Extraction of Semi-volatile Organic Compounds in Drinking Water with NEW Atlantic[®] ReadyDisk DVB Solid Phase Extraction Disks in Compliance with EPA Method 525.3

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Introduction

The U.S. EPA has been regulating semi-volatile organic compounds in drinking water with Method 525 since 1988. There have been several method updates since then, but the most recent was introduced in February of 2012. EPA method 525.3 is an update to 525.2 that includes a few major changes including: discontinuing the use of a C18 disk in favor of specific DVB or SDVB disks for extraction, modifying the sample preservation procedure and requiring the addition of internal standards immediately following the extraction.

Per Method 525.3, an Initial Demonstration of Capability (IDC) must be performed prior to processing any samples. An IDC for Method 525.3 includes a demonstration of low background noise, precision, and accuracy. Each laboratory must also set and test a minimum reporting level (MRL) based on their application needs. In Method 525.3 the Detection Limit (DL) does not have to be determined during the initial demonstration of capability; however, it is a figure of merit which continues to be required by many regulatory bodies¹.

In this application note, Atlantic[®] ReadyDisk DVB solid phase extraction disks will be used in combination with a Biotage[®] Horizon 5000 to extract semi-volatile organic compounds in drinking water samples. All results, based on GC/MS analyses, demonstrate compliance with the performance requirements outlined in EPA Method 525.3

Instrumentation

Table 1. Sample Preparation and Data Collection.

Analysis	
GC Instrument	Agilent 6890 with 5975C Inert GC/MSD
Sample Preparation	
Extraction System	Biotage [®] Horizon 5000
Solvent Evaporation System	XcelVap [®]

Experimental

Method Summary

A summary of the overall sample preparation, extraction, drying and concentration procedure is listed below. A detailed overview of the method run on the Biotage[®] Horizon 5000 is listed in Table 2. The XcelVap[®] and Agilent GC/MS parameters are listed in Table 3 and 4, respectively.

- 1. Obtain 1-liter of drinking water.
- 2. Add 0.10 g L-ascorbic acid and 0.35 g EDTA to each 1-liter sample.
- 3. Buffer each 1-liter water sample to approximately pH 3.8 using 9.4 g potassium dihydrogen citrate.
- 4. Add surrogate and standard compounds into the samples.
- 5. Start extraction method shown in Table 2 and collect extract (~13 mL).
- 6. Dry each extract with sodium sulfate.
- 7. Evaporate each extract to 0.9 mL using the XcelVap[®] using the method listed in Table 3.
- 8. Add internal standards to the extracted solution.
- 9. Quantitatively, bring extract volume to 1.0 mL using ethyl acetate.
- 10. Transfer the extract to a 2.0 mL GC vial.
- 11. Analyze the solution using GC/MS method in Table 4.



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Table 2. Biotage® Horizon 5000 Extraction Program.

Table 2. Diotage Holizon 50	oo Extraction Program							
Step	Solvent		lvent ne (mL)	Purge Time (s)	Pump Rate (#)	Saturation Time (s)	Soak Time (s)	Drain Time (s)
1. Condition SPE Disk	Ethyl Acetate		5	60	2	1	60	30
2. Condition SPE Disk	Methanol		10	60	2	1	60	2
3. Condition SPE Disk	Reagent Water		10	60	2	1	5	2
Step	Sample Pump Speed, # Done Loading Sample Delay (s)							
4. Load Sample		2 (approximate	ely 70 mL/min)		45	
Step	Solvent		lvent ne (mL)	Purge Time (s)	Pump Rate (#)	Saturation Time (s)	Soak Time (s)	Drain Time (s)
5. Wash Sample Container	Reagent Water		10	30	2	1	0	0
Step	Dry 1	Гime (s)	P	oump Speed	(#)		N₂ Blanket	
6. Air Dry Disk Timer		180		6			Off	
Step	Solvent	Solvent Volume (mL)	Purge Time (s)	Pump Speed (#)	N ₂ Blanket	Saturation Time (s)	Soak Time (s)	Elute Time (s)
7. Elute Sample Contain	er Acetone	2	45	2	Off	1	0	30
8. Elute Sample Contain	er Ethyl Acetate	5	30	2	Off	1	60	45
9. Elute Sample Contain	er Methylene Chloride	5	15	6	Off	1	60	60

 Table 3. XcelVap® Concentration Method.

Step	Pressure (psi)	Time (h:mm)	Temperature (°C)
Step 1	16-24	0:12	40
Step 2	24–24	0:17	40

Table 4. GC/MS Parameters.

Parameter	Setting
Injection Volume	1 µL
Inlet Temperature	245 °C
Injection Mode	Splitless
Gas Type	Helium
GC Column	Zebron $$ ZB-Semi Volatiles (Phenomenex), 30 m, 0.25 mm, 0.25 μm
GC Mode	Consistent Flow 1 mL/min
Oven Program	70 °C hold for 0.5 minutes Ramp 16 °C/min to 190 °C Ramp 8 °C/min to 290 °C Ramp 25 °C/min to 325 °C Hold for 3 minutes
MS Ions Monitored	35-550 AMU



Results and Discussion

Per EPA Method 525.3, a series of laboratory reagent blanks (LRBs) were measured to demonstrate a lack of contamination from the extraction system and the Atlantic[®] ReadyDisk DVB, prior to analyzing any samples. Six replicate LRBs were prepared and extracted as described in EPA Method 525.3, following the procedure in the method summary in this note. All blanks were spiked with surrogate and internal standards such that their final concentration in solution was 5 µg/L. The results for the six LRBs are shown in Table 5 below.

To demonstrate an Initial Demonstration of Capability (IDC), six replicates of a laboratory fortified blank (LFB) were prepared and extracted as described in EPA Method 525.3. Each replicate contained all analytes of interest, including internal standards and surrogates, at 5 μ g/L. For each measured analyte and surrogate, the mean accuracy, expressed as a percentage of the true value, should be 70–130 % and the RSD should be less than 30 %, per Method 525.3 Results for the six samples are shown in Table 5 below.

Seven additional laboratory fortified blanks were prepared such that all analytes of interest were present at approximately 0.5 μ g/L. All seven replicates were analyzed to produce data for calculating method detection limits (DL).

Method Detection Limits (MDLs) were calculated based on the measured LFB solutions and are reported in Table 5 below. Results are based on the standard deviation of the replicate measurements, multiplied by the appropriate Student's t value for the 99 % confidence interval. Results are reported Not Detected (ND) if the measured concentration for all samples were below the lowest calibration point of 0.1 μ g/L.

The method detection limits (MDL) were calculated using the formula1:

MDL = S x t (n-1, 1 alpha 0.99)

Where:

t = Student's t value for the 99% confidence level

(n-1,1-alpha = 0.99) with n-1 degrees of freedom

n = number of replicates

S = standard deviation of replicate analyses

Table 5. IDC, Precision, Accuracy, DL and LRB results for the Atlantic® ReadyDisk DVB.

Analyte	Average Recovery (%) n=6	RSD (%) n=6	DL (μg/L) n=7	Blank (µg/L) n=6
1,3-Dimethyl-2-nitrobenzene (SUR)	78.0%	6.7%	N/A	4.23
13C6 Pentachlorophenol (IS)	90.0%	2.0%	N/A	4.23
2,2',3,4,4',5,5'-Heptachlorobiphenyl	77.4%	2.7%	0.16	ND
2,2',3,4,4',5'-Hexachlorobiphenyl	76.7%	2.1%	0.13	ND
2,2',3,4',5',6-Hexachlorobiphenyl	74.2%	2.1%	0.10	ND
2,2',3,5'-Tetrachlorobiphenyl	74.7%	3.1%	0.05	ND
2,2',4,4',5,5'-Hexachlorobiphenyl	76.1%	2.1%	0.10	ND
2,2',5,5'-Tetrachlorobiphenyl	73.9%	3.3%	0.07	ND
2,2',5-Trichlorobiphenyl	69.2%	3.5%	0.07	ND
2,3,3',4',6-Pentachlorobiphenyl	77.6%	2.4%	0.09	ND
2,3',4,4',5-Pentachlorobiphenyl	79.4%	2.4%	0.12	ND
2,3',4',5-Tetrachlorobiphenyl	81.7%	2.6%	0.05	ND
2,4,4'-Trichlorobiphenyl	79.7%	3.2%	0.04	ND
2,4'-Dichlorobiphenyl	77.9%	3.0%	0.01	ND
2,4-Dinitrotoluene	91.4%	2.8%	0.07	ND
2,6-Dinitrotoluene	90.4%	2.6%	0.08	ND
2-Chlorobiphenyl	74.3%	3.5%	0.04	ND
4,4'-DDD	77.0%	2.7%	0.12	ND
4,4'-DDE	79.0%	2.6%	0.07	ND
4,4'-DDT	83.4%	3.0%	0.13	ND
4-Chlorobiphenyl	79.9%	3.2%	0.05	ND
Acenaphthene-d10 (IS)	98.7%	1.1%	N/A	4.81
Acenaphthylene	79.1%	3.7%	0.06	ND
Acetochlor	85.4%	2.7%	0.05	ND
a-HCH	78.4%	2.8%	0.05	ND
Alachlor	81.1%	2.7%	0.07	ND



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•	Ethoprop	89.6%	2.1%	0.10	ND
Etridiazole 77.0% 2.6% 0.06 ND	Ethyl Parathion	92.1%	3.4%		ND
	Etridiazole	77.0%	2.6%	0.06	ND



Analyte	Average Recovery	RSD (%)	DL (μg/L)	Blank (µg/L)
	(%) n=6	n=6	n=7	n=6
Fenarimol	107.0%	3.0%	0.09	ND
Fluorene	83.4%	2.9%	0.04	ND
Fluridone	95.9%	2.2%	0.13	0.04
Heptachlor	84.3%	3.2%	0.04	ND
Heptachlor epoxide	70.4%	3.4%	0.18	ND
Hexachlorobenzene (HCB)	68.7%	2.6%	0.05	ND
Hexachlorocyclopentadiene (HCCPD)	49.2%	13.2%	0.12	ND
Hexazinone	99.1%	2.8%	0.03	ND
Indeno[1,2,3-c,d]pyrene	79.7%	1.9%	0.18	ND
Isophorone	90.9%	3.3%	0.08	ND
Lindane	76.7%	3.4%	0.04	ND
Methoxychlor	74.1%	2.0%	0.22	ND
Metolachlor	80.9%	2.6%	0.12	ND
Metribuzin	76.3%	5.2%	0.08	ND
Mevinphos	92.9%	2.8%	0.06	ND
MGK-264 (a)	85.8%	3.2%	0.06	ND
MGK-264 (b)	85.9%	2.7%	0.09	ND
Molinate	84.8%	3.6%	0.11	ND
Naproamide	115.8%	2.6%	0.19	0.17
Nitrofen	92.8%	3.0%	0.08	ND
Norflurazon	93.4%	2.9%	0.09	ND
Oxyfluorofen	93.2%	3.4%	0.17	ND
Pebulate	92.3%	3.2%	0.18	ND
Pentachlorophenol	91.2%	3.2%	0.08	ND
Phenanthrene	84.7%	2.9%	0.04	ND
Phenanthrene-d10 (IS)	105.6%	1.3%	N/A	5.16
Phorate	31.7%	98.2%	0.52	ND
Phosphamidon	98.2%	2.6%	0.07	ND
Profenofos	88.9%	3.4%	0.18	ND
Prometon	80.6%	3.5%	0.07	ND
Prometryn	71.8%	14.0%	0.13	ND
Pronamide	87.5%	2.8%	0.05	ND
Propachlor	90.5%	2.8%	0.03	ND
Propazine	81.3%	3.2%	0.06	ND
	92.2%	2.9%	0.06	ND
Pyrene Simazine	86.2%	3.0%	0.07	ND
	75.1%	16.3%	0.13	ND
Simetryn Tebuconazole	105.6%			ND
		3.1%	0.08	
Tebuthiuron	102.3%	2.3%	0.03	ND
Terbacil	94.5%	2.5%	0.12	ND
Terbutryn	72.3%	16.4%	0.15	ND
Tetrachlorvinphos	83.0%	3.4%	0.12	ND
Trans-Chlordane	72.7%	2.9%	0.08	ND
Trans-Nonachlor	71.6%	2.9%	0.11	ND
Trans-Permethrin	92.5%	2.2%	0.18	ND
Triadimefon	85.8%	2.2%	0.15	ND
Tribufos	122.1%	3.2%	0.35	ND
Trifluralin	75.0%	2.8%	0.08	ND
Triphenyl phosphate (SUR)	110.4%	3.5%	N/A	5.34
Vernolate	80.1%	3.2%	0.07	ND
Vinclozolin	81.4%	3.5%	0.05	ND



With the exception of BHT, disulfoton, phorate and 2, 2', 5-Trichlorobiphenyl, all analytes of interest were recovered between 70 and 130 % of their true value in compliance with Method 525.3 criterion for spike recoveries. HCCPD was the only exception and recovered within the 60 and 130 % true value limit required by Method 525.3. The average spike (including the low recovery compounds) recovered at 84.0 %

Phorate and disulfoton have been shown to be unstable in ethyl acetate² as well as in aqueous solutions which accounts for their consistently low recoveries. HCCPD is known to be photosensitive as well as thermally sensitive, which makes it susceptible to degradation and negatively impacts its recovery in solution. With the exception of BHT, HCCPD and phorate, and a few of the triazine herbicides, all compounds were recovered with precision below 10 % RSD. Phorate was recovered with a precision value above 30 % RSD.

Bis(2-ethylhexyl)phthalate had a DL concentration higher than the concentrations at which they were spiked and that was due to one of seven replicates being an outlier. The measured blank concentrations were also shown to be sufficiently low with only four compounds being above an average of $0.1 \mu g/L$. Based on the performance data in this application note, the Atlantic[®] ReadyDisk DVB SPE disks meet and exceed the criteria outlined in EPA Method 525.3.

References

- 1. US EPA Method 525.3, US EPA EPA Method 525.3 -Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry (GC/MS), available at www.epa.gov, 2010.
- 2. Storage stability of organophosphorus pesticide residues in peanut and soya bean extracted solutions, Gang Guo, Naiwen Jiang, Fengmao Liu, Yanli Bian, R. Soc. open sci. 2018 5 180757; DOI: 10.1098/rsos.180757. Published 25 July 2018.

Ordering Information

Part Number	Description	Quantity
47-6001	Atlantic [®] ReadyDisk DVB	Pk/24

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