Determining Organic Compounds in Drinking Water Utilizing the Biotage® Horizon 5000 and TurboVap® II

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Introduction

This application note will outline a procedure for the extraction and concentration of organic compounds in drinking water utilizing the Biotage® Horizon 5000, TurboVap® II with End-Point Sensors, and Atlantic® C18 SPE Disks. To demonstrate compliance, the limits set in EPA Method 525.2[1] were used as a guideline and an initial demonstration of capability (IDOC) as well as method detection limit (MDL) study was performed.

When SPE is performed manually, unintentional errors can occur throughout the process. The same can be said when concentrating extracts, such as inconsistent final volumes or uneven nitrogen delivery into sample tubes. These factors can end up in skewed results and relative standard deviations, resulting in possible method criteria failures.

The proposed solution outlined below will eliminate manual intervention throughout the extraction process while delivering a very reliable, efficient and consistent means of concentrating while demonstrating its ability to comply to the EPA Method 525.2 guidelines.

Instrumentation

Biotage Instruments and Consumables

- Biotage® Horizon 5000 Automated Extraction System (P/N SPE-DEX 5000)
- TurboVap® II (P/N 415100)
- TurboVap® II Rack with End-Point Sensors, 6 positions, 200 mL tubes (P/N: 415100)
- Atlantic® C-18 SPE Disk, 47 mm (P/N 47-2346-02)
- Evaporation Tube TurboVap® II, 200 mL, 1 mL End-Point (P/N C42567)

GC/MS

- Agilent 6890 GC
- Agilent 5793 MSD

Method Summary

1. Purge the extractor with the method listed in table 1.
2. Obtain 1 L water samples and adjust to pH 2 using HCl.
3. Add 0.05 g of sodium sulfite (Na_2SO_3) and 5 mL of methanol to each sample.
4. Spike each sample with the appropriate amount of 525.2 Internal Standard Mix that has been diluted to 500 µg/mL.
5. Spike any laboratory control or matrix spike samples with the appropriate amount of standard.
6. Place the sample bottle on the Biotage® Horizon 5000 Extractor System and place the Atlantic C-18 disk in the standard 47mm disk holder. Attach collection vessels to the system.
7. Extract the samples using the method in table 2 and collect the final sample extract.
8. Dry the final sample extract by passing it through sodium sulfate (Na_2SO_4) and transfer to a clean 200 mL TurboVap® 1 mL End-Point tube.
9. Concentrate the extract to a final volume of 1 mL using the TurboVap® II and the settings in table 3.
10. Spike each extract with the appropriate amount of 525.2 External Standard Mix that has been diluted to 500 µg/mL.
11. Transfer the extract to a 2.0 mL GC vial and analyze via GC/MS in scan mode. The conditions in table 4 were used for analysis, however any GC/MS and column capable of producing results equivalent to or better may be used. See manufacturer information for suggested settings specific to the GC/MS or column that is used for analysis.

Table 1. Purge Method for Biotage® Horizon 5000.

Step	Select Solvent	Volume (mL)	Purge (s)	Vacuum	Saturate (s)	Soak (s)	Drain/Elute (s)
Elute Sample Container	Reagent Water	10	60	2	0	0	15
Elute Sample Container	Methanol	10	60	2	0	0	15
Elute Sample Container	Ethyl Acetate	10	60	2	0	0	15
Elute Sample Container	Dichloromethane	10	60	2	0	0	15

Table 2. Extraction Method for Biotage® Horizon 5000.

Step	Select Solvent	Volume (mL)	Purge (s)	Vacuum	Saturate (s)	Soak (s)	Drain/Elute (s)	Sample Delay(s)
Condition SPE Disk	Dichloromethane	15	60	2	1	20	30	
Condition SPE Disk	Ethyl Acetate	11	60	2	1	20	30	
Condition SPE Disk	Methanol	11	60	2	1	60	2	
Condition SPE Disk	Reagent Water	9	30	2	1	5	5	
Condition SPE Disk	Reagent Water	9	60	2	1	30	0	
Load Sample				2				45
Air Dry Disk				6			60	
Elute Sample Container	Ethyl Acetate	8	60	2	1	30	45	
Elute Sample Container	Dichloromethane	8	15	2	1	30	45	
Elute Sample Container	Dichloromethane	8	15	2	1	30	45	
Elute Sample Container	Dichloromethane	8	15	6	2	30	60	

Table 3. TurboVap® II Settings.

Parameter	Setting
Water Bath Temperature	40 °C
Inlet Nitrogen Pressure	87 psi
Gas Flow	2.8 mL/min
Evaporation Mode	End-Point

Table 4. GC Settings.

Step		Oven Temperature Ramp		
Carrier Gas	Helium	Temp (C)	Rate (C/min.)	Hold (min.)
Flow Rate	9 psi			
Flow Mode	Constant	45	0	1:00
Injection Amount	1 µL	270	15	0:00
Injection Temp.	280 °C	320	6	0:00
Split Ratio	1:10	**Total Run Time:**		**24:33**

Results and Discussion

To demonstrate compliance, the limits set in EPA Method 525.2 were used as a guideline and an initial demonstration of capability (IDOC) as well as method detection limit (MDL) study was completed. The IDOC consisted of four replicates spiked at a concentration of 5.0 ppb where percent recovery must fall within 70–130% and the RSD must be ≤ 30%. The MDL consisted of seven replicates spiked at 0.5 ppb which are used to calculate the minimum amount that can be measured with 99% confidence that the reported value is greater than zero. These values are all found within table 5 below.

Excluding hexachlorocyclopentadiene, carboxin, atraton and prometon, all results observed in table 5 fall within a range of 70–130%. Even including the trouble compounds, the average percent recovery for all compounds across the study was 96.9%. The relative standard deviation is < 20%, with a majority of compounds under 5% RSD. The calculated MDLs were all roughly comparable to the MDL values outlined in EPA method 525.2 which this application is based upon.

Hexachlorocyclopentadiene's low recovery can be attributed to the compound's sensitivity to thermal and photochemical degradation. The recovery of carboxin can be attributed to its significant instability in water. The low recoveries for atraton and prometon likely stem from inefficient extraction from the water at pH 2, which causes ionization in solution under acidic conditions.

Table 5. Extraction Results
(4 IDOC samples @ 5.0 ppb, 7 MDL samples @ 0.5 ppb).

Target Compounds	IDOC Avg. % Recovery	IDOC % RSD	Calculated MDL (µg/L)
Acenaphthene d10 **(Internal Standard)**	84.25	10.27%	-
Phenanthrene d10 **(Internal Standard)**	91.45	10.77%	-
Chrysene d12 **(Internal Standard)**	89.95	9.04%	-
Isophorone	96.65	1.41%	0.04
Dichlorvos	95.00	1.90%	0.05
Hexachlorocyclopentadiene	47.35	6.62%	0.12
EPTC	101.30	1.50%	0.02
Mevinphos	102.70	2.46%	0.05
Butylate	100.30	1.39%	0.04
Vernolate	101.85	1.81%	0.04
Dimethyl phthalate	103.65	0.62%	0.05
Pebulate	101.85	1.52%	0.05
Etridiazole	99.75	2.77%	0.02
2,6-Dinitrotoluene	74.15	4.73%	0.04
Chloroneb	105.65	3.17%	0.06
Tebuthiuron	116.45	2.60%	0.09
2,4-Dinitrotoluene	76.25	4.50%	0.03
Molinate	103.95	1.79%	0.05
Diethyl phthalate	108.85	1.29%	0.06
Fluorene	101.85	1.65%	0.06
Propachlor	108.80	1.33%	0.04
Ethoprop	110.20	2.24%	0.07
Cycloate	106.60	0.73%	0.05
Chlorpropham	110.10	1.05%	0.06
Trifluralin	97.85	1.94%	0.04
a-BHC	101.20	1.41%	0.06
Atraton	51.45	2.66%	0.05
Hexachlorobenzene	92.60	3.84%	0.05

Target Compounds	IDOC Avg. % Recovery	IDOC % RSD	Calculated MDL (µg/L)
Prometon	61.85	2.67%	0.05
Lindane (g-BHC)	102.10	2.11%	0.04
Simazine	98.80	1.82%	0.07
Atrazine	109.70	5.17%	0.07
Propazine	110.65	0.90%	0.04
b-BHC	97.10	2.05%	0.11
Pentachlorophenol	105.20	1.58%	0.02
Terbufos	94.65	1.70%	0.14
Pronamide	101.85	1.04%	0.03
Diazinon	75.95	15.23%	0.03
d-BHC	101.35	1.74%	0.04
Phenanthrene	98.95	2.23%	0.03
Disulfoton	76.75	1.99%	0.41
Methyl paraoxon	98.00	1.52%	0.04
Anthracene	71.80	17.46%	0.02
Terbacil	114.75	1.68%	0.05
Chlorothalonil	95.20	4.64%	0.03
Metribuzin	94.45	3.29%	0.06
Simetryn	95.45	2.86%	0.13
Heptachlor	93.45	3.65%	0.08
Ametryn	95.90	1.10%	0.13
Alachlor	103.65	1.32%	0.04
Prometryn	96.80	1.16%	0.14
Terbutryn	97.20	0.98%	0.14
Di-n-butyl phthalate	102.50	1.47%	0.05
Bromacil	98.95	1.53%	0.08
Cyanazine	94.20	1.89%	0.08
Metolachlor	100.65	1.36%	0.04
Chlorpyrifos	96.30	1.77%	0.05
Aldrin	90.10	5.50%	0.08

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Table 5. Continued.

Target Compounds	IDOC Avg. % Recovery	IDOC % RSD	Calculated MDL (µg/L)
Triademefon	102.35	0.49%	0.06
Dacthal	101.25	1.30%	0.05
Diphenamid	103.85	0.99%	0.03
Merphos	104.50	3.90%	0.22
g-Chlordane	97.20	2.92%	0.05
Stirofos	106.95	3.95%	0.16
Disulfoton sulfone	105.10	3.97%	0.05
Butaclor	103.50	3.07%	0.05
a-Chlordane	97.05	2.98%	0.08
Endosulfan I	97.1	4.2%	0.48
Fenamiphos	100.00	5.43%	0.20
Pyrene-d10 (Surrogate)	95.65	2.70%	-
Pyrene	99.45	3.08%	0.06
Napropamide	103.35	2.40%	0.07
trans-Nonachlor	91.70	3.16%	0.07
4,4'-DDE	94.05	4.04%	0.08
Dieldrin	103.40	0.82%	0.07
Tricyclazole	97.10	2.81%	0.05
Terphenyl-d14 (External Standard)	111.80	9.04%	-
Carboxin	42.45	18.37%	0.20
Endrin	103.50	1.57%	0.07
Chlorobenzilate	107.15	3.31%	0.06
Endosulfan II	102.50	2.52%	0.08

Target Compounds	IDOC Avg. % Recovery	IDOC % RSD	Calculated MDL (µg/L)
4,4'-DDD	97.70	3.30%	0.05
Butyl benzyl phthalate	104.40	3.05%	0.06
Norflurazon	104.90	3.30%	0.06
4,4-DDT	97.70	3.30%	0.05
Endosulfan Sulfate	105.15	2.29%	0.07
Bis(2-ethylhexyl)adipate	96.70	4.34%	0.06
Hexazinone	107.95	2.10%	0.07
Triphenylphosphate (Surrogate)	103.40	2.77%	-
Endrin Ketone	100.45	2.64%	0.07
Methoxychlor	98.20	2.60%	0.05
Benz(a)anthracene	94.45	4.73%	0.05
Chrysene	96.50	3.40%	0.05
Bis(2-ethylhexyl)phtha...	101.10	4.50%	0.31
Fenarimol	105.05	2.99%	0.05
cis-Permethrin	98.05	4.28%	0.06
trans-Permethrin	94.90	2.69%	0.06
Benzo(b)fluoranthene	97.55	3.83%	0.06
Benzo(k)fluoranthene	96.30	3.96%	0.06
Benzo(a)pyrene	79.90	6.34%	0.07
Fluridone	108.65	4.33%	0.06
Perylene-d12 (Surrogate)	70.05	10.88%	-
Indeno(1,2,3-cd)pyrene	94.05	4.04%	0.06
Dibenz(ah)anthracene	93.30	4.32%	0.06
Benzo(ghi)perylene	96.40	4.25%	0.08

Conclusion

The results in table 5 demonstrate the Biotage workflow solution is capable of fully automating the extraction of organic compounds from drinking water. The resulting data is both accurate and precise while achieving EPA 525.2 method limits. This solution not only eliminated the sample to sample variation that is experienced with manual techniques, but it frees up time for users to perform other tasks within the laboratory.

The Biotage solution including the Biotage® Horizon 5000, TurboVap® II, and Atlantic® C18 SPE Disks reduce analyst labor, solvent usage, turnaround time, and improves accuracy and precision when compared to manual SPE extraction methods. All of these qualities improved method performance while reducing the total cost of a sample to a laboratory.

References

1. United States Environmental Protection Agency, Method 525.2, Revision 2.0: Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry.

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