SPE for analysis of environmental contaminants in water

Dr. Frank Michel
frank.michel@sial.com

sigma-aldrich.com

© 2012 Sigma-Aldrich Co. All rights reserved.

SIGMA-ALDRICH®

OUTLINE

Extraction Disks

- ENVI Disks vs. Empore
- Applications

Carbons

- ENVI-Carb
- ENVI-Carb Plus

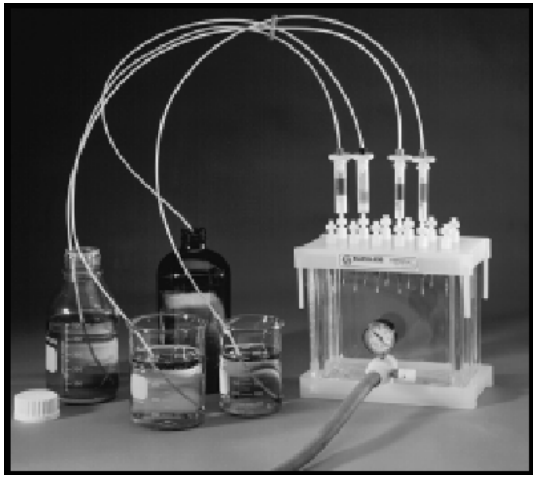
SupelSelect HLB for extraction of PPCPs

© 2012 Sigma-Aldrich Co. All rights reserved.

Useful EPA 500 Series (drinking water) Accessories

Visiprep 5-Port Flask Manifold

ENVI-Disk Holder



Visiprep Lg. Vol. Sampler

ENVI-Disk Holder Manifold

© 2012 Sigma-Aldrich Co. All rights reserved.

Extraction Disks

© 2012 Sigma-Aldrich Co. All rights reserved.

ENVI-Disk™ SPE Disk vs. Empore SPE Disk

- **ENVI-Disk SPE Disk**
  - SPE Particles embedded in glass fiber matrix
  - Available in C18 and C8
  - Better flow properties but more resistant to clogging
  - More prone to fines
- **Empore SPE Disk**
  - SPE particles embedded in a PTFE fiber matrix
  - Available in over 9 x different chemistries
  - Minimal fines but more prone to clogging
    - Clogging addressed via Empore Filter Aid
  - Compatible with ENVI-Disk Holder
- Both products written in dozens of EPA & other environmental methods

© 2012 Sigma-Aldrich Co. All rights reserved.

SPE Disks

3M Empore™ SPE

Reduced SPE bed mass = Reduced solvent & elution volumes

- Minimizes SPE eluate evaporation time
- Potentially allows for direct injection of the SPE eluate

Dense & uniform extraction medium = NO channeling/voiding

- Efficient mass-transfer kinetics allow for faster flow rates

47 mm Disks for Environmental Analysis

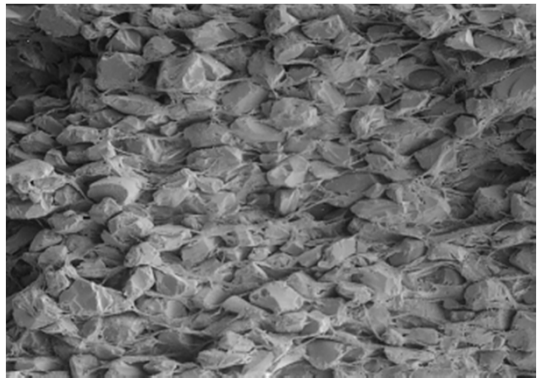
© 2012 Sigma-Aldrich Co. All rights reserved.

Empore SPE Disk Technology

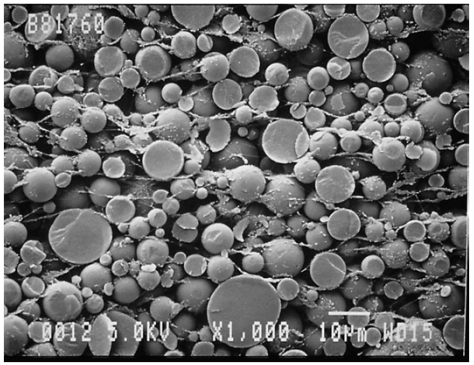
SPE Particles tightly enmeshed in an inert PTFE matrix

- 90% SPE Sorbent; 10% PTFE (by weight)
- Dense particle packing (no void spacing)
- Uniform particle distribution
- Thin membrane, small bed volume
- High Surface Area / No Fines
- Smaller bed weights
- Shorter diffusion paths
- More efficient extractions

Silica-based



Resin-based



© 2012 Sigma-Aldrich Co. All rights reserved.

Official EPA Methods using Empore Disks

- **1664 (Rev. A)** - N-Hexane Extractable Material (HEM; Oil and Grease)
- **506** - Phthalate and Adipate Esters in Drinking Water
- **507** - Nitrogen– and Phosphorus-Containing Pesticides in Water
- **508.1** - Chlorinated Pesticides, Herbicides, and Organohalides in Water
- **512.2** - Chlorinated Acids in Water
- **525.2** - Organic Compounds in Drinking Water
- **549.1** - Diquat and Paraquat in Drinking Water
- **550.1** - Polycyclic Aromatic Hydrocarbons in Drinking Water
- **552.1** - Haloacetic Acids and Dalapon in Drinking Water
- **553** - Benzidines and Nitrogen-Containing Pesticides in Water
- **1613** (Rev.B) - Tetra- Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution e.g. in water
- **SW846 Method 3535** - Test Methods for TCLP Leachates
- **QTM - Aqueous Phases Quick Turnaround Methods**
  - **PAH**
  - **Phenols**
  - **Pesticides & PCBs**

© 2012 Sigma-Aldrich Co. All rights reserved.

Tips to get good performance from extraction disks

Conditioning

- Prepares sorbent to interact with analytes
- Critical to good recovery and reproducibility
- Do not allow disk to go dry

Extraction

- Flow rate is not critical to recovery
- After sample, remove water by pulling vacuum

Elution

- Use multiple aliquots of elution solvent
- Use first aliquot to rinse out sample container
- Soak the disk for one minute with elution solvent prior to pulling vacuum

© 2012 Sigma-Aldrich Co. All rights reserved.

Application:

Polynuclear Aromatic Hydrocarbons

Method: US EPA 550.1

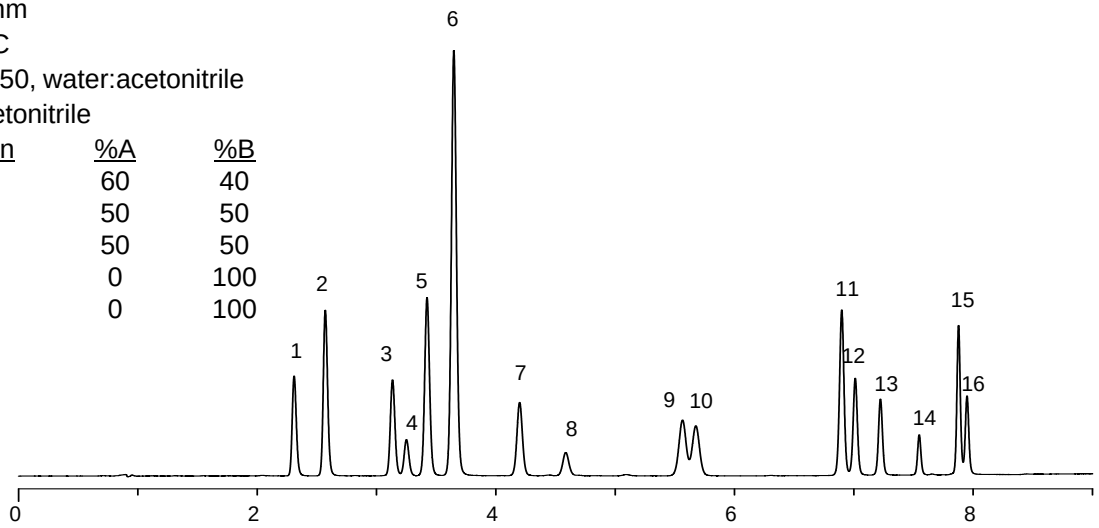
Disk: 47 mm Empore or ENVI-C18

1. Add 5 mL methanol and IS (if used) to 1 L water sample.
2. Wash disk w/5 mL methylene chloride.
3. Condition disk w/ 5 mL methanol followed by 5 mL dei. water.
4. Process sample through disk at a flow rate of 100 mL/min.
5. Elute the disk with 5 mL acetonitrile and 5 X 5 mL methylene chloride
6. Dry eluate through 3 gm anhydrous sodium sulfate.
7. Concentrate to 0.5 mL and analyze by HPLC

Column: Ascentis Express C18, 15 cm x 2.1 mm ID
Detection: UV, 250 nm
Temperature: 35 ° C
Mobile Phase A: 50:50, water:acetonitrile
Mobile Phase B: acetonitrile

Gradient:

min	%A	%B
0	60	40
3	50	50
5	50	50
7	0	100
9	0	100



1. Naphthalene
2. Acenaphthylene
3. Fluorene
4. Acenaphthene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Chrysene
10. Benzo[a]anthracene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Dibenzo[a,h]anthracene
15. Indeno[1,2,3-cd]pyrene
16. Benzo[g,h,i]perylene

© 2012 Sigma-Aldrich Co. All rights reserved.

Application:

Paraquat and Diquat from Water

Method: US EPA 549.2

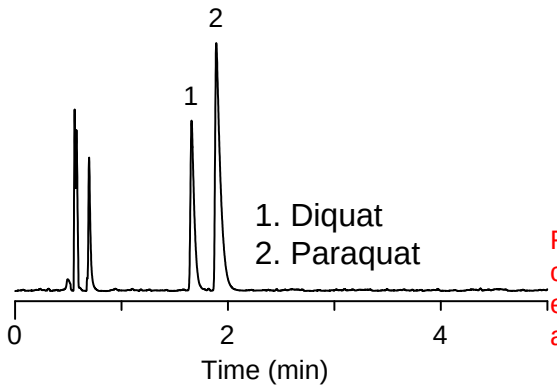
Disk: 47 mm Empore or ENVI-C8

1. Adjust 250 mL water sample to pH 7-9 using 10% NaOH or 10% HCl
2. Condition the disk as follows:
   1. 10 mL methanol
   2. 2 x 10 mL dei. water
   3. 10 mL conditionin soln. A*
   4. 2x 10 mL dei. water
   5. 20 mL conditioning soln. B*
3. Process sample through disk at a flow rate of 100 mL/min.

elution

4. Add 1 mL methanol to disk; soak for 1 min.
5. Add 4 mL eluting solution* to disk, apply slight vacuum, and soak disk for 1 min.
6. Add 4 mL eluting solution* to disk and draw completely through.
7. (Add ion pair reagent concentrate to eluate, adjust final volume (if necessary) and analyze by HPLC/UV)

*see method 549.2 for composition of solution



Fused core column for highly efficient, fast analysis

Column : Ascentis Express HILIC,

10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)

Mobile Phase: 20:80; 200 mM TFA NH_3: acetonitrile

Temperature: 60 °C

Flow Rate: 0.4 mL/min

Detection: UV at 308 (diquat) and 257 (paraquat) nm

Injection Volume: 1 µL

© 2012 Sigma-Aldrich Co. All rights reserved.

Carbon SPE
ENVI-Carb™ and ENVI-Carb™ Plus

© 2012 Sigma-Aldrich Co. All rights reserved.

Carbon SPE

ENVI-Carb™

- Predominately GCB (graphitized carbon black)
- Unique Selectivity & Ideal for polar compounds
- Non-porous (adsorption) = faster flow rates
- Example Applications
  - Chloroacetanilide and Chloroacetamide Herbicide Degradents (535.1)
  - Non-volatile pesticides (carbamate & thiourea) in drinking water
  - BNA Pesticides in ground water
  - Acidic herbicides in drinking water (515.2)
  - Nitrosamines in drinking water (521)

ENVI-Carb Adsorbent	C8- & C18-Modified Silica
graphitized carbon black	silane phase-modified silica gel
hydrophobic	hydrophobic
irregular 40-100µm particles	irregular 40-60µm particles
nonporous	porous (60-300Å)
surface area: 100 m^2/g	surface area: 400-600 m^2/g

© 2012 Sigma-Aldrich Co. All rights reserved.

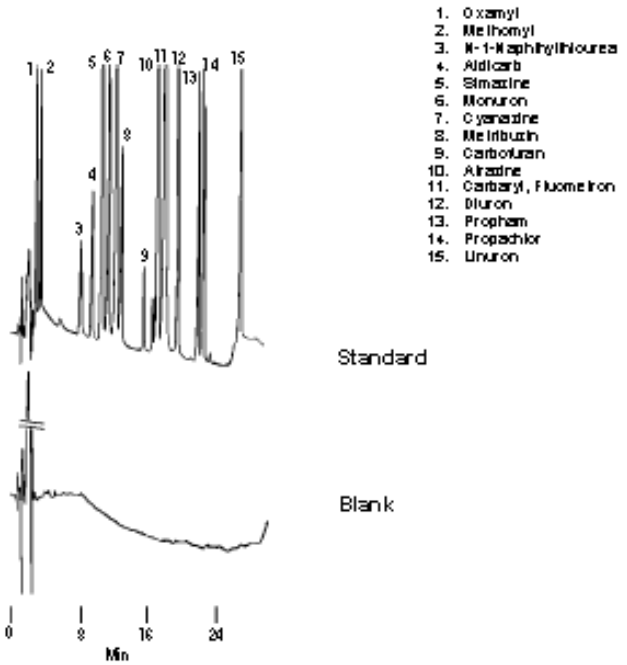
Application:
Use of Envi-Carb for pesticides in water

Pesticide Extraction from Drinking Water, Using ENVI-Carb Solid Phase Extraction Tubes

SPE Tube:
Supelclean ENVI-Carb, 3mL/0.25g packing
Tube Conditioning:
5mL methylene chloride: methanol (80:20)
1mL methanol
10mL 2% acetic acid in water
Draw solutions through the packing bed consecutively.
Keep packing bed wet until sample is added.
Sample Addition:
100mL-1 liter drinking water (pesticide concentration: 10-50µg/L)
Draw sample through packing at a rate of 5mL/min.
Accelerated rates do not have an adverse effect on recovery.
Drying:
1 minute, vacuum suction
Sample Elution (base-neutral fraction):
0.8-1mL methanol
2×3.5mL methylene chloride: methanol (80:20)
Dry eluate under gentle nitrogen purge in a room temperature water bath to approximately 400-500µL. For best recovery, wash inside wall of recovery vial with methanol, again dry to 400-500µL.
Reconstitutes samples to a constant volume of 1mL, using methanol.
Inject 20µL for HPLC analysis. Analysis can be automated.

HPLC Analysis

Column: SUPELCOSIL LC-18-DB, 25cm x 4.6mm (5µm particles) (with Supelguard™ LC-18-DB guard column)
Cat. No.: 58355-U
Mobile Phase: A - water:acetonitrile, 90:10, B - acetonitrile 20% B (5 min) to 70% B in 30 min
Flow Rate: 1.5mL/min
Det.: UV, 220nm
Inj.: 20µL extract (see Table 2)



© 2012 Sigma-Aldrich Co. All rights reserved.

SIGMA-ALDRICH®

Pesticide recovery using ENVI-Carb

	Solid Phase Extraction	
	ENVI-Carb Tubes	C8/C18 Silica♦
	n=5	n=4
Analyte●●	Recovery (% ± standard deviation)	
Oxamyl	111 ±9.6	28 ±46
Methomyl	105 ±5.0	25 ±44
Carbofuran	106 ±6.2	97 ±3.8
Fluometron	106 ±5.7	96 ±4.1
Monuron	99 ±3.2	98 ±6.2
Metribuzin	97 ±3.9	43 ±25
Carbaryl	97 ±3.5	90 ±12
Propham	95 ±3.2	76 ±23
Propachlor	96 ±3.8	90 ±7.5
Aldicarb	96 ±3.5	86 ±14
Cyanazine	90 ±5.4	99 ±10
Atrazine	89 ±5.7	74 ±14
Diuron	88 ±5.7	91 ±7.8
Linuron	88 ±5.4	94 ±4.1

Low recoveries for polar pesticides on C18/C8

●●100mLwater samples, 10-50µg/liter (ENVI-Carb extracts) or 20-500µg/liter (C8/C18 silica), HPLC/UV (ENVI-Carb extracts) or HPLC/MS (C8/C18 silica) analysis.

♦Data from Bellar & Budde, *Anal. Chem.*, 60: 2076-2083 (1988).

© 2012 Sigma-Aldrich Co. All rights reserved.

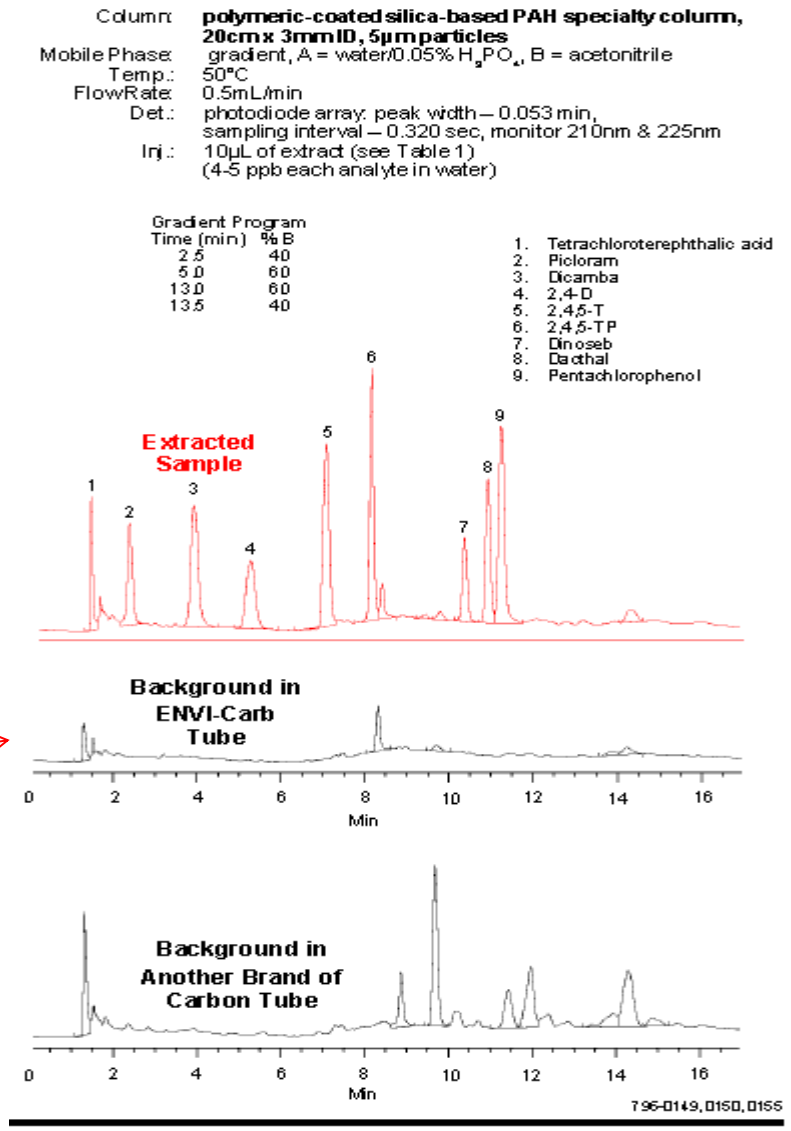
Application: Use of ENVI-Carb for extraction of acidic herbicides

Extraction procedure

(automated using Zymark)

1. Condition 6 mL/250 mg ENVI-Carb tube w/ 10 mL water
2. Pass 900 mL sample through tube
3. Rinse tube w/10 mL water
4. Dry tube for 10 min.
5. Elute with 10 mL 80:20 methylene chloride: methanol containing .01% phosphoric acid

Minimal background from ENVI-Carb tube



Figures provided by A. Lichtman, Nassau County Dept. of Health, Hempstead, New York, USA.

© 2012 Sigma-Aldrich Co. All rights reserved.

SIGMA-ALDRICH®

Acidic herbicide recovery using ENVI-Carb

Results: average recovery of 4 sample replicates spiked at 4-5 ug/L

Lower recovery due to strong retention on carbon

Analyte	% Recovery	Std. Dev.
Tetrachloroterephthalic acid	15.7	-
Picloram	91.5	2.3
Dicamba	91	3.4
2,4-D	88.3	7.1
2,4,5-T	78.7	6.2
2,4,5,-TP	84.7	9.9
Dinoseb	95.7	5.7
Dachtal	89.3	4.7
Pentachlorophenol	69.5	7.6

- The smaller, more polar herbicides show good recoveries from ENVI-Carb.
- Tetrachloroerephthalic acid and pentachlorophenol are large with more planar structures. These are difficult to remove.

© 2012 Sigma-Aldrich Co. All rights reserved.

Carbon SPE

ENVI-Carb™ PLUS

Spherical Carbon Molecular Sieve

- Extraction of highly polar compounds from water samples
- > 70% Absolute Recovery from 0.5 L drinking water (1-100 ng/mL)

Procedure:

1. Condition w/ 10 mL MeOH & 10 mL DI water
2. Load up to 1 L sample
3. Reverse tube & elute w/ 4-5 mL MeOH in opposite direction

© 2012 Sigma-Aldrich Co. All rights reserved.

Application:
Analysis of Glycols In Water Using ENVI-Carb Plus

Current method requires direct aqueous injection (DAI)

- Water has a high vapor volume
  - Requires low injection volume and/or high inlet pressure to contain vapor cloud
- Poor peak shape
  - Especially early eluting compounds
  - Affects detection and quantitation
- Presence of matrix (such as salt) in a sample could easily foul the GC inlet and/or column.

© 2012 Sigma-Aldrich Co. All rights reserved.

Analysis of Glycols

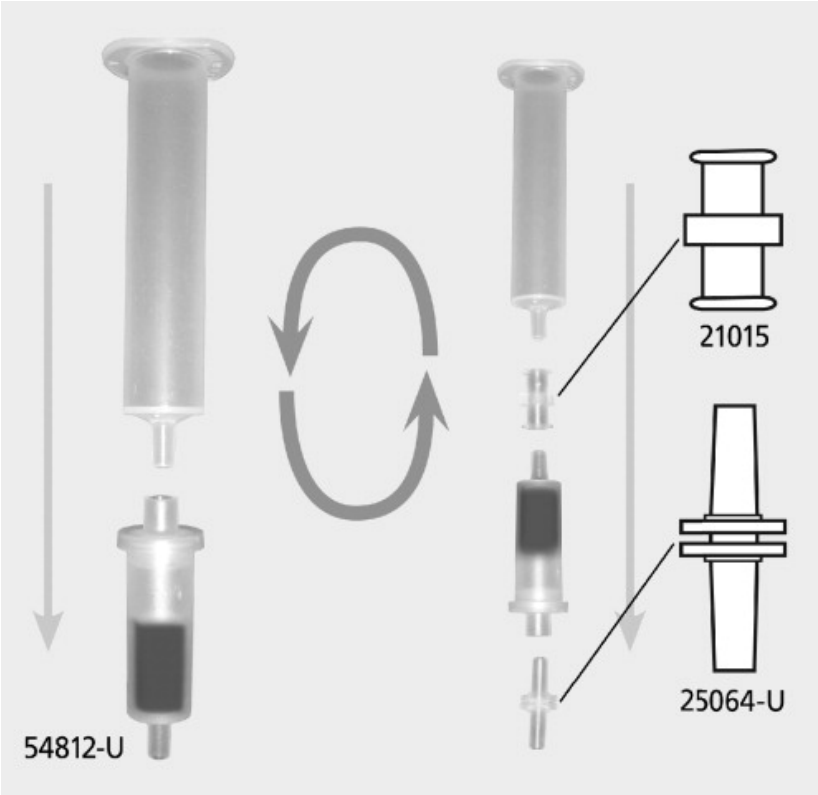
Goals of New Method

- Eliminate direct aqueous injection (DAI) of samples for glycol analysis.
- Lower detection and quantitation levels.
- Use solid phase extraction to extract glycols from water matrices.
  - Achieve elution with an organic solvent
  - Inject sample extract in organic solvent into GC

© 2012 Sigma-Aldrich Co. All rights reserved.

Glycol SPE Extraction Method Using ENVI-Carb Plus

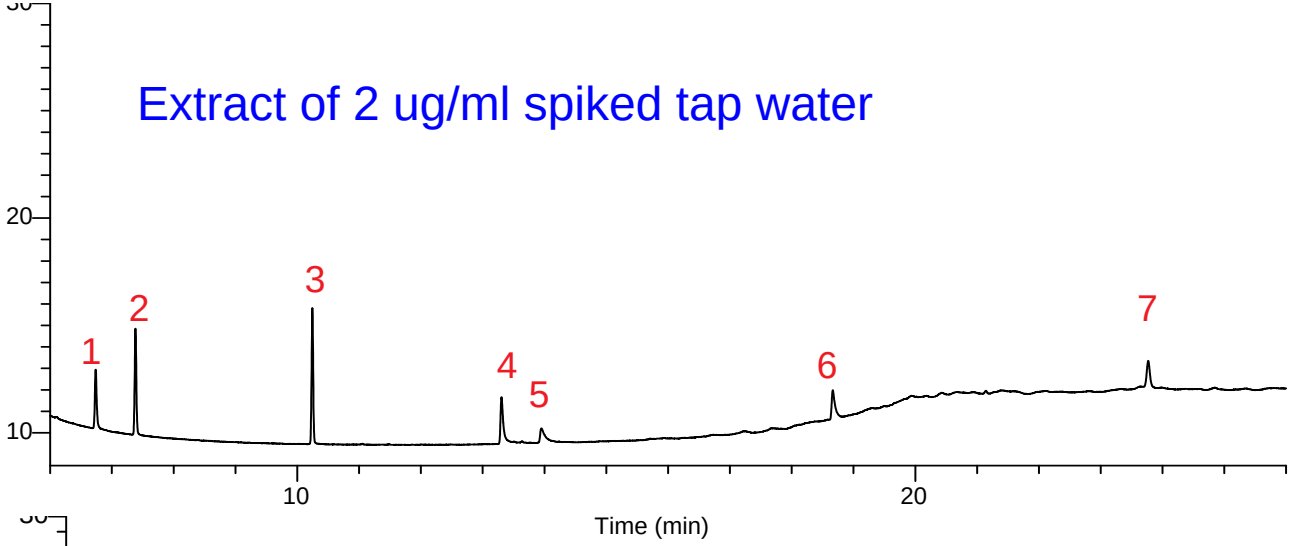
Extraction Cartridge	ENVI-Carb ™ Plus reversible cartridge, 1 ml/400 mg
Cartridge Conditioning	1 ml methylene chloride , 2 x 2 ml aliquots methanol, 3 ml dei. water
Sample	5 mL tap water spiked at 10 ug/ml
Sample extraction	5 ml sample, 5 mm Hg
Dry time	10 minutes, 10 mm Hg
Elution	Cartridge in **forward** direction, 2 ml of methanol:methylene chloride, 80:20 (soak cartridge for 1 minutes prior to pulling through)
Preparation for GC analysis	Added methanol to bring sample to final volume of 2 ml and analyzed directly



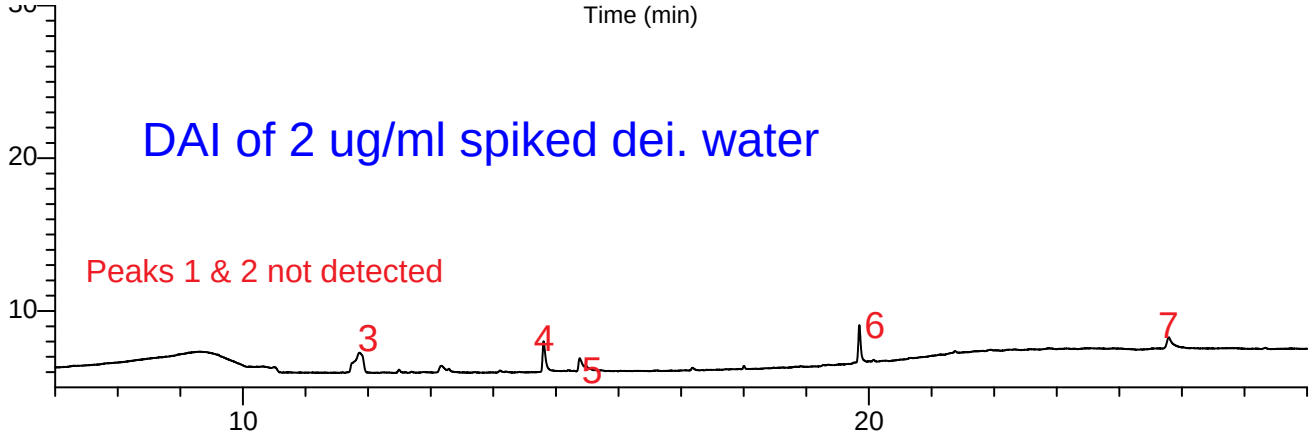
© 2012 Sigma-Aldrich Co. All rights reserved.

SIGMA-ALDRICH®

Extraction method vs. DAI



1. 2-methoxyethanol
2. 2-ethoxyethanol
3. 2-butoxyethanol
4. Propylene glycol
5. Ethylene glycol
6. Diethylene glycol
7. Triethylene glycol



Column: SPB-1000, 30 m x 0.25 mm I.D. x 0.25 µm
Oven: 50 ° C (1 min. or 2.5 min.), 8 ° C/min. to 200 ° C (12 min.)
Injector: 220° C
Carrier gas: Helium, 1.5 ml/min, constant flow
Detector: FID, 220 ° C
Injection: 1 µL, splitless
Liner: 4 mm ID, focus liner with taper (extracts) & 4 mm ID dual taper liner (DAI)

© 2012 Sigma-Aldrich Co. All rights reserved.

Recovery and Reproducibility Evaluation

Comparison of extraction method with DAI

Absolute recovery from tap water spiked at 2 ug/ml, n=7

	DAI		Extraction	
	Avg. % Recovery	%RSD n=7	Avg. % Recovery	%RSD n=7
2-methoxyethanol	Not detected		96%	3
2-ethoxyethanol			88%	3
2-butoxyethanol	34%	30	94%	3
Propylene glycol	106%	26	95%	6
Ethylene glycol	114%	30	53%	16
Diethylene glycol	56%	28	99%	6
Triethylene glycol	77%	26	98%	7

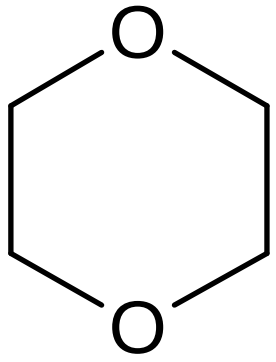
High RSDs

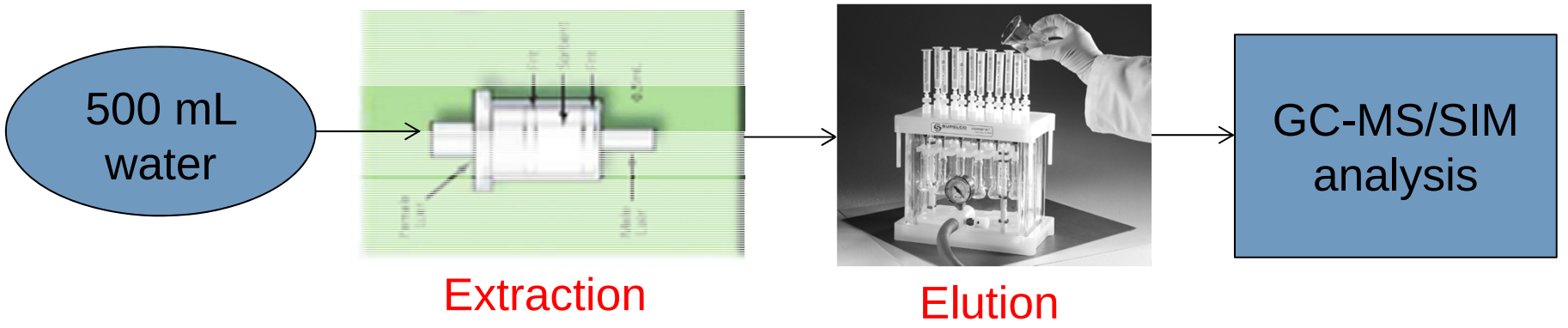
EG, smallest, most hydrophilic – difficult to retain on SPE

© 2012 Sigma-Aldrich Co. All rights reserved.

Application: Extraction of 1,4-Dioxane from water using ENVI-Carb Plus

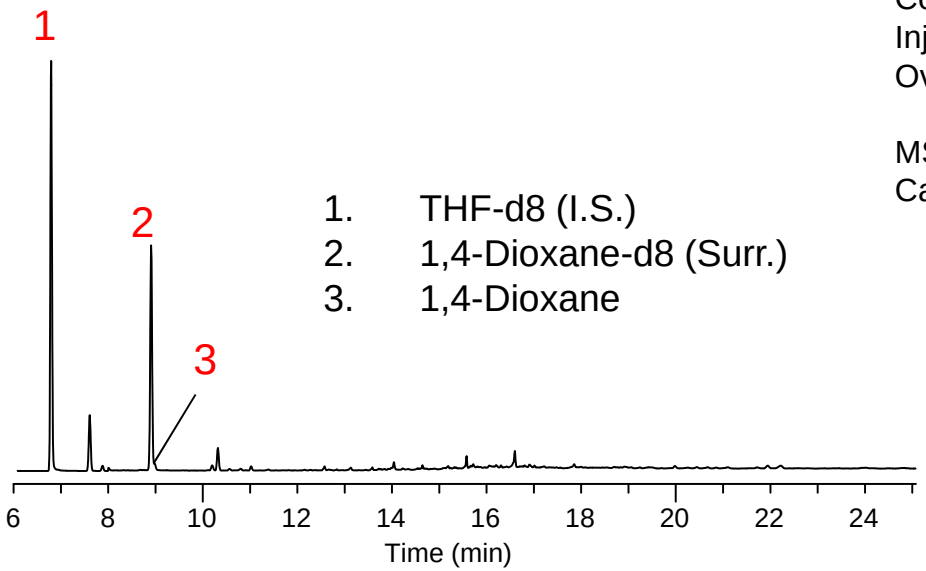
- US EPA Method 522 describes several different sorbents for extraction, including ENVI-Carb Plus.
- Flow through design of cartridge allows it to be connected directly to a continuous flow pump.
- ENVI-Carb Plus cartridges can be reversed (if necessary) for efficient elution of analytes.





© 2012 Sigma-Aldrich Co. All rights reserved.

Analysis of 1,4-dioxane from water, extracted using ENVI-Carb Plus



Column: SPB-624; 30 m x 0.25 mm I.D. x 1.4 µm
Inj. temp.: 200° C
Oven: 30 ° C (1 min.), 13 ° C/min. to 90 ° C,
20 ° C/min. to 200 ° C (15 min.)
MS interface: 220 ° C
Carrier: helium, 1 mL/min constant

	1,4-dioxane-d8 (surrogate)	**1,4-dioxane**
Spike level (ug/L)	10	0.3
Avg. % Recovery	102	83
%RSD, n=9	6	4

© 2012 Sigma-Aldrich Co. All rights reserved.

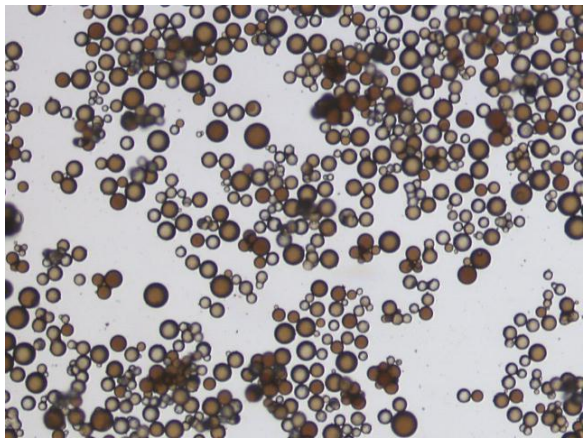
SIGMA-ALDRICH®

Supel™-Select HLB

© 2012 Sigma-Aldrich Co. All rights reserved.

Supel™- Select HLB

- Hydrophilic modified styrene-based polymer
- Can retain lipophilic and hydrophic compounds
- Wide pH compatibility range (0-14)
- Imparts minimal extractables to samples
- Described in US EPA Method 1694 for the extraction of personal care and pharmaceutical compounds from water
- Very reproducible lot-to-lot performance



Optical microscope picture of Supel-Select HLB resin

© 2012 Sigma-Aldrich Co. All rights reserved.

Extraction of Pharmaceutical & Personal Care Products (PPCPs) from Water using SupelSelect HLB

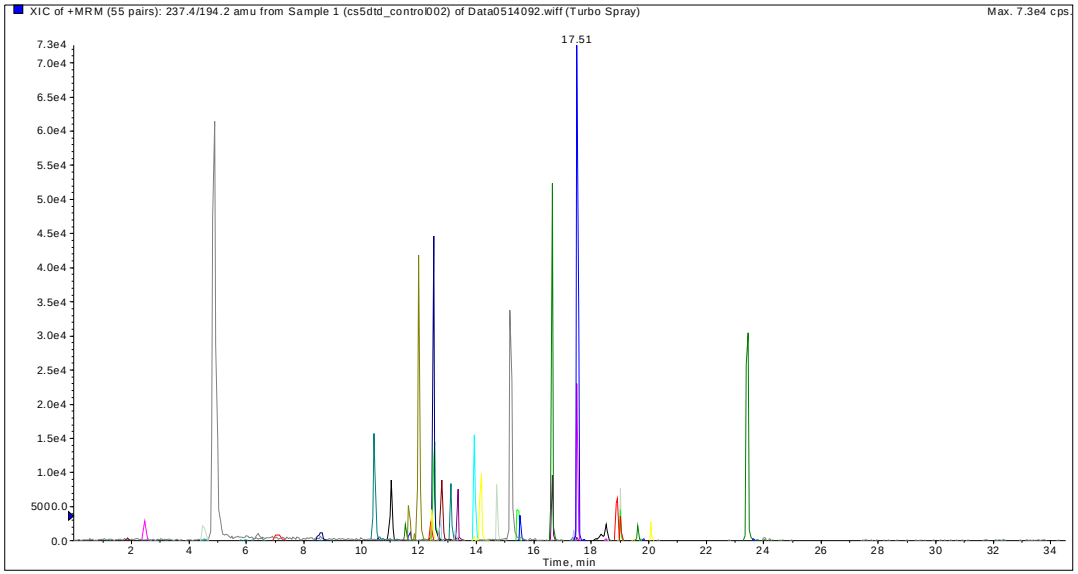
- US EPA Method 1694
- Method uses solid phase extraction (SPE) and LC-MS-MS for analysis of >70 PPCPs
- SPE used is hydrophilic/lipophilic balance (HLB)
  - Supel-Select HLB SPE

Extraction – based on method 1694

Extraction Cartridge	Supelco Select HLB SPE Tube, 500 mg/6 mL
Sample	500 mL of drinking water spiked with Group 1 compounds, adjusted to pH=4 with 6M HCl
Tube Conditioning	20 mL of methanol, 6 mL of water, 6 mL of water at pH 2 (with 6 M HCl)
Sample extraction	10 mL/min through tube
Dry time	5 min
Elution	12 mL, 50:50 methanol:acetonitrile
Dry down	40 ° C, under nitrogen stream
Reconstitution	To final volume of 2 mL using mobile phase

© 2012 Sigma-Aldrich Co. All rights reserved.

LC-MS/MS Analysis



column:	Ascentis Express C18, 10cm x 2.1 mm, I.D., 2.7 µm (53823-U)
mobile phase A:	0.1% formic acid and 0.1 % ammonium formate
mobile phase B:	50:50 methanol:acetonitrile
temp.:	40 ° C
det.:	ESI (+), MS/MS
injection:	5 µL
sample:	CS-5 Concentrations from EPA method 1694

gradient:	min	mL/min	% A	% B
	0	0.15	95	5
	4	0.25	95	5
	22.5	0.30	12	88
	23	0.30	0	100
	26	0.30	0	100
	26.5	0.15	95	5

Fused core column for efficient, fast analysis

© 2012 Sigma-Aldrich Co. All rights reserved.

SIGMA-ALDRICH®

Recoveries of some PPCPs from spiked water samples - extracted using Supel-Select HLB SPE

Compound	MRM Transition	SPE Spiking Level (ug/L)	% Recovery
Azithromycin	749.9-591.6	1	95
Caffeine	195.0-138.0	10	129
Carbadox	263.2-231.2	1	123
Ciprofloxacin	332.2-314.2	3,5	105
Clinafloxacin	366.3-348.1	4	48
Digoxigenin	391.2-355.2	4	128
Digoxin	781.5-113.1	10	136
1,7-Dimethylxanthine	181.2-124.0	100	86
Diphenhydramine	256.8-168.1	0,4	122
Enrofloxacin	360.0-316.0	2	83
Lomefloxacin	352.2-308.1	2	106
Miconazole	417.0-161.0	1	74
Norfloxacin	320.0-302.0	10	75
Ofloxacin	362.2-318.0	1	83
Ormetoprim	275.3-259.1	0,4	128
Oxolinic acid	244.1-216.1	0,4	77
Sarafloxacin	386.0-299.0	9,12	100
Thiabendazole	202.1-175.1	1	119

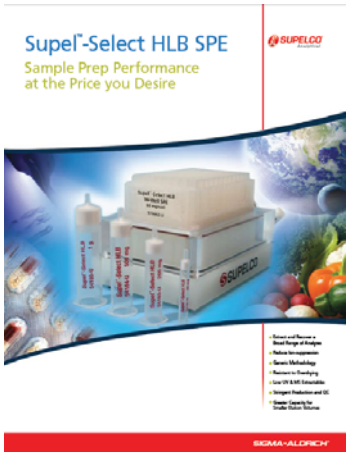
© 2012 Sigma-Aldrich Co. All rights reserved.

Key SPE Literature

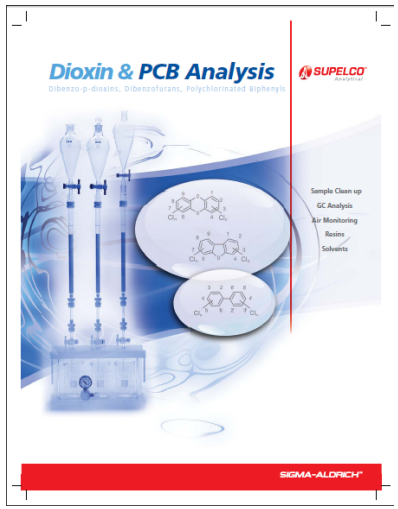
SPE Brochure T402150D

SPE Methodology Wall Poster T409088

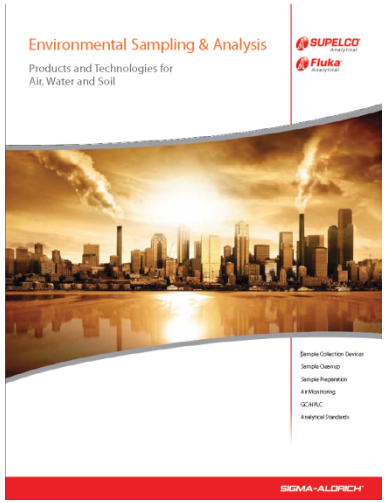
Empore SPE Flyer T407075



Supel Select HLB Brochure T408148



Dioxin & PCB Analysis



Environmental Sampling & Analysis Guide T412043

© 2012 Sigma-Aldrich Co. All rights reserved.

SIGMA-ALDRICH®

Thank you!

© 2012 Sigma-Aldrich Co. All rights reserved.

SIGMA-ALDRICH®

Associated Supelco Publications

1. *Extract Polynuclear Aromatic Hydrocarbons from Water, Using Solid Phase Extraction Disks*: Application Note 54 (T394054A).
2. *Extraction of Paraquat and Diquat from Water, Using ENVI™-8 DSK Solid Phase Extraction Disks:* Application Note 60 (T394060A).
3. *ENVI™-18 SPE Tube Ensures Low Background from Monitoring Organic Compounds in Drinking Water by EPA Method 525*: Application Note 65 (T395065A).
4. *Extract Nonvolatile Pesticides from Drinking Water, Using a Graphitized Carbon Adsorbent*: Application Note 27 (T394027B)
5. *Solid Phase Extraction/HPLC Analysis of Acidic Herbicides in Drinking Water*: Application Note 100 (T396100).
6. Stenerson, K.K.; Sidisky, L.M.; Betz, W.R.; Keeler, M.J.; McCoy, M.; Brown, J.. *The Extraction of Glycols from Water Using ENVI-Carb™ Plus Solid Phase Extraction Cartridges* (T410135)
7. Santasania, C.T.; Stenerson, K.K.; Aurand, C.; Trinh, A.; Shirey R.E. *Using Bonded Silica Solid Phase Microextraction Fibers as a Screening Tool for Pharmaceuticals and Personal Care Products in Drinking Water* (T409139)

© 2012 Sigma-Aldrich Co. All rights reserved.

References

1. Methods for the Determination or Organic Compounds in Drinking Water Supplement 1; EPA/600/R/4-90/020
2. Methods for the Determination of Organic and Inorganic Compounds in Drinking Water Volume 1; EPA 815-R-00-014
3. Munch, J.W.; Grimmett, P.E. 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).* Ver. 1; EPA /600/R-08/101; National Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency: Cincinnati, OH, Sept. 2008.
4. Method 1694: *Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS.* EPA-821-R-08-002; U.S. Environmental Protection Agency Office of Water Office of Science and Technology Engineering and Analysis Division (4303T): Washington, D.C., Dec. 2007.

© 2012 Sigma-Aldrich Co. All rights reserved.