Environmental analysis: Smarter sample prep

How to streamline analysis, meet regulations and achieve laboratory compliance with solid-phase extraction (SPE)

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Introduction

Environmental analysis of pollutants, water, and soil is essential in preserving and protecting our environment and limiting unnecessary exposure to contaminated material that threatens the livelihood of humans and wildlife. There remains no doubt that good water quality is a key contributor to the preservation of a healthy ecosystem, however maintaining healthy ecosystems also requires the analysis of soil, waste, and pollution, as well as fats, oils and grease (FOG).

Monitoring the environment is a challenge because of the growing number of known and emerging contaminants. As society becomes more aware of rising environmental and climatic issues, the demand for robust environmental compliance testing increases.

Updated regulations within the past decade have allowed for new and more flexible analysis techniques to be introduced and applied, providing all quality control requirements are met.

Biotage offers an extensive range of environmental testing solutions to help chemists reliably meet the ever-growing demands in the laboratory testing environment by applying technologically

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advanced solutions to existing methods, which in turn removes complexity, overcomes bottlenecks, and facilitates method flexibility.

Sample preparation continues to be an important step in environmental analysis, where significant progress has already been made toward developing more rapid, safer, greener, and simple analytical techniques, particularly those associated with solid-phase extraction (SPE). To become certified and comply with ongoing regulations, as well as to attain environmental lab excellence, it is essential that accuracy, consistency, and quality of sample preparation processes are achieved. Environmental labs now require the most effective and reliable equipment along with highly efficient methods that incorporate automated extraction and concentration/evaporation.

With a growing demand to better test and monitor new and existing environmental contaminants, laboratories are limited to the methods prescribed. There is the flexibility to use allowable modifications within some methods that comply, whilst adapting to a smarter and more efficient workflow solution. In this eBook, learn how solid-phase extraction, developing instrumentation, and media formats can accommodate increasing compound lists, application-specific media, and custom media for specific application challenges. Also, find out how you can solve the challenges laboratories face by creating smarter sample prep workflow solutions that meet the market requirements and regulatory methods for which they were designed.

Drinking water: Drinking water solutions for semi-volatile organic compounds

Poor water quality will not only negatively impact public health, but also our surrounding environment, including aquatic life and ecosystems. Water analysis is necessary to meet drinking water standards and ensure optimum water quality is realized. Water analyses are carried out to identify the presence or absence of specified parameters within samples. Such parameters can include a collection of physical, chemical, and biological components found in aqueous solutions. Technological advancements have enabled the development of instrumentation and chemistry offerings designed to facilitate the analysis of water, offering extremely low limits of detection and enhanced accuracy at trace levels.

The quality of water supplied by public water systems in the U.S. is regulated by the Environmental Protection Agency (EPA), including the regulation of semi-volatile organic compounds (SVOCs). In February 2012, there were several updates made to EPA Method 525, an analytical method for the monitoring of SVOCs in water by GC/MS and solid-phase extraction. These application notes outline how to carry out the quality control criteria to become certified and stay within method compliance by implementing SPE disks and automation, as well as how to successfully extract semi-volatile organic compounds in drinking water per EPA Method 525.3.

Wastewater: Influent and effluent solutions for semi-volatile organic compounds

Organic pollutants within water and wastewater can include surfactants, organic solvents, alcohols, and aromatic hydrocarbons, as well as pathogenic microorganisms and heavy metals. The EPA closely monitors organic pollutants found within wastewater in accordance with their acceptance criteria given in EPA Method 625.1 According to the update issued on August 28, 2017, laboratories are now permitted to extract samples via solid-phase extraction. Discover more on automated EPA Method 625.1 by solid-phase extraction by applying only one pH change instead of two. Find out how Biotage can help your lab achieve extraction excellence.

Oil and grease: Avoid setbacks and improve workflows

The treatment and removal of fats, oils, and grease (FOG) from wastewater are essential to preserve and protect both the public and underground sewage pipes. Oil and grease analyses are routinely performed in many environmental laboratories, particularly those assessing water quality and extracting analytes via EPA methods. This work presents several challenges, such as meeting regulatory requirements when processing matrices

with high FOG levels. These application notes detail how to perform accurate, consistent and efficient extractions of total n-hexane extractable material (HEM) using solid-phase extraction with an inline extract drying process. This solution uses an inline sodium sulfate cartridge to ensure any residual water does not compromise your results.

Improving the SPE workflow for 1,4 dioxane in drinking water

1, 4-dioxane is a heterocyclic compound found in groundwater at sites throughout the U.S. that is emerging as a potential threat to human health. Short-term exposure of 1, 4-dioxane has been shown to lead to irritation, whereas exposure in large amounts can develop into organ toxicity, such as kidney and liver damage, as well as cancer, with the U.S. EPA declaring that this compound is likely to be carcinogenic to humans. Take a look at this application note to find out how an automated solid-phase extraction system can be applied in your lab to accurately determine the presence of 1,4-dioxane whilst ensuring compliance.

EPH fractionation: Streamline your EPH fractionation process

The analysis of extractable petroleum hydrocarbons (EPH) involves an initial extraction and concentration, as well as the fractionation of the extract into aromatic and aliphatic fractions. To analyze complex mixtures of organic matter, fractionation is often applied. Fractionation is a separation process that divides a multifaceted mixture to separate the aliphatic from the aromatic. This process enables chemists to physically separate mixtures that differ both in particle size and density. EPH fractionation can be a challenging process and has been associated with many bottlenecks. Biotage technology overcomes such limitations by carefully combining dedicated consumables and automation for water and soil extracts. This application note introduces the ISOLUTE™ EPH SPE column that helps you efficiently fractionate EPH into aliphatic and aromatic fractions and highlights simple soil extraction procedures and sample preparation techniques to facilitate optimum results.

Improving throughput and quality in determining diquat and paraquat in drinking water

First produced in the late 1950s, diquat and paraquat are common herbicides used to control weeds and protect cropland worldwide. Strict guidelines have been put in place to limit unnecessary exposure to these herbicides. Learn more about this and how your lab can effectively extract diquat and paraquat by using the ISOLUTE C8 in-line with an automated extraction system that provides excellent flow rates, consistency, and recovery for diquat and paraquat.

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

Authors: *Stephen Panos, Michael Ebitson, Andrew Taylor, Deanna Bissonnette, Biotage, Salem, NH, USA*

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 bodies[1].

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®
Solid Phase Extraction Disk	Atlantic® ReadyDisk DVB

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.

Table 2. Biotage® Horizon 5000 Extraction Program.

Step	Solvent	Solvent Volume (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 (approximately 70 mL/min)	45

Step	Solvent	Solvent Volume (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 Time (s)	Pump Speed (#)	N_2 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 Container	Acetone	2	45	2	Off	1	0	30
8. Elute Sample Container	Ethyl Acetate	5	30	2	Off	1	60	45
9. Elute Sample Container	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

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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 µg/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 µg/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 µg/L.

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

$MDL = S \times t_{(n-1,1\,\alpha\,0.99)}$

Where:

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

(n-1,1-α = 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

Analyte	Average Recovery (%) n=6	RSD (%) n=6	DL (µg/L) n=7	Blank (µg/L) n=6
Aldrin	84.8%	3.0%	0.11	ND
Ametryn	72.3%	16.4%	0.11	ND
Anthracene	81.2%	4.7%	0.03	ND
Atraton	85.3%	3.1%	0.05	ND
Atrazine	83.8%	2.6%	0.09	ND
Benzo[a]anthracene	91.4%	3.3%	0.07	ND
Benzo[a]pyrene	78.6%	5.1%	0.12	ND
Benzo[a]pyrene-d12 (SUR)	91.4%	4.3%	N/A	4.16
Benzo[b]fluoranthene	88.8%	2.4%	0.21	ND
Benzo[g,h,i]perylene	80.2%	2.1%	0.16	ND
Benzo[k]fluoranthene	85.5%	2.5%	0.21	ND
b-HCH	82.7%	3.2%	0.04	ND
BHT	61.1%	16.1%	0.13	ND
Bis(2-ethylhexyl)adipate	102.2%	4.2%	0.31	0.24
Bis(2-ethylhexyl)phthalate	92.4%	2.3%	1.45	0.17
Bromacil	92.9%	3.0%	0.16	ND
Butachlor	91.0%	3.1%	0.06	ND
Butyl benzyl phthalate	95.2%	3.2%	0.13	ND
Butylate	74.9%	4.5%	0.09	ND
Chlorfenvinphos	87.6%	2.6%	0.08	ND
Chlorobenzilate	84.0%	3.3%	0.10	ND
Chloroneb	78.9%	2.7%	0.06	ND
Chlorothalonil	80.3%	3.4%	0.14	ND
Chlorpropham	98.2%	2.7%	0.08	ND
Chlorpyrifos	75.9%	3.1%	0.12	ND
Chrysene	81.6%	3.2%	0.15	ND
Chrysened-d12 (IS)	105.0%	1.1%	N/A	4.94
Cis-Chlordane	71.7%	2.6%	0.12	ND
Cis-Permethrin	95.3%	2.2%	0.18	ND
Cyanazine	100.5%	2.8%	0.04	ND
Cycloate	90.7%	3.8%	0.16	ND
Dacthal	75.6%	2.5%	0.10	ND
DEET	93.4%	2.5%	0.07	ND
d-HCH	80.3%	3.2%	0.06	ND
Dibenz[a,h]anthracene	78.3%	1.8%	0.20	ND
Dichlorvos	86.8%	2.8%	0.08	ND
Dieldrin	78.0%	3.3%	0.08	ND
Diethyl phthalate	85.1%	2.3%	0.11	0.03
Dimethipin	63.8%	22.2%	0.25	ND
Dimethyl phthalate	83.7%	2.5%	0.08	ND
DIMP	87.6%	4.1%	0.08	ND
Di-n-butyl phthalate	88.9%	2.8%	0.13	0.17
Diphenamid	104.7%	3.0%	0.10	ND
Disulfoton	17.9%	12.8%	0.17	ND
Endosulfan I	86.5%	3.1%	0.08	ND
Endosulfan II	86.6%	3.1%	0.08	ND
Endosulfan sulfate	79.1%	3.2%	0.15	ND
Endrin	78.0%	3.3%	0.08	ND
EPTC	76.5%	3.5%	0.08	ND
Ethion	85.9%	3.0%	0.15	ND
Ethoprop	89.6%	2.1%	0.10	ND
Ethyl Parathion	92.1%	3.4%	0.06	ND
Etridiazole	77.0%	2.6%	0.06	ND

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Analyte	Average Recovery (%) n=6	RSD (%) n=6	DL (µg/L) n=7	Blank (µg/L) 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
Pyrene	92.2%	2.9%	0.06	ND
Simazine	86.2%	3.0%	0.07	ND
Simetryn	75.1%	16.3%	0.13	ND
Tebuconazole	105.6%	3.1%	0.08	ND
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

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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 acetate[2] 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 µg/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

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Extraction of Semi-volatile Organic Compounds in Drinking Water with NEW Atlantic® ReadyDisk C18 Solid Phase Extraction Disks

Authors: *Andrew Taylor, Michael Ebitson, Biotage, Salem, NH, USA.*

Introduction

Drinking water is one of the primary sources of human exposure to toxic chemicals. The U.S. EPA identifies and regulates a number of compounds in drinking water that could pose health risks, and outlines methods for properly quantifying them. Contaminants can be biological, physical, chemical or radiological, and can exist in a wide range of concentrations. Therefore, the list of EPA methods that are approved for use in testing drinking water is extensive and each method presents its own challenges, based on the specific compounds being quantified.

EPA Method 525.2 is used to quantitate organic compounds found in drinking and source waters. Method 525.2 uses a reversed phase separation mechanism to isolate a large variety of compounds from the sample matrix. The reversed phase separation is achieved using a C18 bonded silica stationary phase which is packed into an SPE cartridge or disk. The C18 stationary phase allows for the extraction of semi-volatile compounds from the sample matrix which are then analyzed by gas chromatography-mass spectrometry (GC-MS).[1]

Instrumentation

All samples were analyzed using the instrumentation listed in Table 1 below.

Experimental

The organic compounds were eluted from the ReadyDisk with small quantities of ethyl acetate, followed by methylene chloride, followed by a 1 to 1 (v/v) mixture of ethyl acetate to methylene chloride. The extracted solution was dried and concentrated via solvent evaporation using the DryVap® with DryDisk® Separation Membranes.

The sample components were separated, identified, and measured using the gas chromatography/mass spectrometry (GC/MS) system listed in Table 1.

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

1. Obtain 1-liter samples of drinking water.
2. Add dechlorinating agent to each 1-liter sample.
3. Acidify each sample to pH <2 using concentrated HCl.
4. Add surrogate and internal standard compounds to each sample.
5. Start extraction method shown in Table 2 and collect all extracts (~20 mL each).
6. Add each extract to the DryDisk® holder and start automated drying and concentration process on the DryVap® system. Evaporate each extract to 0.9 mL using the method listed in Table 3.
7. Quantitatively, bring each extract volume to 1.0 mL using ethyl acetate once evaporated to less than 1 mL.
8. Add external standard to each extract.
9. Transfer the extract to a 2.0 mL GC vial.

Table 1. Sample preparation and analysis systems and consumables.

Sample Preparation	
Solid Phase Extraction Disk	Atlantic® ReadyDisk C18
Extraction System	Biotage® Horizon 5000
Drying/Concentration System	Biotage® Horizon DryVap® with DryDisk® Separation Membranes
Analysis	
GC/MS Instrument	Agilent 6890 with 5975C Inert GC/MSD

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Table 2. Biotage® Horizon 5000 Extraction Program.

Step	Solvent	Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	Saturation Time (s)	Soak Time (s)	Drain Time (s)
1. Condition SPE Disk	Ethyl Acetate	10	60	2	1	0	45
2. Condition SPE Disk	Methylene Chloride	10	60	2	1	0	45
3. Condition SPE Disk	Methanol	10	60	2	1	60	5
4. Condition SPE Disk	Reagent Water	10	30	2	1	60	5

Step	Sample Flow Rate (#)	Done Loading Sample Delay (s)
5. Load Sample	2 (approximately 70 mL/min)	45

Step	Dry Time (s)	Pump Speed (#)	N_2 Blanket
6. Air Dry Disk Timer	600	6	OFF

Step	Solvent	Solvent Volume (mL)	Purge Time (s)	Pump Rate(#)	N_2 Blanket	Saturation Time (s)	Soak Time (s)	Drain Time (s)
7. Elute Sample Container	Ethyl Acetate	5	60	2	Off	2	60	45
8. Elute Sample Container	Methylene Chloride	5	60	2	Off	2	60	45
9. Elute Sample Container	1:1 EtOAc/MeCl	3	15	2	Off	1	60	45
10. Elute Sample Container	1:1 EtOAc/MeCl	3	15	6	Off	1	60	60

Table 3. DryVap® Conditions.

Parameters	Value
Drying Mechanism	DryDisk® (PN: 40-705-HT)
Dry Volume	100 mL
Heater Power	5
Heater Timer	Off (automatic endpoint mode used)
Auto Rinse	Off

Table 4. GC/MS Parameters.

Parameter	Value
Injection Volume	1 µL
Inlet Temperature	280 °C
Mode	Splitless
Gas Type	Helium
Column Conditions	ZB-SemiVol (Phenomenex), 30 m, 0.25 mm, 0.25 µm
Mode	Constant Flow 1 mL/min
Oven Program	60 °C hold for 2 minutes Ramp 20 °C/min to 270 °C Ramp 6 °C/min to 320 °C Hold for 3 minutes

Results and Discussion

Per EPA Method 525.2, a series of laboratory reagent blanks (LRBs) were measured to demonstrate a lack of contamination from the extraction system and the Atlantic® ReadyDisk C18, prior to analyzing any samples. Six replicate LRBs were prepared and extracted as described in EPA Method 525.2, following the procedure in the method summary in this note. All blanks were spiked with internal standards such that their final concentration in solution was 5 µg/L. The results for the six LRBs are shown in Table 5.

To demonstrate an Initial Demonstration of Laboratory Accuracy and Precision (IDA and IDP), five replicates of a laboratory fortified blank (LFB) were prepared and extracted as described in EPA Method 525.2. Each replicate contained all analytes of interest, including internal standards and surrogates, at 5 µg/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 percent, per Method 525.2.[1] Results for the five samples are shown in Table 5.

Seven additional laboratory fortified blanks were prepared such that all analytes of interest were present at approximately 0.5 µg/L. All seven replicates were analyzed on three consecutive days to produce data for calculating method detection limits (MDLs).

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 µg/L.

The method detection limits (MDL) were calculated using the formula[1]:

$MDL = S \times t_{(n-1,1\ \alpha\ 0.99)}$

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. IDA and IDP, MDLs and LRB results for the Atlantic® ReadyDisk C18.

Analyte	Average Recovery (%) n=5	RSD (%) n=5	MDL (µg/L) n=7	Blank (µg/L) n=6
Acenaphthene d10	78.0	8.08	-	4.30
Phenanthrene d10	82.6	7.72	-	4.72
Chrysene d12	81.5	9.59	-	4.72
Isophorone	95.2	6.08	0.13	N.D
2-Nitro-m-xylene	98.1	9.89	-	4.41
Naphthalene	84.6	10.33	0.13	N.D
Dichlorvos	93.8	5.48	0.16	N.D
Hexachlorocyclopentadiene	54.9	20.08	0.15	N.D
EPTC	106.3	5.07	0.08	N.D
Mevinphos	99.2	5.63	0.17	N.D
Butylate	99.1	7.56	0.10	N.D
Vernolate	107.0	6.12	0.10	N.D
Dimethyl phthalate	102.8	7.31	0.13	N.D
Pebulate	106.6	5.33	0.07	N.D
Etridiazole	101.2	8.09	0.06	N.D
2,6-Dinitrotoluene	71.0	7.02	0.18	N.D
Acenaphthylene	97.8	6.47	0.11	N.D
Chloroneb	110.3	7.85	0.11	N.D
Tebuthiuron	110.7	3.65	0.13	N.D
2,4-Dinitrotoluene	70.9	7.60	0.15	N.D
Molinate	111.2	5.98	0.08	N.D
Diethyl phthalate	113.8	7.23	0.20	N.D
Fluorene	103.8	6.91	0.12	N.D
Propachlor	112.2	7.03	0.14	N.D
Ethoprop	112.3	6.14	0.09	N.D
Cycloate	114.7	6.14	0.15	N.D
Chlorpropham	113.3	8.58	0.15	N.D
Trifluralin	102.7	10.39	0.12	N.D
a-BHC	106.7	6.56	0.09	N.D
Atraton	42.0	9.72	0.18	N.D
Hexachlorobenzene	99.9	7.54	0.15	N.D
Prometon	54.6	7.77	0.11	N.D
Lindane (g-BHC)	107.2	9.38	0.12	N.D
Simazine	92.5	6.15	0.22	N.D
Atrazine	102.3	5.99	0.12	N.D
Propazine	101.0	5.37	0.09	N.D
b-BHC	105.2	8.34	0.10	N.D
Pentachlorophenol	101.6	11.08	0.08	N.D
Terbufos	105.7	5.80	0.16	N.D
Pronamide	105.8	5.85	0.09	N.D
Diazinon	92.8	6.19	0.15	N.D
d-BHC	106.5	7.94	0.10	N.D
Phenanthrene	105.8	5.64	0.11	N.D
Disulfoton	105.4	4.66	0.13	0.15
Methyl paraoxon	104.6	6.38	0.11	N.D
Anthracene	95.4	7.30	0.22	N.D
Terbacil	102.3	9.11	0.18	0.30
Chlorothalonil	107.6	7.03	0.06	N.D
Metribuzin	85.8	7.89	0.23	N.D
Simetryn	75.4	9.95	0.17	N.D
Heptachlor	100.6	6.85	0.12	N.D
Ametryn	88.8	9.40	0.19	N.D

Analyte	Average Recovery (%) n=5	RSD (%) n=5	MDL (µg/L) n=7	Blank (µg/L) n=6
Alachlor	106.8	6.43	0.09	N.D
Prometryn	94.2	8.93	0.18	N.D
Terbutryn	93.6	8.97	0.19	N.D
Di-n-butyl phthalate	107.5	6.81	0.07	N.D
Bromacil	97.0	8.92	0.18	N.D
Cyanazine	103.3	7.60	0.16	N.D
Metolachlor	108.0	6.91	0.08	N.D
Chlorpyrifos	105.6	7.06	0.11	N.D
Aldrin	95.8	7.80	0.28	N.D
Triademefon	104.9	5.85	0.08	N.D
Dacthal	105.0	7.21	0.09	N.D
MGK-264-A	103.4	6.75	0.11	N.D
Diphenamid	110.0	6.21	0.08	N.D
MGK-264-B	103.4	6.75	0.11	N.D
Merphos	108.7	11.42	0.18	N.D
Heptachlor epoxide B	103.1	8.56	0.07	N.D
Heptachlor epoxide A	103.8	8.42	0.18	N.D
Fluoranthene	104.1	6.36	0.09	N.D
g-Chlordane	101.2	7.69	0.10	N.D
Stirofos	115.0	8.56	0.11	N.D
Disulfoton sulfone	112.8	8.77	0.10	N.D
Butaclor	107.5	8.87	0.10	N.D
a-Chlordane	101.7	8.75	0.11	N.D
Endosulfan I	102.9	9.88	0.12	N.D
Fenamiphos	116.6	9.55	0.13	N.D
Pyrene-d10	103.5	9.46	-	4.78
Pyrene	104.6	7.05	0.11	N.D
Napropamide	111.5	6.93	0.14	N.D
trans-Nonachlor	96.3	10.26	0.08	N.D
4,4'-DDE	101.8	7.95	0.09	N.D
Dieldrin	105.5	6.63	0.11	N.D
Tricyclazole	95.0	3.38	0.19	N.D
Terphenyl-d14	120.6	7.49	-	5.29
Carboxin	80.8	10.80	0.21	N.D
Endrin	101.4	8.71	0.14	N.D
Chlorobenzilate	104.5	13.57	0.09	N.D
Endosulfan II	105.6	9.63	0.16	N.D
4,4'-DDD	107.2	6.09	0.09	N.D
Endrin Aldehyde	95.6	4.19	0.14	N.D
Butyl benzyl phthalate	111.5	8.20	0.09	N.D
Norflurazon	109.7	6.91	0.10	N.D
4,4-DDT	107.1	6.18	0.09	N.D
Endosulfan Sulfate	109.8	10.63	0.14	N.D
Bis(2-ethylhexyl)adipate	108.5	7.88	0.10	N.D
Hexazinone	108.4	7.22	0.13	N.D
Triphenylphosphate	111.7	11.64	-	5.00
Endrin Ketone	110.8	10.55	0.11	N.D
Methoxychlor	107.4	10.59	0.08	N.D
Benz(a)anthracene	104.1	6.26	0.14	N.D
Chrysene	106.0	7.88	0.10	N.D
Bis(2-ethylhexyl)phthalate	112.1	11.09	0.22	0.02
Fenarimol	110.5	7.72	0.08	N.D
cis-Permethrin	106.3	7.77	0.10	N.D

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Analyte	Average Recovery (%) n=5	RSD (%) n=5	MDL (µg/L) n=7	Blank (µg/L) n=6
trans-Permethrin	107.6	9.75	0.10	N.D
Di-n-octyl phthalate	107.4	8.48	0.08	N.D
Benzo(b)fluoranthene	106.8	7.28	0.09	N.D
Benzo(k)fluoranthene	105.4	6.50	0.09	N.D
Benzo(a)pyrene	98.4	6.97	0.29	N.D
Fluridone	111.3	6.00	0.09	N.D
Perylene-d12	91.7	11.88	-	3.88
Indeno(1,2,3-cd)pyrene	102.4	6.76	0.10	N.D
Dibenz(ah)anthracene	102.3	6.08	0.12	N.D
Benzo(ghi)perylene	103.7	7.86	0.12	N.D

Conclusion

With the exception of hexachlorocyclopentadiene, atraton and prometon, all analytes were recovered within 70–130% of the known value, in compliance with Method 525.2 criterion for spike recoveries. The average spike (including the low-recovering compounds) recovered at 101.1%. Hexachlorocyclopentadiene's low recovery (54.9%) can be attributed to the compound's sensitivity to thermal and photochemical degradation, as well as its propensity to react with acetone. The low recoveries for atraton and prometon (42.0% and 54.6%, respectively) likely stem from inefficient extractions from the water at pH 2, which causes ionization in solution under acidic conditions.[1] The relative standard deviation for all compounds ranged from 3.38– 20.08%, below the method's RSD criteria of <30%. The NEW Atlantic® ReadyDisk C18 provided excellent analyte accuracy and precision in an easy-to-use, plug-n-play format.

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.

Ordering Information

Part Number	Description	Quantity
47-6005	Atlantic® ReadyDisk C18	Pk/24
40-705-HT	DryDisk® 65 mm	Pk/50

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Literature Number: AN917

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Extraction of EPA Method 625.1 Semi-Volatile Analytes from Wastewater Using the Biotage® Horizon 5000, DryDisk® Solvent Drying System and TurboVap® II

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***Key Words**: Extraction, Concentration, EPA, 625.1, Wastewater, TurboVap® II, Biotage® Horizon 5000.*

Introduction

The EPA has been monitoring organic pollutants within wastewater matrices since 1984 by developing their own methods and acceptance criteria. The basic, neutral and acidic extractions in EPA Method 625.1 are a part of the revised version of EPA Method 625.

EPA Method 625.1 organizes the target analytes into three different tables. Table 1 lists non-pesticide/PCB basic/neutral analytes and table 2 lists the acidic analytes which may be extracted from a sample matrix to determine analytes quantitatively and qualitatively. Table 3 contains additional analytes that can be extracted via 625.1. Since there are basic and acidic analytes that need to be recovered during the extraction, the method requires two pH adjustment steps when extracting the semi-volatile analytes from a sample. Initially, the water sample is adjusted to a pH less than 2 to extract the acidic and neutral analytes. Following the acidic extraction, the pH is adjusted to greater than 11, extracting the basic analytes from the sample. The large number of analytes in Tables 1–3 of this method makes testing difficult if all analytes are determined simultaneously. Therefore, it is necessary to determine and perform quality control (QC) tests for the "analytes of interest" only. Analytes of interest are those required to be determined by a regulatory/control authority or in a permit, or by a client. If a list of analytes is not specified, the analytes in Tables 1 and 2 must be determined, at a minimum, and QC testing must be performed for these analytes. The analytes in Tables 1 and 2, and some of the analytes in Table 3 have been identified as Toxic Pollutants (40 CFR 401.15), expanded to a list of Priority Pollutants (40 CFR 423, Appendix A).[1]

Within the revision of EPA Method 625.1, laboratories are allowed to extract samples via solid phase extraction. After extraction, the extracts must be dried and concentrated.

This application note will show recoveries of all the analytes within tables 1–2 of 625.1 and a few analytes from table 3 obtained using the Biotage® Horizon 5000 with Atlantic® 8270

One Pass solid phase extraction disks (P/N 47-2346-11), 8270 Carbon Cartridges Max-Detect cartridges (P/N 49-2620-01), and 1.0 Micron Atlantic® Fast Flow Pre-Filters (P/N FFAP-100-HS1) for analyte extraction. The DryDisk® Solvent Drying System and the TurboVap® II are used for solvent drying and concentration and analysis is by GC-MS.

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Experimental

Each 1 L deionized water sample was prepared by adding 1 mL of hydrochloric acid, bringing the sample to a pH less than 2. A total of two method blanks were analyzed. The first method blank contained 50 µg/L of surrogates and the second method blank contained 100 µg/L of surrogates. A total of twelve samples were spiked with a 625.1 spike mix in addition to surrogates. Six of these samples were spiked at a concentration level of 50 µg/L with the remaining six spiked at a concentration level of 100 µg/L. Using the Biotage® Horizon 5000 (P/N SPE-DEX 5000), all the samples were extracted using the method outlined in table 1. Atlantic® 8270 One-Pass Disks in conjunction with the 8270 Carbon Cartridge Max-Detect were used as the solid phase extraction consumables. The Atlantic® 8270 One-Pass Disk consists of a mixed-mode chemistry, eliminating the need for the second adjustment to the pH of the sample. Instead, a 1% ammonium hydroxide rinse adjusts the pH of the disk. After the analytes that were retained on the disk have been collected, the 8270 Carbon Cartridge Max -Detect (P/N 49-2620-01) is eluted to recover the more volatile analytes within a sample.

Upon completion of the extraction, the samples were dried using the DryDisk® Solvent Drying System (P/N SDS-101-19/22) in conjunction with DryDisks® (P/N 40-705-HT) utilizing the parameters outlined in table 2. The dried extracts were transferred to 200 mL evaporation tubes with an endpoint of 0.9 mL (P/N C128506) for use in the TurboVap® II (P/N 415001). Because the final volume of the dried extracts exceeded 200 mL, the samples were added to the evaporation tubes in two portions. The first portion was concentrated to approximately 15 mL before adding the final extract volume as well as glassware rinses. The samples were evaporated using the parameters outlined in table 3.

After evaporation on the TurboVap® II, the samples were brought to 1 mL with methylene chloride and transferred to GC-MS vials. Internal standard was added to an aliquot of each of the completed 1 mL extracts and analyzed using the GC/MS using instrument parameters outlined in table 4.

Table 1. Biotage® Horizon 5000 extraction method.
Note: The method below was written in such a way as to be able to run those samples that may require a 5-µm Pre-filter, glass wool, and a fine mesh screen.

Step	Operation	Message	Attachment
1	Pause with Message	Part 1 of 3: Neutrals and Acids Elution. Have the Fast Flow Disk Holder (FFDH) with One-Pass disk, 1 um filter, 5 um filter, top screen over the filters, 250 mL collection flask, and carbon cartridge installed. The down spout of the water in valve must push down on the top screen in the FFDH. Click "Continue" to start Part 1.	None

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	Saturation Time	Soak Time (s)	Drain Time (s)
2	Condition SPE	Acetone	40	60	4	2	60	60
3	Condition SPE	*Reagent Water 2	20	60	4	2	60	60

Step	Operation	Sample Flow Rate (#)	Done Loading Sample Delay (s)
4	Load Sample	5	45

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	N_2 Blanket	Saturation Time	Soak Time (s)	Drain Time (s)
5	Wash Sample Container	Reagent Water	20	30	4	Off	2	5	30
6	Air Dry Disk Timer			360	6	Off			
7	Elute Sample Container	Acetone	20	20	4	Off	2	180	180
8	Elute Sample Container	Methylene Chloride	17	15	4	Off	2	180	180

*"Reagent Water 2" is added to the list of configured solvents with the Waste Destination of "Solvent Waste" rather than using the factory programmed "Reagent Water", which is sent to "Water Waste".

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	N_2 Blanket	Saturation Time	Soak Time (s)	Drain Time (s)
9	Elute Sample Container	Methylene Chloride	17	15	4	Off	2	120	120
10	Elute Sample Container	Methylene Chloride	17	15	4	Off	2	120	120
11	Elute Sample Container	Methylene Chloride	17	15	6	Off	2	120	180

Step	Operation	Message	Attachment
12	Pause with Message	Part 2 of 3: Ion Exchange Elution. Remove the 250 mL collection flask containing the neutrals and acids elution. Stopper the flask and set aside for part 3. Then install a clean 125 mL flask to collect the ion exchange elution. Click "Continue" to start part 2.	None

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	N_2 Blanket	Saturation Time	Soak Time (s)	Drain Time (s)
13	Elute Sample Container	Acetone	20	20	4	Off	2	0	180
14	Elute Sample Container	1% Ammonium Hydoxide	20	30	4	Off	2	120	120
15	Elute Sample Container	Acetone	20	20	4	Off	2	180	120
16	Elute Sample Container	Methylene Chloride	17	15	4	Off	2	180	180
17	Elute Sample Container	Methylene Chloride	16	15	4	Off	2	120	180
18	Elute Sample Container	Methylene Chloride	16	15	4	Off	2	120	180
19	Elute Sample Container	Methylene Chloride	16	15	6	Off	2	120	180

Step	Operation	Message	Attachment
20	Pause with Message	Part 3 of 3: Carbon Cartridge Elution. Remove the carbon cartridge from the tubing lines. Connect the tubing ends together. Using a 20 cc syringe, plunge the carbon cartridge with air through the cap adapter to reseat the carbon bed on the frit. Replace the cap adapter with the funnel on the cartridge. Replace the disk holder with the cartridge. Replace the 125 mL flask with the 250 mL flask containing the neutrals and acids elution from part 1. Stopper the 125 mL flask. Click "Continue" to start part 3.	None

Step	Operation	Dry Time (s)	Pump Rate (#)	N_2 Blanket
21	Air Dry Disk Timer	60	6	Off

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	N_2 Blanket	Saturation Time	Soak Time (s)	Drain Time (s)
22	Elute Sample Container	Acetone	25	20	4	Off	3	60	60
23	Elute Sample Container	Methylene Chloride	17	15	4	Off	3	60	20
24	Elute Sample Container	Methylene Chloride	17	15	4	Off	3	60	20
25	Elute Sample Container	Methylene Chloride	17	15	4	Off	3	60	20
26	Elute Sample Container	Methylene Chloride	17	15	4	Off	3	60	20
27	Elute Sample Container	Methylene Chloride	17	15	6	Off	3	60	60

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Table 2. Drying Parameters via the DryDisk® Solvent Drying System.

Parameter	Setting
Vacuum:	-8 ”Hg

Table 3. Evaporation Parameters for Drying via the Biotage TurboVap® II.

Parameter	Setting
Inlet Nitrogen Pressure:	87 psi
Gas Flow:	2.8 mL/min
Water Bath Temperature:	40 °C

Table 4. GC/MS Method.

Parameter	Setting
Injection Volume	1 µL
Inlet Temperature	280 °C
Injection Mode	Split
Split Ratio	10:1
Split Flow	12.5 mL/min
Gas Type	Helium
GC Column	Zebron™ ZB-Semi Volatiles (Phenomenex), 30 m, 0.25 mm, 0.25 µm
GC Mode	Constant Flow: 1.3 mL/min
Oven Program	45 °C hold for 1.0 minutes Ramp 15 °C/min to 270 °C Ramp 6 °C/min to 318 °C
MS Ions Monitored	35–550 AMU

TurboVap® II solvent evaporator.

Biotage® Horizon 5000.

Results and Discussions

Table 5 shows the total concentration time for each sample using the TurboVap® II.

Table 5. Concentration Times on the TurboVap® II.

Sample	Concentration (µg/L)	Time (Hour:Min.:Sec.)
1	50	1:51:56
2	50	1:42:00
3	50	1:56:36
4	50	1:37:25
5	50	1:47:41
6	50	1:55:53
7	100	1:48:43
8	100	1:39:12
9	100	1:48:30
10	100	1:53:53
11	100	1:46:59
12	100	1:55:49

When concentrating the samples, aluminum foil was used to cover each sample vial in order to limit interactions between the evaporating acetone and the water vapor molecules present from the warm water bath. Two holes were pierced on either side of the foil caps to allow the nozzle (N_2 flow) to enter, and evaporated solvent to escape, the vials. The concentration times only varied slightly between the samples. On average, the samples that initially contained approximately 303 mL of solvent were concentrated on the TurboVap® II to approximately 0.9 mL in 1 hour and 49 minutes.

Data for the method blanks and spiked samples, including average percent recovery and %RSD for each level (50 µg/L and 100 µg/L) are outlined in table 6. When reading table 6, the analytes are color coded based upon the legend outlined in the header. The list of analytes in table 6 below contains all the analytes from tables 1 and 2 and some from table 3 of EPA Method 625.1.

Table 6. Average Percent Recovery for 625.1 Analytes Spiked at 50 µg/L and 100 µg/L Using the Biotage® Horizon 5000 and the TurboVap® II.

Legend	Table 1 Analytes	Table 2 Analytes	Table 3 Analytes	Table 4/6	Table 8 Analytes	Not in Table 8

Analyte	Blank 1: Surrogate level 50 µg/L	50 µg/L Spike Average Percent Recovery (n=6)	50 µg/L Spike %RSD	Blank 2: Surrogate level 100 µg/L	100 µg/L Spike Average Percent Recovery (n=6)	100 µg/L Spike %RSD
NDMA	N.D	65.43	6.34	N.D	51.38	25.51
Pyridine	N.D	38.26	11.03	N.D	36.87	8.63
2-Picoline	N.D	60.47	4.99	N.D	58.43	2.82
N-Nitrosomethyl ethylamine	N.D	80.59	5.77	N.D	75.02	7.06
Methyl methanesulfonate	N.D	66.34	7.60	N.D	57.53	11.85
2-Fluorophenol	39.63	73.03	9.34	76.36	57.43	7.23
N-Nitroso-diethylamine	N.D	81.11	5.04	N.D	78.78	0.95
Ethyl methanesulfonate	N.D	83.04	6.55	N.D	78.15	3.58
Phenol-d6	38.85	65.90	6.91	67.25	57.63	4.58
Phenol	N.D	68.00	4.23	N.D	60.05	6.35
Aniline	N.D	69.16	12.69	N.D	61.89	9.35
Bis(2-chloroethyl)ether	N.D	83.57	2.38	N.D	81.99	1.78
Pentachloroethane	N.D	71.28	7.23	N.D	68.72	2.91
2-Chlorophenol	N.D	83.36	5.62	N.D	77.78	3.18
Benzyl alcohol	N.D	85.15	3.52	N.D	88.84	10.41
o-Cresol	N.D	85.33	3.83	N.D	77.89	6.06
Bis(2-chloroisopropyl)ether	N.D	79.32	2.86	N.D	80.04	1.68
N-Nitroso-pyrrolidine	N.D	85.00	4.09	N.D	84.56	3.23
N-Nitroso-morpholine	N.D	79.90	6.72	N.D	83.69	7.41
Acetophenone	N.D	80.92	2.58	N.D	79.83	2.19
m+p-Cresol	N.D	83.14	3.98	N.D	74.41	6.77
N-nitrosodi-n-propylamine	N.D	83.02	2.56	N.D	82.18	2.16
Hexachloroethane	N.D	67.98	6.65	N.D	67.34	2.22
o-Toluidine	N.D	59.84	15.34	N.D	53.31	15.61
Nitrobenzene-d5	39.59	81.37	6.49	82.35	79.50	1.99
Nitrobenzene	N.D	79.48	6.30	N.D	77.96	2.45
N-Nitroso-piperidine	N.D	85.00	6.74	N.D	84.06	2.17
Isophorone	N.D	87.08	3.44	N.D	85.94	1.37
2-Nitrophenol	N.D	87.16	2.66	N.D	81.65	5.72
2,4-Dimethylphenol	N.D	87.12	3.56	N.D	86.57	1.82
Bis(2-chlorethoxy)methane	N.D	87.30	3.09	N.D	85.76	1.22
Benzoic acid	N.D	84.91	11.54	N.D	68.76	14.25

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Legend	Table 1 Analytes	Table 2 Analytes	Table 3 Analytes	Table 4/6	Table 8 Analytes	Not in Table 8

Analyte	Blank 1: Surrogate level 50 µg/L	50 µg/L Spike Average Percent Recovery (n=6)	50 µg/L Spike %RSD	Blank 2: Surrogate level 100 µg/L	100 µg/L Spike Average Percent Recovery (n=6)	100 µg/L Spike %RSD
2,4-Dichlorophenol	N.D	88.33	3.60	N.D	87.00	3.53
1,2,4-Trichlorobenzene	N.D	75.61	3.84	N.D	73.59	2.80
Naphthalene	N.D	80.34	3.46	N.D	78.49	2.54
2,6-Dichlorophenol	N.D	86.86	5.29	N.D	84.33	3.07
4-Chloroaniline	N.D	68.70	13.31	N.D	60.09	11.36
Hexachloropropene	N.D	60.52	3.75	N.D	56.92	2.90
Hexachlorobutadiene	N.D	60.93	8.40	N.D	58.24	3.29
N-nitroso-di-n-butylamine	N.D	88.70	2.68	N.D	88.80	2.51
4-Chloro-3-methylphenol	N.D	91.36	3.31	N.D	91.81	4.09
cis-Isosafrole	N.D	83.02	4.66	N.D	81.29	3.15
2-Methylnaphthalene	N.D	79.98	3.21	N.D	77.43	2.99
Hexachlorocyclopentadiene	N.D	47.35	9.12	N.D	49.42	5.13
1,2,4,5 Tetrachlorobenzene	N.D	65.76	4.72	N.D	70.32	3.09
trans-Isosafrole	N.D	88.65	3.42	N.D	91.60	2.16
2,4,6-Trichlorophenol	N.D	90.99	2.61	N.D	93.65	3.42
2,4,5-Trichlorophenol	N.D	88.52	2.99	N.D	93.63	4.31
2-Fluorobiphenyl	37.44	78.78	2.78	77.06	81.22	2.93
Safrole	N.D	85.08	2.64	N.D	88.70	3.27
2-Chloronaphthalene	N.D	77.84	2.91	N.D	81.13	3.11
2-Nitroaniline	N.D	93.55	3.17	N.D	94.87	4.09
1,4-Naphthoquinone	N.D	73.24	4.47	N.D	81.86	7.60
Dimethyl phthalate	N.D	89.18	1.93	N.D	92.34	3.72
1,3-Dinitrobenzene	N.D	92.33	3.12	N.D	94.65	4.46
2,6-Dinitrotoluene	N.D	89.83	1.98	N.D	94.02	3.68
Acenaphthylene	N.D	82.88	2.54	N.D	85.56	2.67
3-Nitroaniline	N.D	85.43	2.68	N.D	87.64	4.49
Acenaphthene	N.D	81.55	2.39	N.D	83.84	2.75
2,4-Dinitrophenol	N.D	102.41	3.04	N.D	106.78	3.00
Pentachlorobenzene	N.D	73.09	1.92	N.D	79.27	2.95
4-Nitrophenol	N.D	94.00	1.57	N.D	97.93	6.46
Dibenzofuran	N.D	82.93	2.21	N.D	84.57	2.81
2,4-Dinitrotoluene	N.D	92.17	1.43	N.D	94.44	3.04
2,3,4,6-Tetrachlorophenol	N.D	93.30	2.31	N.D	95.22	2.77

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Legend	Table 1 Analytes	Table 2 Analytes	Table 3 Analytes	Table 4/6	Table 8 Analytes	Not in Table 8

Analyte	Blank 1: Surrogate level 50 µg/L	50 µg/L Spike Average Percent Recovery (n=6)	50 µg/L Spike %RSD	Blank 2: Surrogate level 100 µg/L	100 µg/L Spike Average Percent Recovery (n=6)	100 µg/L Spike %RSD
1-Naphthylamine	N.D	56.04	16.37	N.D	59.31	14.02
2-Naphthylamine	N.D	57.26	18.08	N.D	57.06	11.75
Diethyl phthalate	N.D	91.04	1.87	N.D	92.85	2.72
Fluorene	N.D	84.46	2.31	N.D	86.09	2.78
4-Chlorophenyl phenyl ether	N.D	80.24	2.18	N.D	83.33	2.81
4-Nitroaniline	N.D	79.77	3.43	N.D	79.50	3.92
5-nitro-o-toluidine	N.D	72.07	11.38	N.D	76.02	6.89
2-Methyl-4,6-dinitrophenol	N.D	96.90	1.92	N.D	99.31	2.38
Diphenylamine	N.D	89.72	1.42	N.D	90.24	2.59
Azobenzene	N.D	86.88	2.58	N.D	88.78	3.11
2,4,6-Tribromophenol	43.13	99.77	6.09	92.32	96.62	3.66
1,3,5,-Trinitrobenzene	N.D	98.89	7.29	N.D	89.97	7.67
Phenacetin	N.D	106.16	5.18	N.D	100.52	3.70
4-Bromophenyl phenyl ether	N.D	90.17	4.99	N.D	89.19	3.01
Hexachlorobenzene	N.D	90.94	5.32	N.D	90.14	3.35
Pentachlorophenol	N.D	108.91	5.60	N.D	104.51	2.28
Pentachloronitrobenzene	N.D	95.32	5.02	N.D	93.37	2.24
4 Aminobiphenyl	N.D	54.51	22.72	N.D	54.37	10.55
Dinoseb	N.D	109.65	4.24	N.D	108.54	2.11
Phenanthrene	N.D	95.09	4.80	N.D	92.82	2.33
Anthracene	N.D	95.34	4.09	N.D	92.98	2.14
Carbazole	N.D	98.91	3.56	N.D	95.59	2.36
Di-n-butyl phthalate	N.D	104.60	3.49	N.D	100.73	1.90
Methapyrilene	N.D	93.73	4.78	N.D	92.66	2.63
Fluoranthene	N.D	97.78	4.10	N.D	93.39	2.67
Benzidine	N.D	29.04	38.08	N.D	48.31	10.12
Pyrene	N.D	95.73	3.18	N.D	92.24	2.33
p-Terphenyl-d14	44.76	101.64	3.42	92.25	98.14	2.36
Dimethylaminoazobenzene	N.D	109.40	4.36	N.D	105.87	2.09
3,3'-Dimethylbenzidine	N.D	29.31	30.84	N.D	31.59	11.28
Butyl benzyl phthalate	N.D	103.33	2.36	N.D	102.38	2.57
Acetylaminofluorene	N.D	118.53	4.03	N.D	117.59	1.72
3,3'-Dichlorobenzidine	N.D	66.92	8.44	N.D	63.95	7.86

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Legend	Table 1 Analytes	Table 2 Analytes	Table 3 Analytes	Table 4/6	Table 8 Analytes	Not in Table 8

Analyte	Blank 1: Surrogate level 50 µg/L	50 µg/L Spike Average Percent Recovery (n=6)	50 µg/L Spike %RSD	Blank 2: Surrogate level 100 µg/L	100 µg/L Spike Average Percent Recovery (n=6)	100 µg/L Spike %RSD
Benzo(a)anthracene	N.D	99.70	3.89	N.D	97.36	1.60
Chrysene	N.D	98.53	3.36	N.D	95.68	1.01
Bis(2-ethylhexyl)phthalate	N.D	110.09	2.98	N.D	106.74	2.43
Di-n-octyl phthalate	N.D	114.93	4.40	N.D	114.18	2.12
7,12-Dimethylbenz(a)anthracene	N.D	93.58	6.28	N.D	90.10	3.32
Benzo(b)fluoranthene	N.D	98.45	5.47	N.D	95.56	2.60
Benzo(k)fluoranthene	N.D	98.19	5.33	N.D	94.00	2.93
Benzo(a)pyrene	N.D	99.67	5.46	N.D	97.25	2.62
3-Methylcholanthrene	N.D	100.25	6.88	N.D	98.79	2.50
Indeno(1,2,3-cd)pyrene	N.D	99.55	8.01	N.D	97.60	4.30
Dibenz(a,h)anthracene	N.D	98.56	8.25	N.D	96.62	3.99
Benzo(ghi)perylene	N.D	99.28	7.65	N.D	96.11	4.17

The EPA acceptance criteria for table 1 and table 2 analytes are found in tables 4 and 5 respectively in EPA Method 625.1. For those compounds in tables 3 and 8, the EPA has elected not to set specific acceptance criteria themselves. Instead, the appropriate acceptance criteria are left to the laboratories to develop in one of several ways: based on laboratory control charts, using the range 60-140%, or using the guidelines outlined in section 8.4.5 of the 625.1 method itself. All the analytes in table 1 and table 2 of the EPA method passed the EPA method acceptance criteria for both concentration levels. The percent relative standard deviations indicated minimal variation in percent recovery for each sample set.

According to section 6.8.1 of EPA Method 625.1, a minimum of three surrogates are required for analysis as long as they do not interfere with target analytes. The six surrogates used in this application note passed the acceptance criteria set by the EPA method for all of the samples at 50 µg/L and three of the samples at 100 µg/L. The recovery of two surrogates, 2-fluorophenol and phenol-d6, from the other three samples at 100 µg/L fell below the lower passing limit of 60%, lowering the average. However, the EPA only requires a minimum of three surrogates for each sample. Even though two surrogates fell below the lower limit, four surrogates fell within the limits. The calculated percent relative standard deviation for each surrogate in the order found in table 7 was 7.23%, 5.48%, 1.99%, 2.93%, 3.66%, and 2.36%, indicating minimal variation between the samples.

Two method blanks were extracted with surrogates at different levels. The concentration of the surrogates for the first blank was 50 µg/L and the concentration of surrogates for the second blank was 100 µg/L. Neither showed any false positives for target analytes above the detection limit of 10 µg/L which is denoted by N.D, meaning “not detected.”

References

1. United States Environmental Protection Agency, Method 625.1: Base/Neutrals and Acids by GC/MS, available at www.epa.gov, December 2016.

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Literature Number: AN930

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Improving the 90 mm Disk Oil & Grease Method Using the Horizon 5000 (EPA 1664B)

Author: *Matt Harden and Michael Ebitson, Biotage, Salem, NH, USA*
Key Words: *EPA, 1664B, Oil and Grease, HEM, Wastewater, Biotage® Horizon 5000, Extraction, 90 mm.*

Introduction

The treatment and removal of oil and grease from wastewater is imperative because it can negatively affect the biological and aquatic life that encounter it. Not only that, but since the oil and grease are not miscible with water, it leaves an unappealing layer on top of the water. Even before the oil and grease can reach the aquatic life, it will solidify on the inner walls of pipes, causing blockage over time.

Oil and grease analysis is routinely performed in many labs that also extract and analyze a variety of analyte suites using different EPA methods, so time management and flexibility are a necessity. The Biotage® Horizon 5000 offers these labs a flexible solution that can extract a wide variety of analytes as well as oil and grease. In these circumstances it is imperative to have a streamlined solution with the minimum amount of manual operation required.

The purpose of this application note is to optimize the solution for the removal and testing of total n-hexane extractable material (HEM) using solid phase extraction in combination with the Biotage® Horizon 5000 automated extraction system. Using the Speed-Vap® IV Solvent Evaporation System, the extracts will be evaporated with gentle heat and consistent air flow through precisely drilled holes.

Instrumentation

Biotage Products:

- Biotage® Horizon 5000 (P/N SPE-DEX 5000)
- Speed-Vap® IV – Solvent Evaporation System (P/N 200-1000-04)
- Pacific® Premium Oil & Grease Disk, 90 mm (P/N 1664-100-PHT)
- Pacific® O&G Fast Flow Pre-Filter, 90 mm (P/N FFP-90-HT)
- Oil and Grease Standards, 40 mg (P/N 50-021-HT)
- Oil and Grease Standards, 26 mL (P/N 50-003-HT)
- Oil & Grease Aluminum Weighing Pans, 105 mm, 125 mL (P/N 50-002-02-HT)

Other Instruments:

- Analytical Balance

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Method Summary

1. Obtain 1L DI water samples and acidify to pH ≤ 2 using concentrated HCl.
2. Spike any relevant IPR samples with the 40 mg Oil & Grease Standard and any MDL samples with 500 µL of the 26 mL Oil & Grease Standard vial for a final concentration of 4.0 mg.
3. Attach the sample bottle to the water inlet valve using cap adapters as necessary, then place them onto the extractor.
4. Load the disk holder with a 90 mm Pacific® Premium disk and place onto the disk holder platform.
    a. If needed, 90 mm Pacific O&G Fast Flow Pre-Filters may be used when working with high particulate samples, however the entire study must be completed using prefilters.
5. Place a clean 125 mL separatory funnel or equivalent onto the collection vessel adapter, securing it with a clip.
6. Extract the sample using extraction method found in Table 2.
7. Dry the final extract with phase separation paper (DryDisk® from Biotage) or Na_2SO_4 and thoroughly rinse the collection vessel with hexane to collect any residual HEM.
8. Pre-weigh 105 mm pans and transfer the dried extract into each pan rinsing collection flask three times.
9. Using the Speed-Vap® IV – Solvent Evaporation System, evaporate the extracts utilizing the parameters in Table 1.
    a. Be sure to remove the sample from the Speed-Vap® IV as soon as the extract evaporates. If needed complete further evaporation under a hood and then transfer to a desiccator.
10. Weigh each pan and calculate the HEM recovery in mg.
11. To complete the IPR study, extract four replicates using steps one through ten. To pass this study, average % recovery should be between 83–101% and precision should be ‹ 11%.
12. To complete the MDL study, extract seven replicates using steps one through ten over three separate days. To pass this study, the calculated MDL must be ‹ 1.4 mg/L.

Table 1. Speed-Vap® IV Parameters.

Step	Operation
Temperature (˚C)	40
Compressed Air Inlet Pressure (psi)	80

Table 2. 90 mm Pacific No-Prefilter 1664 Extraction.

Step	Select Solvent	Volume (mL)	Purge (s)	Vacuum	Saturate (s)	Soak (s)	Drain/Elute (s)	Sample Delay (s)
Condition SPE Disk	Hexane	16	60	2	1	60	60	
Condition SPE Disk	Methanol	16	60	2	1	60	2	
Load Sample				5				45
Air Dry Disk				6			180	
Elute Sample Container	Hexane	21	35	5	1	10	15	
Elute Sample Container	Hexane	15	35	5	1	45	45	
Elute Sample Container	Hexane	15	60	6	1	45	60	
Wash Sample Container	Methanol	8	60	6	1	20	60	
Elute Sample Container	Hexane	9	35	5	1	45	45	
Elute Sample Container	Hexane	9	35	5	1	45	45	
Elute Sample Container	Hexane	9	60	6	1	45	60	

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Table 4. IPR Results.

Sample	Recovery (mg)	Recovery (%)
1	39.2	98.0
2	39.7	99.2
3	39.7	99.2
4	39.0	97.5
Average % Recovery		98.5
Standard Deviation		0.89

Table 5. Sample Blank Results.

Sample	Initial Weight (g)	Final Weight (g)
1	6.4548	6.4549
2	6.4416	6.4415
3	6.4626	6.4626
All < Calculated MDL of 0.3360 mg/L		

Table 6. MDL Results.

Sample	Recovery (mg)	Recovery (%)
1	3.7	92.5
2	3.5	87.5
3	3.7	92.5
4	3.6	90.0
5	3.6	90.0
6	3.6	90.0
7	3.4	85.0
Standard Deviation		0.1069
Calculated MDL (mg/L)		0.3360

Discussion

The acceptable recovery limit for an IPR study outlined in EPA Method 1664B is 83–101%. As you can see in table 4 the recoveries all fall within that range, confirming that this method passes the IPR guidelines.

Table 5 shows that there is no background contamination that would skew any of the results.

Furthermore, Method 1664B sets the acceptance criteria for an MDL study to be 1.4 mg/L or less, where the calculated value in table 6 concludes that our study passes.

Conclusion

This application note demonstrates that the final extract volume has been optimized, in turn shortening the extraction and evaporation times. Previous methods for this application on the Horizon 5000 resulted in a 45-minute extraction time and a final extract volume close to 120 mL. Unfortunately, because the final volume was greater, it exceeded the capacity of the 105mm pan, resulting in a two-step and longer evaporation process. The newly optimized method produces a final extract volume of roughly 78 mL with a 35-minute extraction time. The most important conclusion is that the entire volume of the sample extract, as well as any rinses, fit into a single 105 mm aluminum weighing pan, reducing the manual labor associated with the previous method. The end goal was achieved by implementing the optimizations for method 1664B using the Biotage® 5000 and 90 mm disk. This workflow will reduce extraction times, solvent usage and evaporation times which all leads to improved data quality and sample throughput.

References

Method 1664, Revision B: n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry, available at www.epa.gov, (2010).

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Literature Number: AN942

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EPA Method 1664B Extractions Using the Biotage® Horizon 3100 with In-Line Drying Using ISOLUTE® Sodium Sulfate Drying Cartridges

Authors: *Deanna Bissonnette and Michael Ebitson Biotage, Salem, NH, USA.*
Keywords: *EPA, 1664B, Oil and Grease, HEM, Wastewater, Biotage® Horizon 3100, Extraction, ISOLUTE® Sodium Sulfate Cartridge, Drying, Speed-Vap®*

Introduction

The treatment and removal of oil and grease from wastewater is imperative because it can negatively affect the biological and aquatic life that encounter it. Not only that, but since the oil and grease are not miscible with water, it leaves an unappealing layer on top of the water. Even before the oil and grease can reach the aquatic life, it will solidify on the inner walls of pipes, causing blockage over time.

The purpose of this application note is to provide an in-line extraction and drying solution for measuring hexane extractable material (HEM) using solid-phase extraction. The extracts will be dried in-line during extraction using ISOLUTE® Sodium Sulfate Drying Cartridges. Using the Speed-Vap®, the extracts will be concentrated with gentle heat and consistent air flow through precisely drilled holes.

Instrumentation

Biotage Instruments

- Biotage® Horizon 3100 Oil and Grease Extraction System (P/N SPE-DEX 3100)
- Speed-Vap® Automated Solvent Evaporation System (P/N 200-1000-04)

Biotage Consumables

- Pacific® Premium Oil & Grease Disk, 90 mm (P/N 1664-100-PHT)
- ISOLUTE® Sodium Sulfate Drying Cartridge (P/N 802-0250-M)
- Oil and Grease Standards, 40 mg (P/N 50-021-HT)
- Oil and Grease Standards, 26 mL (P/N 50-003-HT)
- Oil & Grease Aluminum Weighing Pans, 105 mm, 125 mL (P/N 50-002-02-HT)

Other Instruments

- Analytical Balance: Sartorius

Method Summary

90 mm Pacific® Premium Disk Extractions:

1. Obtain 17 individual liters of DI water. Acidify to a pH <2 with HCl.
2. Place a 90 mm Pacific Premium disk in a 90 mm disk holder and place on the 3100. Repeat for each sample.
3. Blank sample preparation is complete.
4. Complete the following spiking procedures for the method detection limit (MDL) and initial precision and recovery measurement (IPR).
    a. MDL: Spike each acidified liter (10 total) with 4.0 mg/L of HEM (500 µL of the Oil and Grease Standard, 26 mL, PN: 50-003-HT).
    b. IPR: Spike each acidified liter (4 total) with 40 mg/L of HEM (one 10 mL Oil and Grease Standard package, PN: 50-021-HT).
5. Attach the water inlet valve to each sample bottle and place them on the 3100 system. Attach an ISOLUTE® Sodium Sulfate Drying Cartridge onto the end of the check valve, being sure to secure the cartridge as far as it will go. Attach a 24/40 taper flat bottom round flask onto each active station and secure with the clip. Repeat for each sample.
    a. Ease of flask attachment is not restricted by the cartridge.
6. Extract the samples using the extraction method found in Table 1.
7. Pre-weigh each 105 mm aluminum pan and quantitatively transfer one extract to each pan.
    a. Pour the final extract from the 3100 system into the designated pan and rinse the collection vessel vigorously with n-hexane. Swirl slightly to collect all the HEM in the vessel and pour into the designated pan.
8. Using the Speed-Vap® Evaporation System, concentrate the extracts per the parameters in Table 2. Remove the pan from the Speed-Vap® when there is a thin layer of hexane left in the pan because the hexadecane can evaporate if heated too long.
    b. Allow the extract to finish evaporating in the hood and transfer to a desiccator.
9. Weigh each pan and calculate the HEM recovery in mg/L for each sample.

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Table 1. Extraction conditions using 90 mm Pacific® Disk with ISOLUTE® Sodium Sulfate cartridge on the Biotage® Horizon 3100.

Step	Step Description	Solvent	Dispense (s)	Saturate (s)	Soak (s)	Drain Solvent (s)
1:	Condition	Hexane	10	1	30	60
2:	Condition	Methanol	10	1	30	5
3:	Load Sample					
4:	Air Dry 240s					
Step	**Step Description**	**Solvent**	**Rinse (s)**	**Saturate Elute (s)**	**Soak (s)**	**Elute (s)**
5:	Rinse and Elute	Hexane	10	1	10	15
6:	Rinse and Elute	Hexane	7	1	45	45
7:	Rinse and Elute	Hexane	7	1	45	45
Step	**Step Description**	**Solvent**	**Rinse (s)**	**Saturate (s)**	**Soak (s)**	**Drain Solvent (s)**
8:	Wash	Methanol	4	1	20	180
Step	**Step Description**	**Solvent**	**Rinse (s)**	**Saturate Elute (s)**	**Soak (s)**	**Elute (s)**
9:	Rinse and Elute	Hexane	1	0	0	0
Step	**Step Description**	**Solvent**	**Rinse (s)**	**Saturate (s)**	**Soak (s)**	**Drain Solvent (s)**
10:	Wash	Methanol	0	1	10	180
Step	**Step Description**	**Solvent**	**Rinse (s)**	**Saturate Elute (s)**	**Soak (s)**	**Elute (s)**
11:	Rinse and Elute	Hexane	4	1	45	45
12:	Rinse and Elute	Hexane	4	1	45	45
13:	Rinse and Elute	Hexane	4	1	45	60

Table 2. Speed-Vap® Evaporation Parameters.

Parameter	Value
Temperature (°C)	40
Compressed Air Inlet Pressure (psi)	80

Results and Discussion

The extraction process for quality control samples takes approximately 25 minutes and the evaporation time takes approximately 30 minutes. With one system, three quality control samples can be extracted and evaporated in about an hour.

Below are the results from all the extractions completed in the method summary of this application note.

Table 3. 90 mm Disk IPR results.

Sample	HEM (mg/L)	Recovery (%)
1	35.0	87.50
2	34.9	87.25
3	35.4	88.50
4	35.5	88.75
	Average Percent Recovery (X)	88.00
	Precision (s)	0.74

Table 4. 90 mm Disk MDL results.

Sample	HEM (mg/L)
1	2.5
2	2.2
3	2.4
4	3.0
5	3.2
6	3.0
7	2.2
8	2.5
9	2.7
10	2.6
Precision (s)	0.3406
MDL Value < 1.4 mg/L	0.9608

Table 5. Blank results.

Sample	HEM (mg/L)
1	0.1
2	0.7
3	0.6
All blanks < 0.9608 mg/L	

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The ten replicate samples used for the MDL calculation show little variation keeping the calculated MDL below 1.4 mg/L, the required MDL per EPA Method 1664B. The MDLs were calculated using 9 degrees of freedom and a student t-value of 2.821 with a 99% confidence interval.

The recoveries for the four IPRs are well within the acceptable range of 83–101% recovery per EPA Method 1664B as well. The IPR resulted in a precision value of 0.3406. This is well below the method requirement of 11.0.

The blank extractions also pass EPA Method 1664B requirements all recovering below the calculated MDL.

Conclusion

This application note demonstrates that HEM can be effectively recovered within the guidelines of EPA Method 1664B with the use of Oil and Grease product solutions from Biotage. In addition to that, the blanks indicate virtually no contamination of HEM from consumables or instrumentation.

Automated solid-phase extraction improves the precision of HEM recoveries by eliminating operator bias. With the option of the ISOLUTE® Sodium Sulfate Drying Cartridge, installed in-line with extraction, it reduces operator interaction with the sample extracts, but also stays within compliance of EPA Method 1664B.

References

EPA Method 1664, Revision B: n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry, available at www.epa.gov, (2010).

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Determination of 1, 4-Dioxane Using Automated Solid Phase Extraction (SPE), Compliant with US EPA 522 for Drinking Water

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Key Words: *UCMR-3, 1, 4-dioxane, drinking water, Method 522*

Introduction

1, 4-dioxane is a compound that has become known as an emerging contaminant which may cause negative health effects in humans. The US Agency for Toxic Substances and Disease Registry (ATSDR) states that exposure to 1, 4-dioxane at high levels may cause liver and kidney damage. 1,4-dioxane is also reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in animals.[1] The US EPA has also classified 1,4-dioxane as "likely to be carcinogenic to humans" by all routes of exposure.[2] Recent research has evaluated exposure through drinking water and food, generating a comprehensive picture of possible carcinogenicity.

1,4-dioxane exposure occurs from a variety of sources, it's used as a stabilizer in certain chlorinated solvents, therefore it can be found in many products that are known to use chlorinated solvents such as; paint strippers, dyes, greases, anti-freeze and aircraft deicing fluids. Dioxane was also used as a solvent to facilitate SN2 reactions in chemical synthesis because of its polar aprotic nature. Dioxane is also a by-product of ethoxylation reactions, many of which are carried out on a regular basis in cosmetic products that contain sodium laureth sulfate.[2] This reagent is so common among cosmetic products that detectable amounts of 1,4-dioxane can be found in nearly 57% of baby shampoos and 97% of hair relaxers. The FDA and the EU Scientific Committee on Consumer Safety, working on the advice of the International Cooperation on Cosmetics Regulation (ICCR), recommended the limit for 1, 4-dioxane in finished cosmetic products be less than 10 ppm.[3]

Since the main source of 1,4-dioxane is currently cosmetic products, it is no surprise that it can be found in both drinking water and ground water tables. Japan has observed levels in surface water up to 42.8 µg/L and found up to 79 µg/L in groundwater samples. In this case, a high correlation was observed with the presence of 1,1,1-trichloroethane. 1,4-Dioxane was found at a concentration of 0.2–1.5 µg/L in tap water samples from six cities in Kanagawa, Japan, in 1995–1996.[4] In the US, 1, 4-dioxane was included in the third Unregulated Contaminant Monitoring Rule (UCMR-3), a list of candidate contaminant compounds based on toxicity and occurrence. The list of 30 compounds is monitored in large public water supplies and selected small water supplies to better understand the occurrence and magnitude in drinking water to aid in deciding if regulation is warranted.[5] The results of UCMR-3 have not resulted in a regulated maximum contaminant level of 1, 4-dioxane, but some states are beginning to set regulations. These regulations can be observed in Table 1[2] The EPA risk assessments indicate that the drinking water concentration representing a 1×10^{-6} cancer risk level for 1,4-dioxane is 0.35 µg/L.[6]

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Table 1. State Regulations for 1,4-dioxane concentration in various water sources (2017).

State	Guideline (µg/L)	Source
Alaska	77	AL DEC 2016
California	1.0	Cal/EPA 2011
Colorado	0.35	CDPHE 2017
Connecticut	3.0	CTDPH 2013
Delaware	6.0	DE DNR 1999
Florida	3.2	FDEP 2005
Indiana	7.8	IDEM 2015
Maine	4.0	MEDEP 2016
Massachusetts	0.3	MADEP 2004
Mississippi	6.09	MS DEQ 2002
New Hampshire	0.25	NH DES 2011
New Jersey	0.4	NJDEP 2015
North Carolina	3.0	NCDENR 2015
Pennsylvania	6.4	PADEP 2011
Texas	9.1	TCEQ 2016
Vermont	3.0	VTDEP 2016
Washington	0.438	WA ECY 2015
West Virginia	6.1	WV DEP 2009

Several concerns have arisen about measurement of 1, 4- dioxane in water samples due to dioxane's high affinity for water. The compound is completely miscible in water and although it is volatile, it is difficult to purge from water. Evaluation of 1,4-dioxane can be done by a number of existing US EPA methods employing liquid-liquid extraction or purging to remove 1, 4-dioxane from water for GC/MS measurement but these methods have proved to have worse detection limits than desired. US EPA method 522 from the drinking water program specifies solid phase extraction (SPE) and GC/MS analysis using single ion monitoring (SIM) and is the most successful method to date.[2]

This application note will evaluate the performance of the Biotage® Horizon 5000 automated solid phase extraction system in conjunction with US EPA Method 522.

Experimental

The extraction was performed using the Biotage® Horizon 5000 automated solid phase extraction system, using the extraction program displayed in Table 2. A 500 mL water sample size was extracted at a neutral pH. To improve method performance, the consumable used for this application note was a 3-gram, 6 cc coconut charcoal cartridge. This change not only demonstrated optimal recovery rates but it also allowed the 5000 system to be operated at a sample loading speed of 3. This operational change allowed for the sample loading rate to be increased to approximately 25 mL/min from 10 mL/min and is method compliant due to the language in section 1.6 of EPA method 522. This saved up to approximately 20 minutes per sample. The analytical step was performed using GC/MS in the single ion mode (SIM) for the best sensitivity. The conditions for the Agilent 7890A GC coupled with the Agilent 5975C mass spectrometer are presented in Table 3.

Table 2. Extraction program used on the Biotage® Horizon 5000 system.

Step	Operation	Solvent	Solvent Volume (mL)	Vent Purge Time (s)	Vacuum Pump Rate (s)	Saturation Time (s)	Soak Time (s)	Drain Time (s)	Done Loading Sample Delay (s)	Dry Time (s)	N2 Blanket
1	Condition	Methylene chloride	5	30	3	4	10	60			
2	Condition	Methylene chloride	5	30	3	4	10	60			
3	Condition	Methanol	5	30	3	3	10	60			
4	Condition	Methanol	5	30	3	3	10	6			
5	Condition	Water	5	15	3	3	10	4			
6	Condition	Water	5	15	3	3	10	4			
7	Condition	Water	5	15	3	3	10	4			
8	Load sample				3				45		
9	Air dry disk timer				6					600	OFF
10	Elute sample container	Methylene chloride	3	15	3	3	120	60			OFF
11	Elute sample container	Methylene chloride	3	15	3	3	120	60			OFF
12	Elute sample container	Methylene chloride	3	15	3	3	120	90			OFF

Table 3. GC/MS parameters.

Injection	
Amount	1 µL
Inlet Temperature	280 °C
Mode	Splitless
Gas Type	Helium
Column Conditions	**Zebron™ ZB-5 (Phenomenex), 30 m, 0.25 mm, 0.25 µm**
Mode	Consistent Flow
Oven Program	30 °C hold for 2 minutes Ramp 5 °C/min to 50 °C Ramp 50 °C/min to 200 °C Hold for 6 minutes
MS Ions Monitored	Tetrahydrofuran-d_8–46, 78, 80 1,4-dioxane-d_8 – 62, 64, 96 1,4-dioxane – 58, 88

Figure 1. Two Biotage® Horizon 5000 extractors equipped with carbon cartridges for extraction. Both extractors are controlled using the PC in the middle of the image.

Results and Discussion

Table 6 in EPA Method 522 lists the initial demonstration of capability (IDC) requirements as well as the quality control requirements for the analysis of 1,4-dioxane. Table 7 in Method 522 lists the ongoing quality control requirements that must continually be met.

The method states that a low background of the system and the reagents must be determined by examining a lab reagent blank (LRB). A surrogate is added to the reagent blank to ensure that the extraction was performed to the standard of the method. The 1, 4-dioxane and background interferences must be less than or equal to 1/3 of the MRL in order to continue with the IDC requirements. The results for one LRB sample are presented in Table 4.

Table 4. Method blank data.

Analyte	Target Conc. (µg/L)	Recovery (µg/L)	Recovery (%)
1,4-Dioxane	N/A	ND	ND
1,4-Dioxane-d8	500.0	515.7	103.1

A set of four laboratory fortified blanks (LFBs) was extracted on the Biotage® Horizon 5000 to determine the initial demonstration of precision (IDP). The precision (relative standard deviation (RSD)) of all four samples must be ≤20%. The precision results are presented in Table 5.

Table 5. Initial demonstration of precision (IDP).

Analyte	Target Conc. (µg/L)	LFB 1 (µg/L)	LFB 2 (µg/L)	LFB 3 (µg/L)	LFB 4 (µg/L)	RSD
1,4-Dioxane	10.0	8.6	8.8	8.5	8.2	2.93
1,4-Dioxane-d8	500.0	420.0	430.0	410.0	395.0	3.61

The initial demonstration of accuracy (IDA), presented in Table 6, uses the same four LFBs that were used for determining the IDP. The method specifies that in order to demonstrate accuracy, the mean recovery of the LFBs must be +/- 20% of the true value. The true value for each of the four samples was 10 µg/L.

Table 6. Initial demonstration of accuracy (IDA).

Analyte	Target Conc. (µg/L)	LFB 1 (µg/L)	LFB 2 (µg/L)	LFB 3 (µg/L)	LFB 4 (µg/L)	Mean Recovery (µg/L)	Average Recovery %
1,4-Dioxane	10.0	8.6	8.8	8.5	8.2	8.53	85.25
1,4-Dioxane-d8	500.0	420.0	430.0	410.0	395.0	413.8	82.75

Seven LFBs were extracted to confirm the minimum reporting level (MRL) and determine the half range for the prediction interval of results (HR_{PIR}). This data set provides an RL for the Biotage® Horizon 5000 automated solid phase extraction instrument. The MRL and HR_{PIR} data is presented in Table 7. The equation for calculating HR_{PIR} is as follows:

HR_{PIR} = 3.963 S

Where S is the standard deviation and 3.963 is a constant value for seven replicates

Data from Table 7 was also used to confirm the upper and lower prediction interval of results (PIR). These two limits must be met in order to confirm that the MRL is valid. The upper PIR limit must be less than or equal to 150% while the lower PIR limit must be greater than or equal to 50%. The data for the upper and lower PIR limits is presented in Table 8. The equations for calculating Upper PIR and Lower PIR are as follows:

Upper:

(Mean + HR_{PIR} / Fortified Concentration) *100

Lower:

(Mean - HR_{PIR} / Fortified Concentration) *100

Table 7. MRL data for seven replicates used to calculate the HR_{PIR}. The reported values (µg/L) account for the 500 mL starting volume and the final extract volume of 10 mL.

Analyte	Target Conc. (µg/L)	MRL 1 (µg/L)	MRL 2 (µg/L)	MRL 3 (µg/L)	MRL 4 (µg/L)	MRL 5 (µg/L)	MRL 6 (µg/L)	MRL 7 (µg/L)	Mean (µg/L)	Std. Dev.	HR_{PIR}
1,4-Dioxane	0.150	0.155	0.153	0.137	0.150	0.144	0.140	0.137	0.145	0.008	0.032

Table 8. Upper and Lower PIR limit calculations.

Analyte	Target Conc. (µg/L)	Mean (µg/L)	HR_{PIR}	Upper PIR (%)	Lower PIR (%)
1,4-Dioxane	0.150	0.145	0.032	118%	75%

A method detection limit (MDL) (optional for an IDC) was calculated using the procedure in 40CFR, part 136 for an initial MDL. Eight LFBs were spiked at low concentration (0.15 µg/L) and extracted through the Biotage® Horizon 5000 over a period of one month. The standard deviation of the eight replicates was multiplied by the Student's T value of 2.998 to calculate the MDL. The results for the MDL study are presented in Table 9.

Table 9. MDL determination.

Analyte	Target Conc. (µg/L)	MDL 1 (µg/L)	MDL 2 (µg/L)	MDL 3 (µg/L)	MDL 4 (µg/L)	MDL 5 (µg/L)	MDL 6 (µg/L)	MDL 7 (µg/L)	MDL 8 (µg/L)	Std. Dev.	Calculated MDL (µg/L)
1,4-Dioxane	0.150	0.155	0.153	0.137	0.150	0.144	0.140	0.137	0.159	0.009	0.026

Table 10. Recovery values for Laboratory Fortified Blank samples.

LFB From Three Batches	Target Concentration (µg/L)	Measured 1,4-Dioxane (µg/L)	Recovery (%)
High LFB (05/22/2019	10.0	8.70	87.0
Med LFB (05/23/2019)*	1.00	1.00	100.0
Low LFB (05/22/2019)	0.150	0.149	99.0

*Within 24 hours of the samples analyzed on 05/22/2019.

Method 522 specifies that an analytical batch containing between 10–20 samples must contain a laboratory fortified blank (LFB) as a quality control check. The concentration of the LFB should rotate between low, medium and high concentrations. The acceptance criteria for a low LFB is +/-50% of the true value while a medium and high LFB must fall within +/-30% of the true value. The data for three LFBs, one at each concentration, is presented in Table 10.

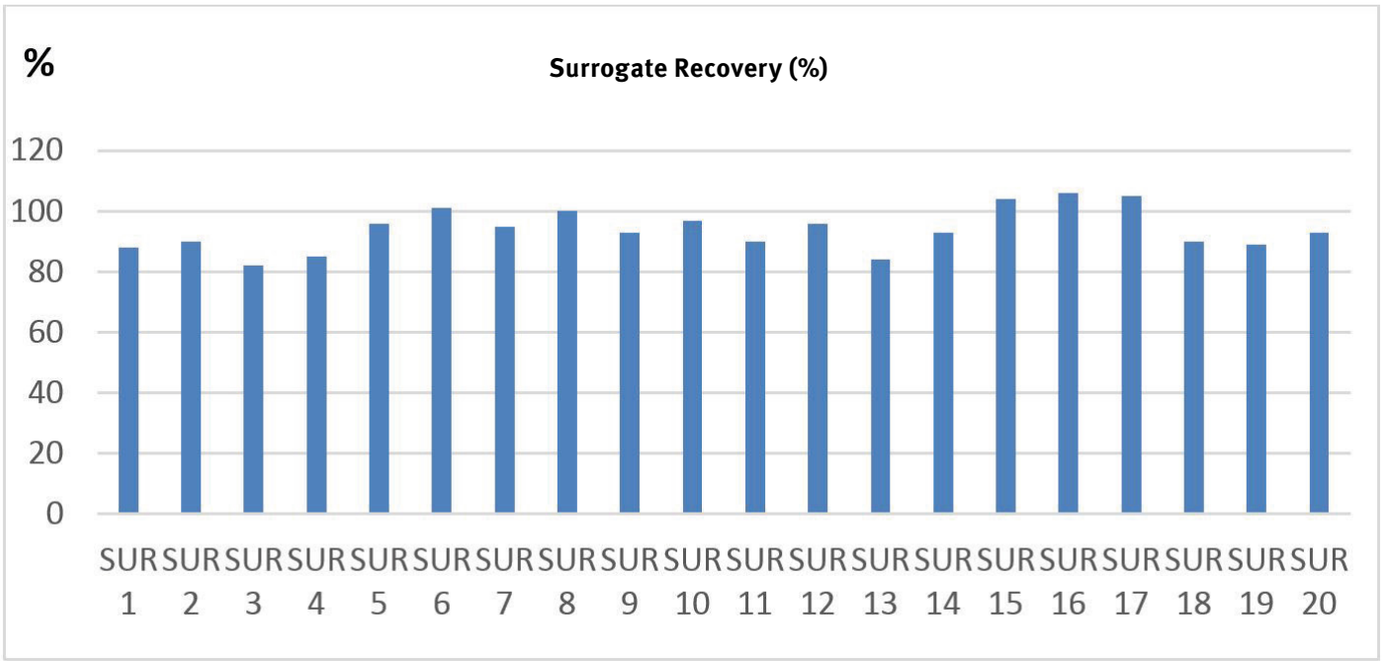

Figure 2. Recovery values for twenty samples spiked with surrogate.

The three-gram cartridges performed exceedingly well and passed all IDC and ongoing QC requirements. The three-gram cartridge allowed for faster sample processing because the larger sorbent volume allows the sample to be pulled through faster while preventing breakthrough. The measured recovery of twenty surrogates is presented in Figure 2.

A blind performance testing sample (PT) was analyzed in order to make sure that the extraction process, as well as the analytical method, are capable of quantitation. A sample was received from an accredited provider and extracted using the Biotage® Horizon 5000. The results as well as the acceptance criteria are presented in Table 11.

Table 11. Recovery values for the performance testing sample.

Sample	True Value (µg/L)	Measured Value (µg/L)	Recovery (%)	Acceptable Range (µg/L)
PT Sample 1	16.0	18.1	113.1	6.4–25.6

Conclusion

This application note proves that EPA method 522 can be successfully implemented in a laboratory using the Biotage® Horizon 5000 automated solid phase extraction system. Four LFB samples were analyzed for precision and accuracy, yielding an average recovery value of 85.25% with an RSD of 2.93%. Both values meet the acceptance criteria of the method. The batch to batch quality control requirements set by the EPA method are easily met using this extraction method. A blind performance test sample validated the accuracy of results obtained for drinking water. The automation of this method provides less analyst intervention which reduces any possible outside contamination. The 10 mL final extract volume eliminates any losses due to evaporation while the larger sorbent bed allows for faster flow rate with better performance. All of these factors lead to an increase in productivity while easily meeting all of the quality control requirements for EPA Method 522.

References

1. Agency for Toxic Substances and Disease Registry, Toxicological Health Profile for 1, 4-Dioxane (2012), https://www.atsdr.cdc.gov/toxprofiles/tp187-c2.pdf, accessed July 5, 2018.
2. US EPA Technical Fact Sheet –1,4-Dioxane, November 2017, https://www.epa.gov/sites/production/files/2014-03/documents/ffrro_factsheet_contaminant_14-dioxane_january2014_final.pdf, accessed July 5, 2018.
3. Scientific Committee on Consumer Safety (SCCS), The Report of the ICCR Working Group: Considerations on Acceptable Trace Level of 1,4-Dioxane in Cosmetic Products, December 2015. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_194.pdf, accessed July 5, 2018.
4. 1,4-Dioxane in Drinking-water, Background document for development of WHO Guidelines for Drinking-water Quality, World Health Organization 2005. https://www.who.int/water_sanitation_health/dwq/chemicals/14dioxane0505.pdf, accessed July 8, 201 8
5. Third Unregulated Contaminant Monitoring Rule Fact Sheet, https://www.epa.gov/sites/production/files/2015-10/documents/ucmr3_factsheet_general.pdf, accessed July 5, 2018.
6. 2018 Edition of the Drinking Water Standards and Health Advisories Tables, https://www.epa.gov/sites/production/files/2018-03/documents/dwtable2018.pdf, accessed July 5, 2018.
7. David T. Adamson, Elizabeth A. Piña, Abigail E. Cartwright, Sharon R. Rauch, R. Hunter Anderson, Thomas Mohr, John A. Connor, 1,4-Dioxane drinking water occurrence data from the third unregulated contaminant monitoring rule, Science of the Total Environment 596–597 (2017) 236–245.
8. US EPA Method 522, Method 522_Determination of 1,4-Dioxane in Drinking Water by Solid Phase Extraction (SPE) and Gas Chromatography/ Mass Spectrometry (GC/MS) with Selected Ion Monitoring (SIM) Sep, 2008, https://nepis.epa.gov/Exe/ZyPDF.cgi?Dockey=P100EQ8A.txt, accessed July 6, 2018.

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Literature Number: AN927

Automated Fractionation of Extractable Petroleum Hydrocarbons (EPH) in Soil Using ISOLUTE® EPH SPE Columns on Biotage® Extrahera™

Introduction

ISOLUTE® EPH SPE columns and associated methodologies have been optimized to efficiently fractionate EPHs into aliphatic and polycyclic aromatic (PAH) fractions (C8–C40 aliphatics, C10–C22 aromatics).

The principle is similar to the approach taken by the Massachusetts Department of Environmental Protection (MADEP) and TPH criteria working group (TPHCWG) methods. However, compared to these methods, the ISOLUTE EPH fractionation column has been significantly reduced in size and the sorbent has been optimized. This allows an automation-compatible method of fractionation which overcomes the common problem of PAH breakthrough into the aliphatic fraction, in addition to reduced solvent volumes.

This application note describes the operating conditions for the automated fractionation of EPH into aliphatic and PAH fractions using the ISOLUTE EPH column in conjunction with the Biotage® Extrahera™ automation system.

Application Analytes

Table 1. Aliphatics: C8-C40.

octane	eicosane	dotriacontane
decane	docosane	tetratriacontane
dodecane	tetracosane	hexatriacontane
tetradecane	hexacosane	octatriacontane
hexadecane	octacosane	tetracontane
octadecane	triacontane	

Table 2. Aromatics: C10-C22

naphthalene	fluoranthene	benzo(a)pyrene
acenaphthalene	pyrene	indeno(123,c,d)pyrene
acenaphthene	benz(a)anthracene	dibenz(ah)anthracene
fluorene	chrysene	benzo(ghi)perylene
phenanthrene	benzo(b)fluoranthene	
anthracene	benzo(k)fluoranthene	

Method Overview

Soil Extraction Procedures

Any of the MADEP or TPHCWG approved soil extraction procedures can be used with the ISOLUTE EPH method. The only requirements for successful fractionation are that the final solvent containing the soil extract should be either pentane or hexane. The presence of any polar modifier in the final extract will compromise the fractionation process. For example, if soil is extracted using dichloromethane, this solvent must be exchanged for hexane or pentane prior to fractionation.

Up to 1 mL soil extract in hexane or pentane can be loaded onto the ISOLUTE EPH columns.

Sample Preparation Procedure

Format

ISOLUTE® EPH 1.45g/3 mL, part number 928-0145-B.

Sample

Soil extract in hexane or pentane.

Column Conditioning

6 mL Hexane (4 x 1.5 mL).

Sample Loading

1 mL Hexane or Pentane extract. Collect column eluate.

Aliphatic Elution

1.5 mL Hexane. Add to column eluate from load step.

Aromatic Elution

4.5 mL DCM (3 x 1.5 mL). Collect in a separate tube to the aliphatic elution.

Post Elution

Gently vortex and homogenize both fractions for each sample and transfer 1 mL of each fraction to separate GC vials for analysis.

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GC Conditions

Instrument

Agilent 7890A with QuickSwap

Column

Agilent J&W DB-5, 30 m x 0.25 mm ID x 0.25 µm

Carrier

Helium 1.2 mL/min (constant flow)

Inlet

300 °C, Splitless, purge flow: 50 mL/min at 1.0 min

Injection

2 µL

Wash Solvents

Methanol and DCM

Oven Temperature

Described in Table 3.

Table 3. Methods for the aliphatic and aromatic hydrocarbon analysis.

Aliphatics	Aromatics
Initial temperature 45 °C, hold for 1 minute	Initial temperature 45 °C, hold for 1 minute
Ramp 35 °C to 115 °C	Ramp 10 °C to 350 °C
Ramp 70 °C to 350 °C, hold for 6.5 minutes	

Post Run

Backflush for 2.4 minutes (3 void volumes)

Transfer Line

300 °C

Mass Spectrometry Conditions

Instrument

Agilent 5975C

Source

230 °C

Quadrupole

150 °C

MSD Mode

SIM

SIM Parameters

Ions were acquired in the Selected Ion Monitoring (SIM) mode.

Aliphatic ions acquired: 41, 43, 57, 71, 85

Aromatic ions acquired displayed in Table 4.

Table 4. Aromatic fraction ions acquired.

SIM Group	Analyte	Target (Quant) Ion	Target (Quant) Ion 1	Target (Quant) Ion 2
1	Naphthalene	128	102	126
2	Acenaphthalene	152	76	150
3	Acenaphthene	153	76	154
4	Fluorene	166	163	165
5	Phenanthrene	178	152	176
5	Anthracene	178	152	176
6	Fluoranthene	202	101	200
6	Pyrene	202	101	200
7	Benz(a)anthracene	228	113	226
7	Chrysene	228	113	226
8	Benzo(b)fluoranthene	252	126	250
8	Benzo(k)fluoranthene	252	126	250
8	Benzo(a)pyrene	252	126	250
9	Indeno(123,c,d)pyrene	276	137	138
9	Dibenz(a,h)anthracene	278	139	
9	Benzo(ghi)perylene	276	137	138

Results

The optimized ISOLUTE® EPH protocol on Biotage® Extrahera™ provided typical recoveries as demonstrated in Figures 1 and 2, with RSD values <10% for all analytes.

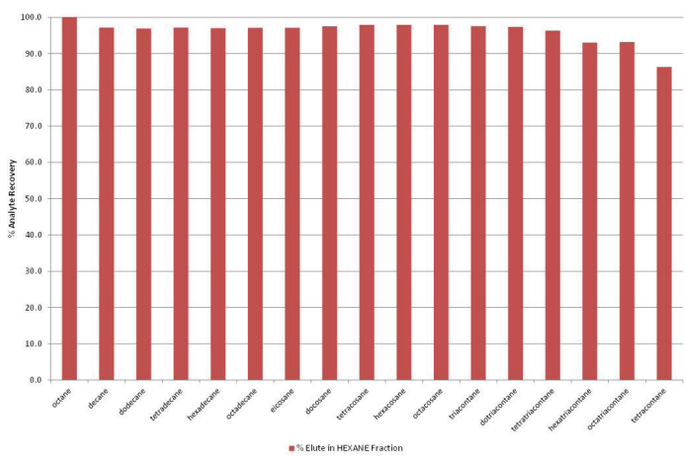

Figure 1. Chart demonstrating typical recoveries of aliphatic analytes in the hexane fraction.

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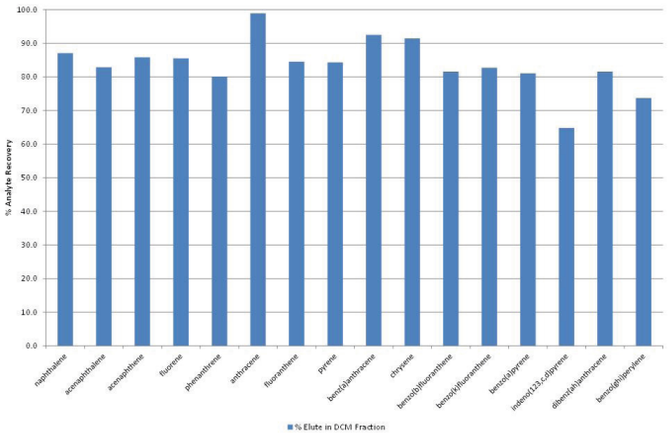

Figure 2. Chart demonstrating typical recoveries of aromatic analytes in the dichloromethane fraction.

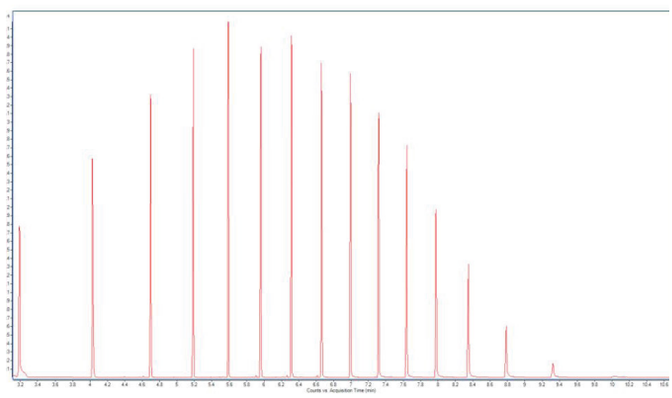

Figure 3. Total Ion Chromatogram of aliphatic analytes in the hexane fraction.

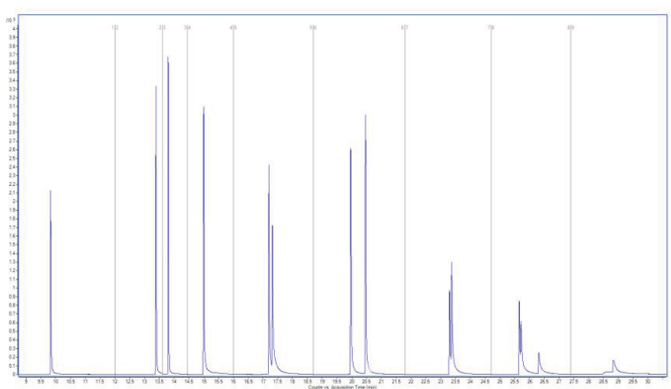

Figure 4. Total Ion Chromatogram of aromatic analytes in the dichloromethane fraction.

Reduction in Solvent Consumption

ISOLUTE® EPH columns allow a significant reduction in solvent consumption compared to the existing MADEP or TPHCWG procedures as is illustrated in the following Table:

Table 5. Solvent consumption of each fractionation method.

Method	Solvent Consumed per Sample During Fractionation Step	
	Hexane/Pentane	Dichloromethane
ISOLUTE EPH	7.5 mL	4.5 mL
MADEP	50 mL	20 mL
TPHCWG	32 mL	30 mL

Additional Information

- All solvents were HPLC grade.
- For fully automated workflow, an additional 12x75mm x 24 position collection rack (part 414511SP) is required to collect separate fractions.

Ordering Information

Part Number	Description	Quantity
928-0145-B	ISOLUTE® EPH 1.45 g/3 mL	50
For Automated Processing		
414001	Biotage® Extrahera	1
414008	Configuration Kit 24 positions	1
414511SP	Collection Rack 12 x 75 mm, 24 positions	1

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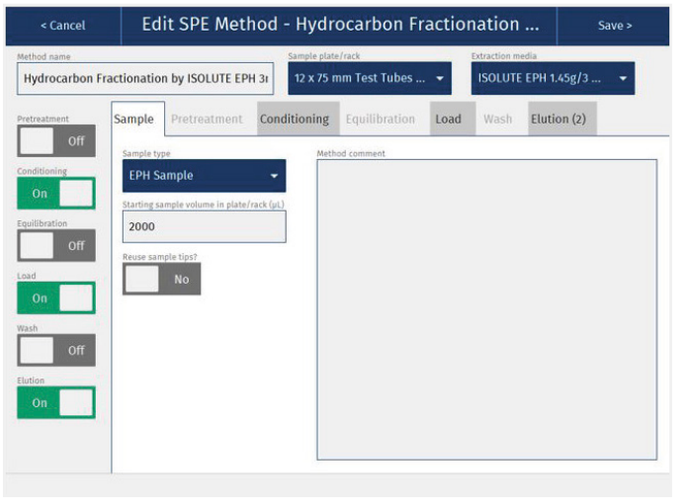
Appendix

Biotage® Extrahera™ Settings

The method described in this application note was automated on the Biotage® Extrahera. This appendix contains the software settings required to configure Extrahera to run this method. An importable electronic copy of this method for Extrahera can be downloaded from www.biotage.com.

When two collection racks are present, sample processing and fractionation can be fully automated and 24 samples can be processed in a total of 42 min 30 secs.

Method Name:	Hydrocarbon Fractionation by ISOLUTE® EPH 1.45 g/3 mL
Sample Plate/Rack:	12 x 75 mm Test Tubes EPH
Extraction Media:	ISOLUTE® EPH 1.45 g/3 mL



Settings

"Sample" Tab

Sample Type:	EPH Sample
Starting Sample Volume (µL)	2000
Reuse sample tips?	No
Method comment:	

	Pre-treatment	Not Activated
	No. of steps	
	Pause after last step	
	Dispose tips after last step	

	Solvent
1	
2	
3	
4	

	1	2	3	4
Volume (µL)				
Time				

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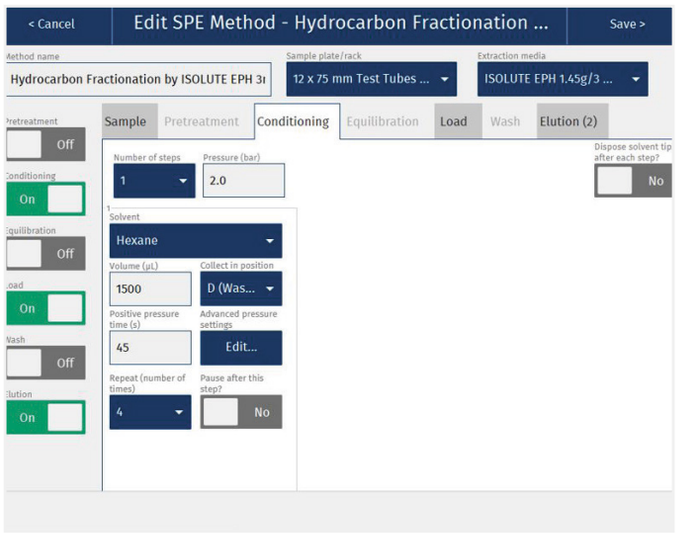
Screenshot



Settings

Conditioning	Activated
No. of steps	1
Pressure (Bar	2.0
Dispose tips after last step	No

	Solvent
1	Hexane
2	
3	
4	

	1	2	3	4
Volume (µL)	1500			
Collect in position	D			
Pressure time (s)	45			
Repeat	4			
Pause after this step	No			

'Advanced Settings'

Equilibration	Activated
No. of steps	
Pressure (Bar	
Dispose tips after last step	

	Solvent
1	Solvent
2	
3	
4	

	1	2	3	4
Volume (µL)				
Collect in position				
Pressure time (s)				
Repeat				
Pause after this step				

'Advanced Settings'

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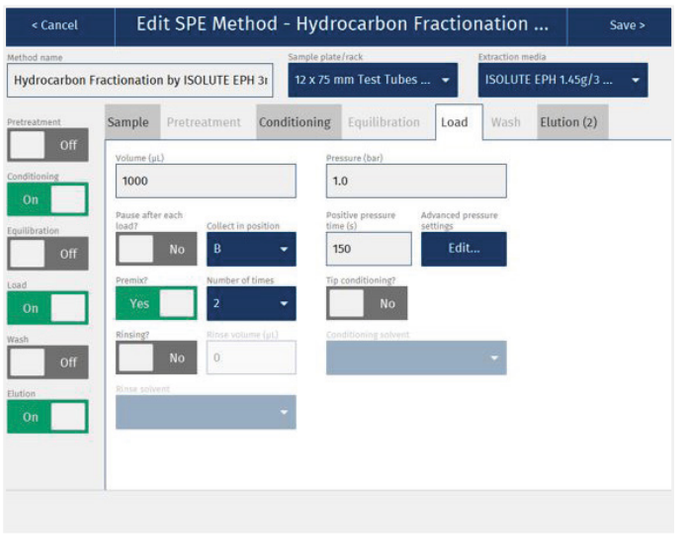

Load	Activated
Pressure (Bar)	1.0
Pause after each load	No
Volume (µL)	1000
Collect in position	B
Positive pressure time (s)	150
Premix	Yes
Number of times	2
Rinsing	No
Rinse volume (µL)	N/A
Rinse solvent	N/A
Tip Conditioning	No
Conditioning solvent	N/A

'Advanced Settings'

Wash	Not Activated
No. of steps	
Pressure (Bar)	
Plate dry after last wash	
Plate dry time (s)	
Dispose tips after each step	

	Solvent
1	Solvent
2	
3	
4	

	1	2	3	4
Volume (µL)				
Collect in position				
Pressure time (s)				
Repeat				
Pause after this step				

'Advanced Settings'

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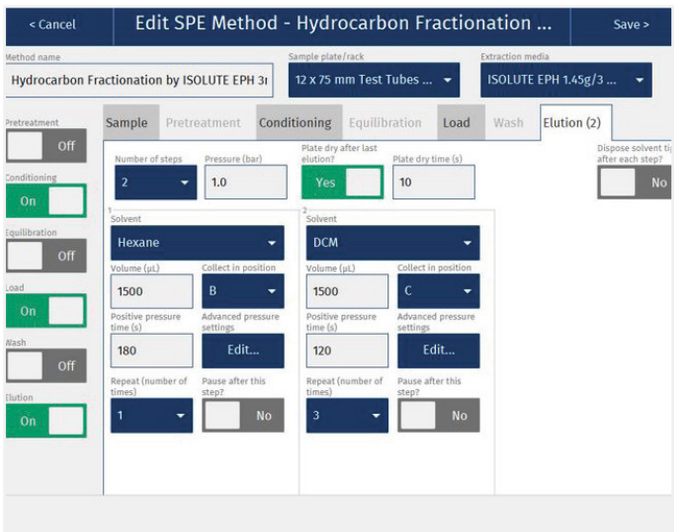

Elution	Activated
No. of steps	2
Pressure (Bar)	1.0
Plate dry after last elution	Yes
Plate dry time (s)	10
Dispose tips after each step	No

	Solvent
1	Hexane
2	DCM
3	
4	

	1	2	3	4
Volume (µL)	1500	1500		
Position	B	C		
Pressure time (s)	180	120		
Repeat	1	3		
Pause after this step	No	No		

'Advanced Settings'

Solvent Properties

	Solvent Description
1	Hexane
2	DCM
3	
4	
5	
6	
7	
8	
9	
10	

Solvent	1	2	3	4	5	6	7	8	9	10
Reservoir Type	**Refillable**					**Non Refillable**				
Capacity	N/A	N/A	N/A	N/A	N/A					
Aspiration flow rate (mL/min)	10	10								
Dispense flow rate (mL/min)	20	10								
Lower air gap flow rate (mL/min)	20	10								
Lower air gap volume (µL)	5	5								
Upper air gap flow rate (mL/min)	120	120								
Upper air gap volume (µL)	100	100								
Upper air gap dispense pause	300	300								
Conditioning?	Yes	Yes								
Conditioning number of times	3	2								
Conditioning flow rate (mL/min)	20	10								
Conditioning volume (%)	100	100								
Aspirate post dispense	Yes	Yes								
Chlorinated	No	Yes								
Serial dispense	No	No								

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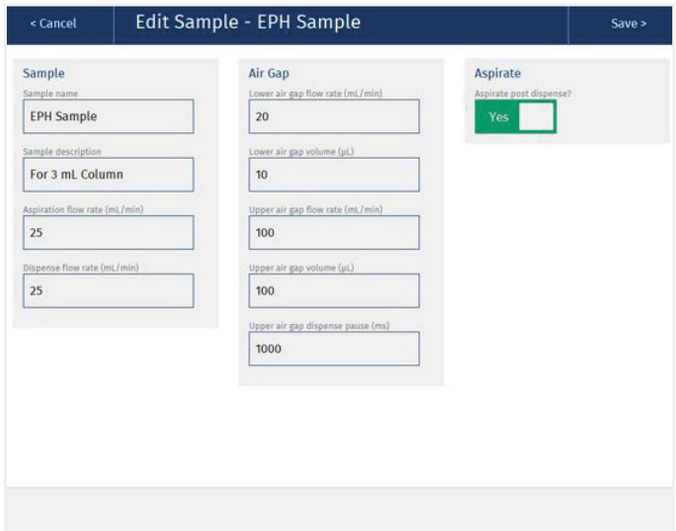
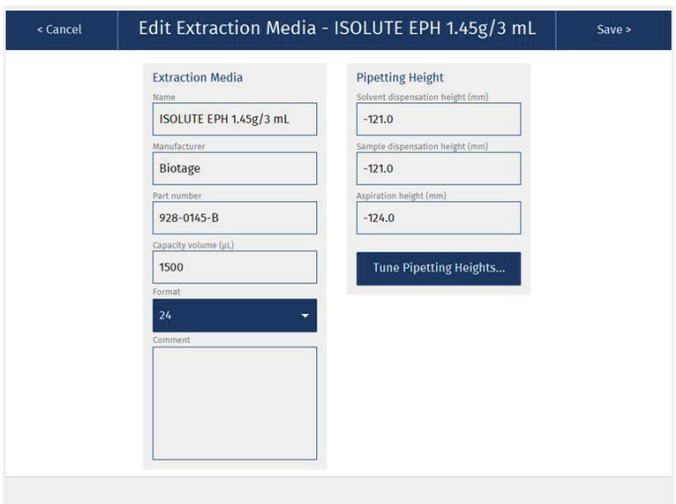
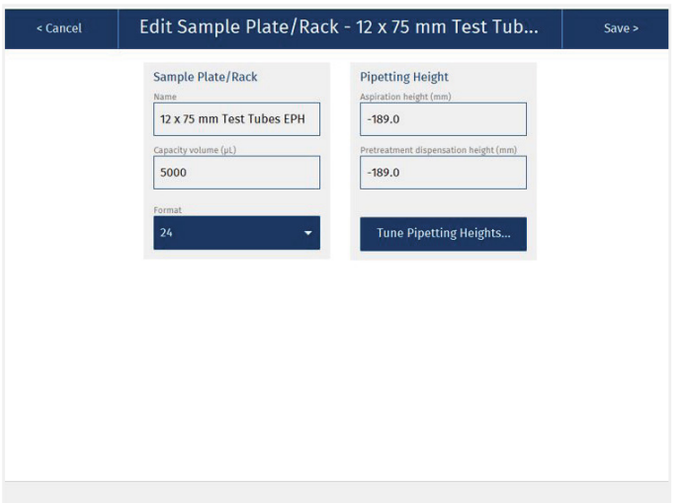


"Sample" Screen	
Sample name	EPH Sample
Sample description	For 3 mL Column
Aspiration flow rate (mL/min)	25
Dispense flow rate (mL/min)	25
Lower air gap flow rate (mL/min)	20
Lower air gap volume (µL)	10
Upper air gap flow rate (mL/min)	100
Upper air gap volume (µL)	100
Upper air gap dispense pause	1000
Aspirate post dispense	Yes



"Extraction Media" Screen	
Name	ISOLUTE EPH 1.45 g/3 mL
Manufacturer	Biotage
Part number	928-0145-B
Capacity volume (µL)	1500
Format	24
Comment	
Solvent dispensation height (mm)	-121.0
Sample dispensation height (mm)	-121.0
Aspiration height (mm)	-124.0



"Sample Plate/Rack" Screen	
Name	12 x 75 mm Test Tubes EPH
Capacity volume (µL)	5000
Format	24
Aspiration height (mm)	-189.0
Pre-treatment dispensation height (mm)	-189.0

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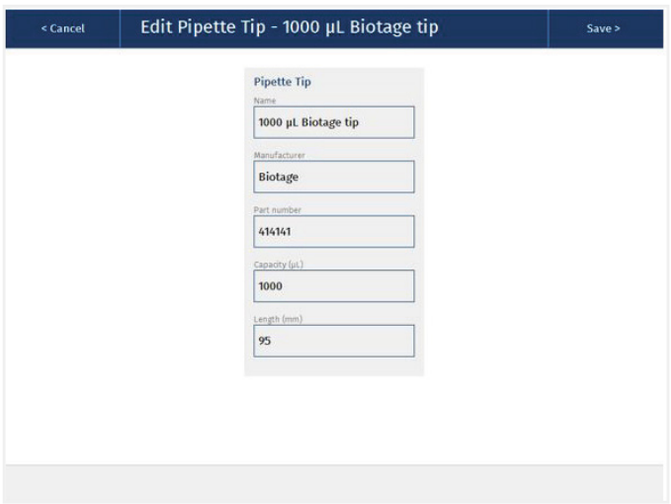

"Pipette tip" Screen	
Name	1000 µL Biotage Tip
Manufacturer	Biotage
Part number	414141
Capacity (µL)	1000
Length (mm)	95

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Quantitative Determination of Diquat and Paraquat in Drinking Water via EPA Method 549.2

Andrew Taylor, Michael Ebitson, Biotage, Salem, NH USA
Key Words: Diquat, Paraquat, EPA Method 549.2

Introduction

Diquat and paraquat are some of the most widely used and commercially available herbicides in the world. Both compounds are fast-acting, non-selective quaternary amines used primarily in the agricultural industries to control the penetration of invasive plants and increase crop yield. While these compounds have proven to be effective in herbicides, they also have been proven to be toxic to humans upon exposure. This toxicity and widespread availability has led to instances where individuals have issued fatal doses to humans. In turn, this has led to strict guidelines worldwide involving the use of diquat and paraquat in the agricultural community.[1]

The scope of this application note is to demonstrate the process used to extract diquat and paraquat from reagent water using the Biotage® Horizon 5000 in tandem with the ISOLUTE® C8(EC) 500 mg/6 mL solid phase extraction cartridge. The procedure will outline the extraction of the two herbicides in reagent water, following guidelines from the initial demonstration of capability (IDC) within US Environmental Protection Agency (EPA) method 549.2.

Table 1. Biotage® Horizon 5000 extraction method.

Step	Operation	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	Sat. Time (s)	Soak Time (s)	Drain Time (s)
1	Condition SPE	Reagent water	5	15	6	1	10	1
2	Condition SPE	Methanol	5	15	6	2	15	3
3	Condition SPE	Reagent water	5	15	6	2	10	2
4	Condition SPE	Conditioning solution A*	5	15	6	2	15	5
5	Condition SPE	Reagent water	5	15	6	2	0	2
6	Condition SPE	Methanol	10	15	6	2	15	10
7	Condition SPE	Reagent water	5	15	6	2	0	3
8	Condition SPE	Conditioning solution B**	20	15	6	5	90	18

Step	Operation	Sample Flow Rate (#)	Done Loading Sample Delay (s)
9	Load Sample	1	60

Step	Operation	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Drain Time (s)
10	Wash SPE Disk	Methanol	5	15	2	Off	0	0	140
11	Elute SPE Disk	Disk eluting solution***	1	15	1	Off	2	90	90
12	Elute SPE Disk	Disk eluting solution***	1	15	1	Off	2	90	90

* Conditioning solution A found in EPA method 549.2 section 7.14.1
** Conditioning solution B found in EPA method 549.2 section 7.14.2
*** Disk eluting solution found in EPA method 549.2 section 7.14.5

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Experimental

The extraction was performed using the Biotage® Horizon 5000 automated solid phase extraction system, using the extraction program displayed in Table 1. A 250 mL sample size (1 L sample size for laboratory reagent blank sample) was extracted at a pH between 7 and 9. The consumable used for this application note was an ISOLUTE® C8(EC) 500 mg/6 mL solid phase extraction cartridge (p/n 291-0050-C). The instrument provided extracts with approximately 4.5 mL of solvent. 100 µL of the ion-pair concentrate (section 7.14.6 in EPA method 549.2) was added to each extract and the final volume was brought up volumetrically to 5 mL with disk eluting solution (section 7.14.5 EPA method 549.2) and vortexed to ensure the ion-pair concentrate was dispersed throughout the mixture. The analytical step was performed using an Agilent 1260 Infinity II HPLC instrument outfitted with Diode Array Detector. The conditions for the HPLC-DAD analysis are presented in Table 2.

Table 2. HPLC-DAD parameters.

Parameter	Value
Column	YMC AQ12S03-1546WT (4.6 x 150 mm)
Column Temperature	35.0 °C
Flow Rate	2.0 mL/min
Mobile Phase	Ion-Pair Mobile Phase (section 7.16 in EPA Method 549.2)
Run Time	5 min
Wavelength Range	210–370 nm
Quantitation Wavelengths	Parquat: 257 nm Diquat: 308 nm

Results and Discussion

Section 9 in EPA Method 549.2 lists the quality control requirements for the analysis of diquat and paraquat. This section of the method states that a low system background must be demonstrated, an initial demonstration of capability (IDC) study must be performed, and finally a method detection limit (MDL) must be determined. All three of these tasks must be completed for both diquat and paraquat.

Section 9.2 of the method states that a low background of the system, the deactivated glassware or plasticware, as well as the reagents must be demonstrated by examining a lab reagent blank (LRB). The results for one LRB sample are presented in Table 3.

Table 3. Demonstration of low system background.

Sample	Paraquat (µg/L)	Diquat (µg/L)
Lab Reagent Blank (LRB)	0.00	0.00

The initial demonstration of capability (IDC), presented in Table 4, is demonstrated through extracting four laboratory fortified blank (LFB) samples. The method specifies that in order to demonstrate accuracy the recovery values must fall within ± 30% of the true value. The method also specifies that the samples must be spiked at 100µg/L. The relative standard deviation for the mean of all four replicates must be lower than 30%. The statistical data for these four replicates is presented in Table 5.

Table 4. Initial demonstration of capability recovery data.

Sample	Paraquat recovery (%)	Diquat recovery (%)
LFB 1	95.43	88.80
LFB 2	93.60	90.46
LFB 3	93.95	93.16
LFB 4	96.79	91.68

Table 5. Initial demonstration of capability statistical data.

Paraquat			Diquat		
Average Recovery (%)	**Standard Deviation**	**RSD (%)**	**Average Recovery (%)**	**Standard Deviation**	**RSD (%)**
94.94	1.46	1.54	91.68	1.85	2.03

Table 6. Paraquat and diquat MDL.

Analyte	Target Conc. (µg/L)	MDL 1 (µg/L)	MDL 2 (µg/L)	MDL 3 (µg/L)	MDL 4 (µg/L)	MDL 5 (µg/L)	Std. Dev.	Calculated MDL (µg/L)
Paraquat	0.80	0.75	0.74	0.73	0.90	0.87	0.079	0.298
Diquat	0.80	0.62	0.60	0.66	0.73	0.74	0.063	0.237

The method detection limit (MDL) was calculated according to the procedure in section 9.3.3 of EPA method 549.2. The method specifies that minimum of 4-7 replicates must be analyzed at a low concentration. Five LFBs were spiked at 0.80 µg/L and extracted using the Biotage® Horizon 5000. The standard deviation of the five replicates was multiplied by the Student's T value of 3.747 to calculate the MDL. The results for the paraquat and diquat MDL studies are presented in Table 6.

Conclusion

According to the quality control section of EPA method 549.2, all the reported values fall well within the acceptance criteria. A low demonstration of background was confirmed using a laboratory reagent blank sample that resulted in values that were too low to quantify for both paraquat and diquat. The initial demonstration of capability was performed by extracting four laboratory fortified blank samples (spiked at 100 µg/L), resulting in average percent recovery values of 94.94 and 91.68 for paraquat and diquat respectively. The calculated RSD values for paraquat and diquat are 1.54% and 2.03% respectively. The method detection limits were established for both paraquat and diquat by analyzing five low level laboratory fortified blank samples. The calculated MDL values for paraquat and diquat are 0.298 µg/L and 0.237 µg/L respectively.

References

1. Munch, J.W. and Bashe W.J., "Method 549.2- Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection", National Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 45268,1997.

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