Using Empore™ C18 SPE Disk to Extract SVOCs in Drinking Water Followed by GC-MS Analysis for EPA Method 525.2

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Application Note
Environmental

Abstract
CDS Empore™ (formerly 3M™ Empore™) C18 Solid Phase Extraction (SPE) disks facilitate rapid and reliable sample preparation and provide excellent analyte recovery for clean chromatograms. This application note demonstrates the performance of such disk in the monitoring of drinking water samples under EPA Method 525.2.

Introduction
The target analyte list for EPA Method 525.2 is comprised of 110 compounds that are representative of four organic compound classes as pesticides, polynuclear aromatic hydrocarbons, PCBs, phthalates and adipates. Method detection limits (MDLs), as published in the method, ranges from 0.03-2.4 µg/L and the recovery rate varies from 20 – 180% for each individual compound. However, after averaging each compound within the four compound classes, the averaged recovery rate for each class is:

Pesticides	108%
PCBs	108%
Phthalates & Adipates	116%
PAHs	112%

EPA Method 525.2 specified SPE disks as the sample preparation tool for the cleanup and concentration of organic contaminants from drinking water samples[1,2]. There are two challenges in the methods in the sample preparation as (1) large sampled volume to 1 liter, and (2) low pH around 2. Empore™ C18 disks can consistently tackle with these challenges without loss of C18 phase from the silica support in the disks. EPA Method 525.2 specially warned that stripping C18 phase in the extraction disk packing will complicate the chromatographic analysis with high background, which could obscure the testing results on compounds of interests.

In this application note, a one-liter water sample was passed through a 47mm C18 Empore™ disk and eluted with ethyl acetate and methylene chloride under negative pressure. Then the extract was dried and reduced in volume down to 1.0 mL and further analyzed by GC/MS.

The validation data presented herein was determined on three repeats of the same lot of C18 disks. MDLs were not determined as part of this validation.

Experimental Setup

Sample Pre-treatment:
40 mg of sodium sulfite was added to 1 L of tap water to reduce free chlorine. The water sample was adjusted to pH=2 by using 6M HCl and 5 mL of methanol was added as a wetting agent. Each sample was fortified with 2 µg of each internal standard and surrogate. For recovery data, each sample was fortified with 2 µg of each method analyte. The CDS Empore™ 47mm C18-bonded silica disks (MilliporeSigma Cat. No. 66883-U) were used for the extraction with repeated number n=3.

Method:
1.Assemble an all glass filtration assembly using a 47 mm C18 Empore™ disk. Use of a manifold for multiple extractions is acceptable.

2. Wash the extraction apparatus and disk by adding 5 mL of a 1:1 mixture of ethyl acetate (EtAc): methylene chloride ($MeCl_2$) to the reservoir. Pull a small amount through the disk with a vacuum; turn off the vacuum and allow the disk to soak for about one minute. Pull the remaining solvent through the disk and allow the disk to dry.

3. Condition the disk by adding approximately 5 mL of methanol to the reservoir, pulling a small amount through the disk then letting it soak for about one minute. Pull most of the remaining methanol through the disk, leaving 3 to 5mm of methanol on the surface of the disk.

4. Add 5 mL of reagent water to the disk and using the vacuum pull most through, again leaving 3 to 5 mm of water on the surface of the disk.

5. Add 5 mL of methanol to the water sample and mix well. Add the water sample to the reservoir and, under vacuum, filter as quickly as the vacuum will allow. Drain as much water from sample bottle as possible.

6. Remove filter assembly and insert suitable sample tube for eluate collection.

7. Add 5 mL of EtAc to the sample bottle. Rinse bottle thoroughly and transfer solvent to the disk with dispo-pipet, rinsing sides of filtration reservoir in the process.

8. Pull half of solvent through disk then release the vacuum. Allow the remaining solvent to soak the disk for about one minute, then draw remainder through under vacuum.

9. Repeat the solvent rinse of the sample bottle and apparatus using 5 mL of $MeCl_2$.

10. Using a disposable pipette, rinse down the sides of the filtration glassware with two 3 mL aliquots of 1:1 EtAc/$MeCl_2$.

11. Dry the combined eluant with 5-7 grams granular anhydrous sodium sulfate. Rinse the collection tube and sodium sulfate with two 3 mL portions of 1:1 EtAc/$MeCl_2$ and place combined solvent into a concentrator tube.

12. Concentrate extract to 1 mL under gentle stream of nitrogen (may be warmed gently). Do not concentrate to <0.5 ml or loss of analytes could occur.

GC/MS Analysis:
The extract analysis was performed on a Shimadzu GC-2010 Gas Chromatograph with a split/splitless injection portal interfaced to a Shimadzu GC-MS QP2010 (Kyoto, Japan). GC-MS parameters are shown below:

GC Parameters:
Recommended Column: SLB®-5ms (30 m × 0.25 mm, ID × 0.25 µm df)

Inlet Temp:	230°C
TransferLine:	250°C
Injection Mode:	Splitless
Injection Volume:	1 µL
Carrier Gas:	He at 33 cm/sec (constant flow)
Oven Program:	45°C hold for 1 minute,45°C to 130°C at 45°C/min,130°C to 180°C at 12°C/min, 180°C to 240°C at 7°C/min, and 240°C to 320°C at 12°C/min Hold for 4 minutes.

Mass Spectrometer Parameters

Solvent Delay:	3.0 minutes
Threshold:	0
Scan Range:	45-450
EM Voltage:	870
Sampling Rate:	2
Scans/sec:	3.3

Results and Discussions
Figure 1 showed the GC chromatogram of 102 semi-volatile compounds from EPA Method 525.2. It can be seen from Figure 1 that these compounds are well separated at the current experimental conditions.

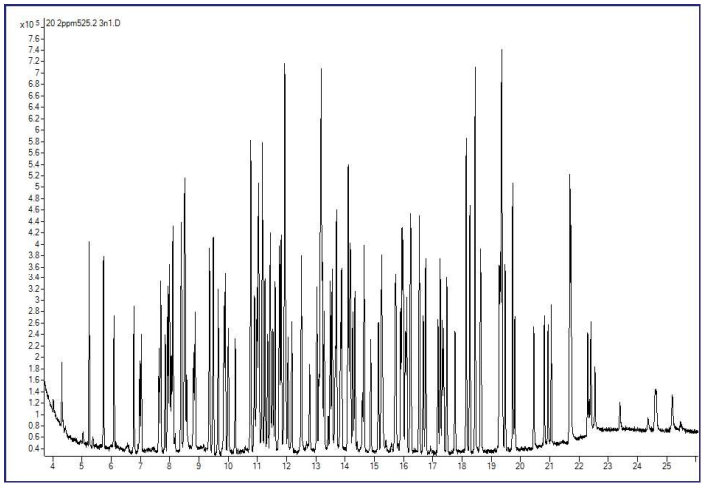

Figure 1. Chromatogram of 102 semi-volatile compounds from EPA 525.2 method.

Table 1 showed the recovery data of the 102 compounds in EPA Method 525.2 list studied in this experiment. The average recovery for 89 compounds exceeded 85% with average relative standard deviation (RSD) of 4.7%. The other 9 compounds had recovery between 70% to 84%, with average RSD of 7.8%. Together, 98 of 102 compounds in this study have the recovery rates falling into the range of 70% to 130%, required by EPA Method 525.2.

There are only 4 compounds with recovery less than 70%: atraton-58%, 2,4-dinitrotoluene-42%, 2,6-dinitrotoluene-45%, and simetryn-65%. For Atraton, the recovery reported from EPA Method 525.2 is 44%, due to the low pH=2 condition for this extraction method. The recovery reported here is a little improved from that of the EPA Method, but to accurately determine its level in water samples, a separated method with pH neutral during the extraction is necessary to get recovery >90%. For 2,4-Dinitrotoluene and 2,6-dinitrotoluene, the low recoveries are suspected from the breakthrough of C18 phases. Mark Krigbaum has done an excellent investigation on this phenomenon, and his explanation for this issue is credible[3]. The polarity of both dinitrotoluenes caused their poor retentions on the reversed C18 phases. The exact same extraction conditions in this note have been applied to Empore™ SDB-RPS disks (MilliporeSigma Cat. No. 66886-U), and both compounds showed recoveries >80% (results not shown here). SDB-RPS is a mixed phase combining reversed phase and strong cation exchange phase (SCX) together. The SCX portion of the phase has better retention of these polar compounds through ionic interactions, thus improving the recovery dramatically. This result is consistent with the results observed by Mark Krigbaum[3]. The low recovery of simetryn is due to the similar reason: the polar groups in diamino-1,3,5-triazine type compounds.

Conclusions:

A simple and effective method to extract organic compounds from large volume 1L drinking water sample by Empore™ C18 47mm disks has been validated per EPA Method 525.2. 102 organic compounds listed in the method have been effectively extracted from drinking water samples, and then quantified by GC-MS with concentration at 2.0 ppb. 89 compounds spiked into the water samples had the recovery rate exceeded 85% with average RSD of 4.7%, and 9 compounds have the recovery in the range of 70% to 84% with RSD around 7.8%, which are still good for a water quality test method. Together 98 of 102 compounds in this study have recoveries in the range of 70% -130% per the request of EPA Method 525.2. There are only 4 compounds with recovery less than 70% observed in this study, and the reasons caused the low recovery for each compound have been reasonably explained, respectively.

In summary, excellent analyte recovery and very clean chromatograms can be obtained by using Empore™ C18 disks. The data supports that CDS Empore™ C18 disks are qualified for screening drinking water samples according to EPA Method 525.2, as well as monitoring phthalates, organochlorine pesticides, triazine herbicides, or PAHs in drinking water.

References:

1. Method 525. Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry (Revision 2.1), Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH USA 45268.

2. National Primary Drinking Water Regulations; Analytical Techniques 40 CFR Parts 141 and 143 (Final Rule), Federal Register 53 (No. 33), 5142-5147 (Feb. 19, 1988)

3. Krigbaum, M., 1997, Evaluation of automated solid phase extractions of agrochemicals and industrial organic compounds from drinking water using U.S. EPA Method 525.2: American Environmental Laboratory, v. 9, no. 4, p. 12–14.

Table 1. Average recovery and RSD for compounds in EPA 525.2

compound	avg.%R (n=3)	(%RSD)	Recommended MilliporeSigma Cat. No.
.alpha.-Lindane	89	(5.2)	74142 CRM
.beta.-Hexachlorocyclohexane	87	(4.4)	48494 Pestanal
.delta.-Lindane	91	(3.2)	48495 Pestanal
1,1'-Biphenyl, 2,2',3,3',4,4',6-heptachloro-	91	(3.6)	BCR298 CRM
1,1'-Biphenyl, 2,2',3,3',4,5',6,6'-octachloro-	90	(5.2)	none
1,1'-Biphenyl, 2,2',3',4,6-pentachloro-	93	(3.0)	none
2,2',4,4'-Tetrachlorobiphenyl	94	(2.9)	none
2,2'.4.4'.5.6-Hexachlorobiphenyl	93	(4.0)	BCR297 CRM
2,3-Dichlorobiphenyl	94	(3.5)	35588 (PCB No 5) analytical standard
2,4,5-Trichlorobiphenyl	95	(3.4)	31093 (PCB No 29) analytical standard
2-Chlorobiphenyl	92	(3.4)	35586 (PCB No 1) analytical standard
Acenaphthylene	86	(4.1)	92549CRM
a-Chlordane	88	(3.5)	442449 Pestanal
Alachlor	91	(3.7)	08288 CRM
Aldrin	84	(5.2)	08573 CRM
Ametryn	85	(7.5)	45321 Pestanal
Anthracene	92	(3.4)	07671 CRM
Atraton	58	(15.3)	31206 Pestanal
Atrazine	90	(3.8)	90935 CRM
Benz[a]anthracene	93	(1.9)	75451 CRM
Benz[a]pyrene	105	(2.4)	51968 CRM
Benzene, 1-methyl-2,4-dinitro-	42	(6.7)	18191 CRM
Benzene, 2-methyl-1,3-dinitro-	45	(5.6)	none

compound	avg.%R (n=3)	(%RSD)	Recommended MilliporeSigma Cat. No.
Benzo(k)fluoranthene	97	(5.9)	33323 CRM
Benzo[b]fluoranthene	96	(2.8)	30958 CRM
Benzo[ghi]perylene	121	(12.1)	55488 CRM
Benzyl butyl phthalate	120	(9.4)	442503 analytical standard
Bis(2-ethylhexyl) phthalate	101	(5.3)	67261 CRM
Bromacil	81	(18.2)	69402 CRM
Butachlor	91	(3.3)	37887 Pestanal
Butylate	91	(3.3)	45363 Pestanal
Carboxin*	54	(12)	45371 Pestanal
Chlorobenzilate	90	(6.7)	69151 RM
Chloroneb	90	(4.2)	36125 Pestanal
Chloropropham	93	(3.9)	CRM45393 CRM
Chlorothalonil	89	(3.5)	36791 Pestanal
Chlorpyrifos	94	(2.4)	94114 CRM
Chrysene	92	(1.8)	94035 CRM
cis-Permethrin	90	(8.2)	45614 Pestanal, mix of isomers
Cyanazine	89	(5.2)	45407 Pestanal
Cycloate	92	(3.7)	69034 RM
DCPA	90	(4.2)	59708 RM
Diazinon*	109	(6.8)	68486 CRM
Dibenz(a,h)anthracene	120	(9.3)	91861 CRM
Dibutyl phthalate	88	(8.7)	none
Dichlorvos	81	(6.7)	18185 RM
Dieldrin	91	(3.3)	44959 RM
Diethyl Phthalate	80	(5.3)	53008 Pestanal
Dimethyl phthalate	89	(6.4)	41320 Pestanal
Diphenamid	93	(2.8)	64128 RM

Spike levels = 2.0 µg / L
*Analyte recovery reported is from EPA published method and was not included as part of this independent validation.

Table 1. Average recovery and RSD for compounds in EPA 525.2

compound	avg.%R (%RSD) (n=3)	Recommended MilliporeSigma Cat. No.
Disulfoton*	96 (9.4)	49784 RM
Disulfoton Sulfone*	164 (2.8)	49056 RM
Disulfoton Sulfoxide*	136 (8.9)	05207 RM
Endosulfan II	88 (2.9)	40828 CRM
Endosulfan sulfate	86 (3.2)	36676 Pestanal
Endosulfan I	90 (9.2)	74119 CRM
Endrin	91 (3.7)	32014 Pestanal
Endrin aldehyde	88 (6.5)	442578 analytical standard
Eptam	91 (3.1)	45469 Pestanal
Ethoprophos	93 (3.6)	53161 RM
Etridiazole	90 (3.8)	01342 RM
Fenamiphos	99 (4.7)	67114 RM
Fenarimol*	150 (5.5)	45484 Pestanal
Fluorene	94 (3.7)	56849 CRM
Fluridone	113 (4.9)	45511 Pestanal
g-BHC	97 (7.3)	36141 Pestanal / NMIP1332 CRM-NMI Australia
g-Chlordane	87 (4.0)	45519 Pestanal
Heptachlor	88 (4.6)	90426 CRM
Heptachlor epoxide	89 (3.6)	49042 Pestanal, mix of isomers
Hexachlorocyclopentadiene	86 (3.5)	40051 as solution, CRM
Hexanedioic acid, bis(2-ethylhexyl) ester	108 (9.5)	442492 analytical standard
Hexazinone	92 (4.4)	36129 Pestanal
Indeno(1,2,3-cd)pyrene	118 (5.8)	48669 as solution, CRM
Isophorone	88 (5.3)	78345 RM
Methoxychlor	90 (3.0)	CRM64156 CRM
Metolachlor	91 (2.1)	36163 Pestanal
Metribuzin	75 (8.8)	75296 CRM
Mevinphos	80 (7.0)	68435 Pestanal
Molinate	91 (4.0)	36171 Pestanal
Napropamide	93 (3.5)	36175 Pestanal
Norflurazon	94 (3.9)	34364 Pestanal
p,p'-DDD	89 (5.6)	43923 RM
p,p'-DDE	88 (4.3)	43537 CRM
p,p'-DDT	87 (2.0)	80076 CRM
Pebulate	90 (3.4)	49366 RM
Phenanthrene	95 (3.3)	73338 CRM
Phenol, pentachloro-	132 (7.8)	48555-U analytical standard
Dimethyl 4-nitrophenyl ester phosphate	91 (8.3)	46192 Pestanal
Prometon	84 (8.2)	45635 Pestanal
Prometryn	90 (7.0)	45636 Pestanal
Propachlor	92 (4.8)	45637 Pestanal
Propazine	91 (4.4)	45640 Pestanal
Propyzamide	90 (3.2)	95700 CRM
Pyrene	96 (4.2)	18868 CRM
Simazine	83 (6.8)	32059 Pestanal
Simetryn	65 (12.2)	45660 Pestanal
Tebuthiuron	92 (5.8)	45671 Pestanal
Terbacil	78 (8.7)	45675 Pestanal
Terbufos*	123 (4.2)	45313 Pestanal
Terbutryn	89 (4.5)	45677 Pestanal
Toxaphene*	Not Determined	48103 as solution, CRM
Tetrachlorvinphos	93 (3.8)	none
trans-Nonachlor	87 (3.9)	53747 CRM
trans-Permethrin	91 (8.3)	45614 Pestanal, mix of isomers
Triadimefon	97 (3.8)	45673 Pestanal
Tricyclazole	97 (7.2)	45808 Pestanal

Spike levels = 2.0 µg / L

*Analyte recovery reported is from EPA published method and was not included as part of this independent validation.

Ordering Information:

Product	Recommended MilliporeSigma Cat. No.
Empore™ 47mm C18 SPE Disk	66883-U
Empore™ 47mm C18 SPE Disk	66886-U
Methanol	320390
Ethyl acetate	319902
Methylene chloride	34856
Sodium sulfate	1003052203
EPA 525 Fortification Solution B	48099
EPA 525 Internal Standard Mix	48242
EPA 525 PCB Mix	48246
EPA 505/525 Update Pesticides Mix A	47727-U
EPA 505/525 Update Pesticides Mix B	47728-U
Capillary GC Column	
SLB®-5ms (30 m × 0.25 mm, ID × 0.25 µm df)	28471-U