DryDisk®-R glass reservoir and open the stopcock. Allow the extract to flowthrough completely before rinsing the collection vessel three times with methylene chloride.
5. Transfer dried extract to a concentration tube and concentrate using the TurboVap® II.
6. Concentrate to end-point (approximately 0.7 mL) following the methylene chloride concentration parameters found in Table 2.
7. Add 10 mL of hexane directly into the tip of the evaporation tube using a glass pipette and swirl well for the solvent exchange step.
8. Concentrate to end-point again following the hexane concentration parameters found in Table 2 and bring extracts up to 2 mL with hexane.
9. For PCB extracts only, perform a sulfuric acid cleanup.
10. For pesticides and PCB MS extracts, perform a copper cleanup.
11. For pesticides and PCB Method Detection Limit (MDL), Laboratory Control Spike (LCS), and MS extracts, perform a florisil cleanup.
12. Analyze by GC/ECD.

Image 1. *Biotage® Horizon 5000 (left), Atlantic® C18 ReadyDisk (center), and TurboVap® II (right)*

Table 1. *Biotage® Horizon 5000 Extraction Method.*

Step	Description	Solvent	Volume (mL)	Purge (s)	Pump Speed	Saturate (s)	Soak (s)	Drain (s)	Delay (s)
1	Condition SPE Disk	Methylene Chloride	20	60	4	1	60	60	
2	Condition SPE Disk	Acetone	20	60	2	1	60	30	
3	Condition SPE Disk	Methanol	20	60	2	2	60	10	
4	Condition SPE Disk	Reagent Water	20	60	2	1	45	10	
5	Load Sample				2				45
6	Wash Sample Container	Reagent Water	15	60	6	2	20	30	
7	Air Dry				6			360	
8	Elute Sample Container	Acetone	3	15	2	1	60	45	
9	Elute Sample Container	Methylene Chloride	5	15	2	1	60	45	
10	Elute Sample Container	Methylene Chloride	5	15	6	1	60	60	

Table 2. *TurboVap® II Concentration Parameters. EP = End-Point*

Settings					
Methylene Chloride Concentration Parameters (Pre-Solvent Exchange)			Hexane Concentration Parameters (Post-Solvent Exchange)		
Gradient	EP Detection	Bath Temp	Gradient	EP Detection	Bath Temp
Ramp	On	60 °C	Ramp	On	60 °C
Method	Flow (L/min)	Time (min)	Method	Flow (L/min)	Time (min)
Step 1	3.0	9	Step 1	4.2	2
Step 2	5.0	1	Step 2	5.0	1

GC Conditions

GC: 6890 HP Dual GC (Agilent Technologies Inc.)
Column 1: Restek Rtx-CLPesticides, 30 m, 0.32 mm ID, 0.32 µm film thickness
Column 2: Restek Rtx-CLPesticides2, 30 m, 0.32 mm ID, 0.32 µm film thickness
Carrier Gas: Helium 4.9 mL/min (constant flow)
Inlet: 50 °C, Pulsed Splitless, Pulse Pressure: 28.0 psi; Pulse Time: 1.0 min; Purge Flow: 60.4 mL/min at 0.75 min
Injection volume: 1 µL (Pesticides); 4 µL (PCB)
Oven conditions: Initial temperature 125 °C, hold for 0.5 minute. Ramp 45 °C/min to 200 °C hold for 0 minutes, 12.5 °C/min to 230 °C hold for 0 minutes, 30 °C/min to 300 °C hold for 1.5 minutes.
Oven Post Temperature: 120 °C

Micro ECD Conditions

ECD: Micro ECD (Agilent Technologies Inc.)
Micro ECD 1 and 2 Temperature: 350 °C
Micro ECD 1 Makeup Flow Setpoint: 150.0 mL/min
Micro ECD 2 Makeup Flow Setpoint: 105.0 mL/min
Makeup Flow Gas Type: Nitrogen

Results

To demonstrate that acceptable method detection limits (MDLs) can be achieved with 100 mL reduced sample volume extractions, laboratory defined practical quantitation limits (PQLs) were utilized as a benchmark for acceptance criteria (Table 3). To determine the accuracy and precision of the sample preparation process, four samples were prepared at mid to high level concentrations. Due to the varying recovery values of EPA method acceptance criteria for each analyte, the interim acceptance criteria of 60-140% (Section 8.4.5) was used as the limits on Figure 1 for ease of observation. In addition, the lowest percent standard deviation %SD limit within EPA method 608.3 is 22%. This percent was used as the limit on Figure 2 for ease of observation.

Table 3. *Method Detection Limits (n=7)*

Analyte	Spike Amount (ng/L)	Column 1 - MDL (ng/L)	Column 2 - MDL (ng/L)	Maximum PQL (ng/L)	Acceptance Criteria
PCB 1016	100	32	60	65	PASS
PCB 1260	100	42	39	65	PASS
PCB 1221	200	68	116	130	PASS
PCB 1254	100	16	22	65	PASS
PCB 1242	100	22	29	65	PASS
PCB 1248	100	12	12	65	PASS
PCB 1268	100	19	23	65	PASS
4,4´-DDD	100	14	29	100	PASS
4,4´-DDE	100	13	17	100	PASS
4,4´-DDT	100	26	21	100	PASS
Aldrin	100	14	12	50	PASS
alpha-BHC	100	35	14	50	PASS
alpha-Chlordane	100	23	14	50	PASS
beta-BHC	100	33	29	50	PASS
delta-BHC	100	11	30	50	PASS
Dieldrin	100	13	11	100	PASS
Endosulfan I	100	10	10	50	PASS
Endosulfan II	100	22	20	100	PASS
Endosulfan sulfate	100	28	13	100	PASS
Endrin	100	29	13	100	PASS
Endrin aldehyde	100	37	31	100	PASS
Endrin Ketone	100	64	11	100	PASS
gamma-BHC	100	17	15	50	PASS
gamma-Chlordane	100	14	36	50	PASS
Heptachlor	100	21	15	50	PASS
Heptachlor epoxide	100	19	10	50	PASS
Methoxychlor	100	47	31	500	PASS
Chlordane	200	32	44	50	PASS
Toxaphene	1000	281	273	1000	PASS

Figure 1. *Initial Demonstration of Accuracy (n=4)*

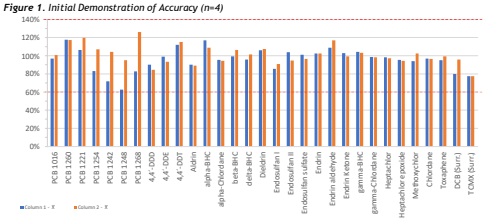
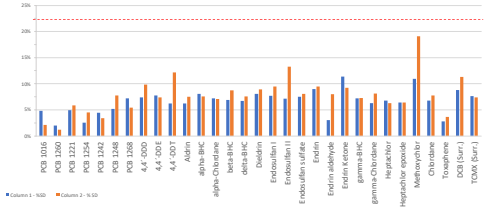


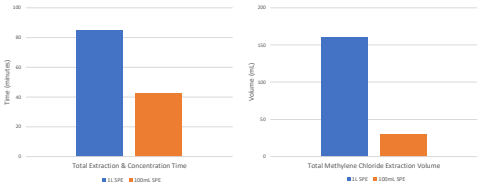
Figure 2. *Initial Demonstration of Precision (n=4)*



Reduced Sample Volume Benefits

When reducing sample volumes from 1 L to 100 mL, the extraction, concentration time and solvent volumes were significantly reduced. Figure 3 illustrates the differences in time and volume between extracting 1 L and 100 mL samples.

Figure 3. *Processing Time and Solvent Consumption*



Conclusion

This work demonstrates the ability to extract concentrate and analyze 100 mL samples while meeting the requirements of EPA Method 608.3. Precision & Accuracy achieved at reduced sample volumes far exceeded the acceptance criteria found within EPA method 608.3. The Biotage® Horizon 5000 system provides automated disk based solid phase extraction for challenging matrices. The reduction of sample volume further improves automated extractions with decreased sample processing time as well as solvent usage.

Biotage® Horizon 5000 **TurboVap®** **EPA 608.3 RSV**