Sample Preparation in N Parts

Dwight R. Stoll

Part I – Introduction to sample preparation, and introduction to Solid Phase Extraction (SPE)

Part II – SPE Details

Part III – Liquid-Liquid Extraction (LLE)

Part IV – QuECheRS, and other techniques

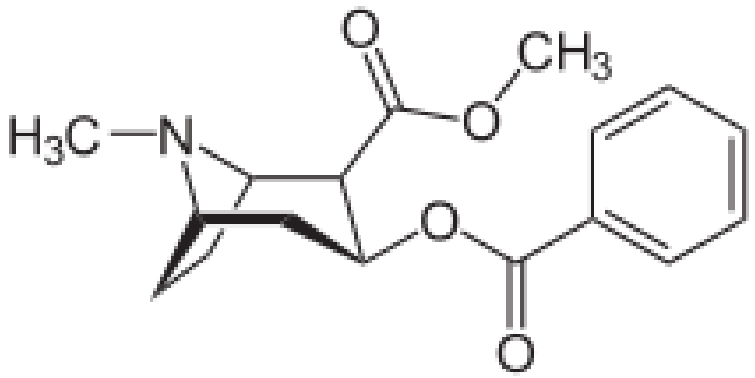
Part V – Solid Phase Microextraction (SPME)

Part VI – Applications and wrapup

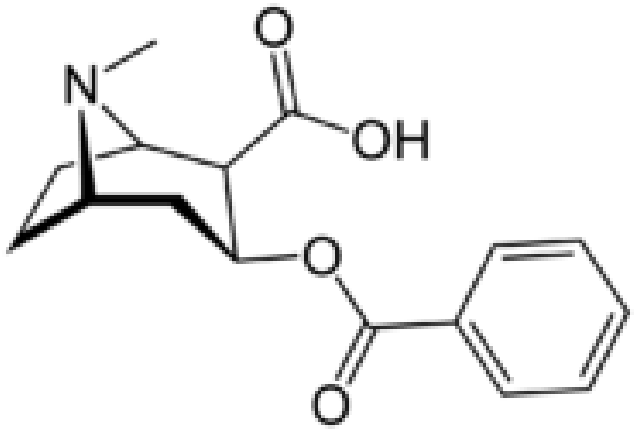
Objectives for our Discussion of Sample Preparation

1. Describe primary goals of sample preparation
2. Recognize circumstances when sample preparation is needed
3. Given circumstances, suggest an appropriate sample preparation method
4. Outline the specific steps of a SPE method, given target analyte/sample matrix combination
5. Predict behavior of a target analyte given a sample preparation method and conditions

Part I – Introduction to sample preparation, and introduction to Solid Phase Extraction (SPE)



Cocaine



Benzoylecognine (BE)

Sample Treatment Examples – Is treatment required? If yes, what type?

1. Determination of the extinction coefficient (UV) of a novel antipsychotic drug that you have just synthesized

2. Analysis of dioxins at parts-per-trillion levels (ng/L) in Minnesota River water

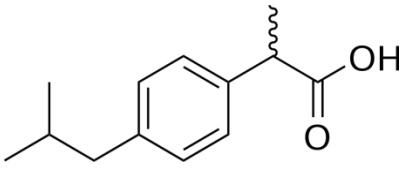
3. Quantitative determination of formic acid in a 1.0 M solution in water, sold by Sigma-Aldrich

4. Analysis of Clara Cell Secretory Protein in human serum at ng/mL levels

SPE Examples

1. Suppose you want to extract pyridine from an aqueous sample using an ion-exchange SPE material. What functional group on the SPE material will be useful? What solvents will you use at each of the four steps of the SPE process?

2. Now Suppose you are interested in extracting ibuprofen from blood by SPE, using a reversed-phase material. What solvents will you use at each step in this case?



Amphetamines in Urine, Oxidation with Periodate for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column *(Part Number: 60108-742)*

Prepare Sample

1. To 2mL of urine add internal standard(s)*, 1mL of 100mM phosphate buffer (pH 6.0) and 1mL of 0.35 M sodium periodate
   Mix/vortex
   Incubate at room temperature for 20 minutes
   Sample pH should be 6.0±0.5
   Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

2. 3mL of CH_3OH then aspirate
3. 3mL of DI H_2O then aspirate
4. 1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: *Aspirate at <3 "Hg to prevent sorbent drying*

5

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

6. 3mL of DI H_2O
   1mL of 100mM acetic acid
7. 3mL of CH_3OH
   Dry column (5 minutes at >10"Hg)
8

Elute Amphetamines

9. 3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)
   Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (pH 11-12)*

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Amphetamines in Urine, Oxidation with Periodate for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column *(Part Number: 60108-742)*

Concentrate Eluate

Add 30µL silylation grade DMF*** to eluate

Evaporate to 30µL at <40°C

Fluoroacylate with PFPA (PFAA)

10

Add 50µL PFPA (PFAA)****

Overlay with N_2 and cap

Improve derivatization by addition of 50µL PFPOH

React for 20 minutes at 70°C

Evaporate to dryness at <40°C

11

Quantitate

Inject 1 to 2µL onto gas chromatograph

For mass spectrometry monitor the following ions:

Chlorophenoxy Acid Herbicides in Water

Using 1g 6mL HyperSep C18 Extraction Column *(Part Number: 60108-301)*

1

Sample Preparation

Adjust pH of 1L of water sample to pH 1.0 with hydrochloric acid

2

Condition C18 Extraction Column

10mL of hexane/acetone (50:50)

10mL of acidified methanol (5% HCl in methanol)

3

10mL of DI H_2O

Apply Sample

Load 1 liter of sample at a rate of 8 to 10mL/minute

Wash Column

4

10mL of DI H_2O adjusted to pH 1.0 with HCl

Dry Column

Use maximum vacuum pressure for 15 to 30 minutes

5

Chlorophenoxy Acid Herbicides in Water

Using 1g 6mL HyperSep C18 Extraction Column *(Part Number: 60108-301)*

Elute Chlorophenoxy Acid Herbicides

10mL of hexane/acetone (50:50)

Concentrate/Evaporate

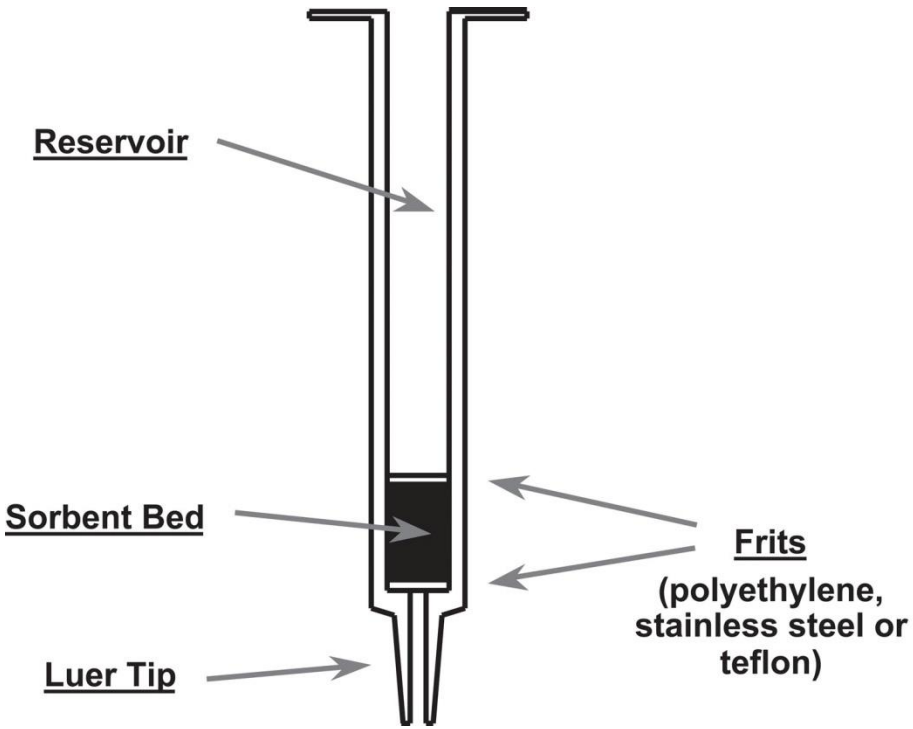
Add 500µL of a keeper solvent (methanol, DMF, other)

Evaporate to 500µL under a nitrogen stream at room temperature

Injection/Analysis

Reconstitute with 100µL of TCTEF and inject at 1 to 2µL onto GC column

Part II – SPE Details



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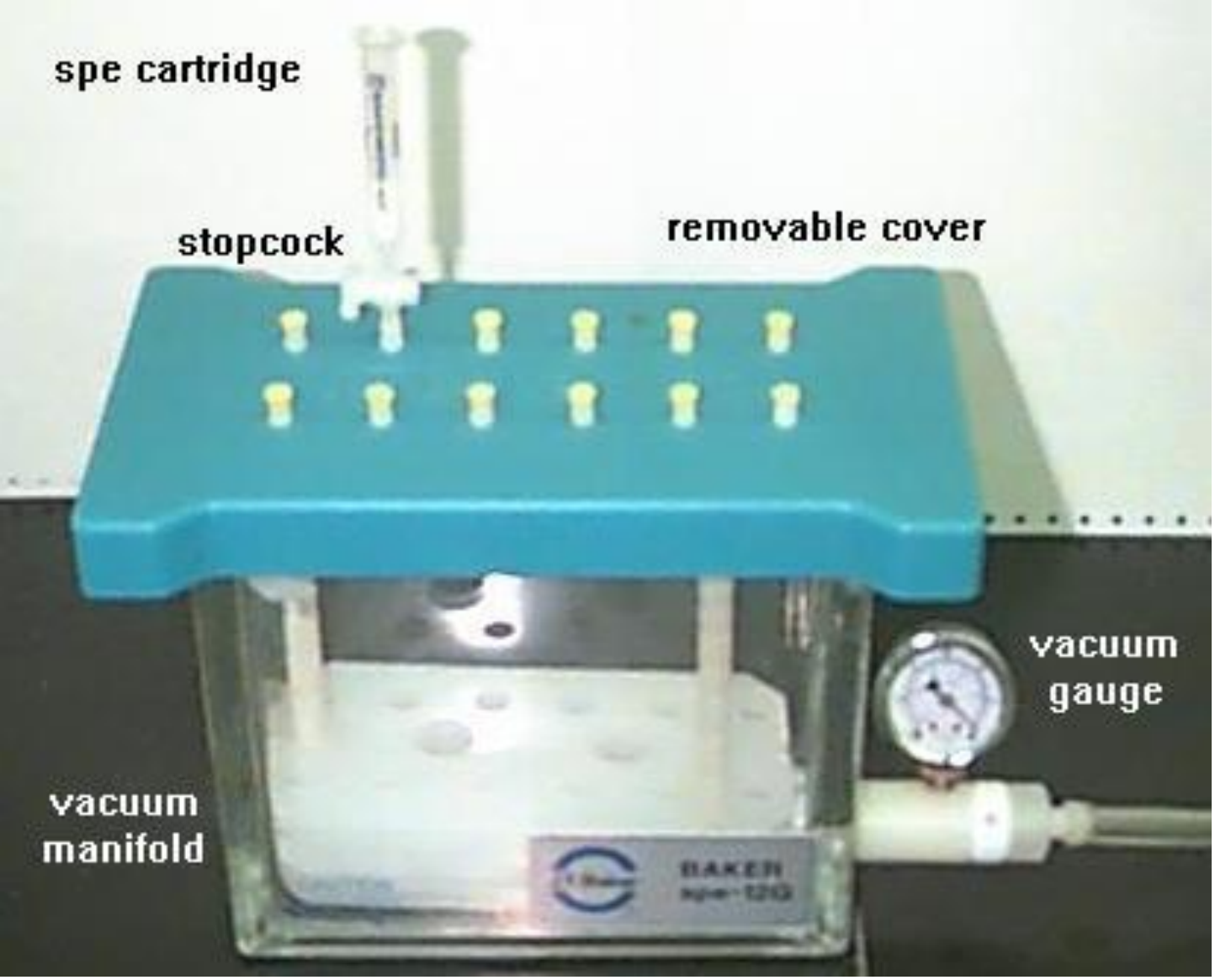
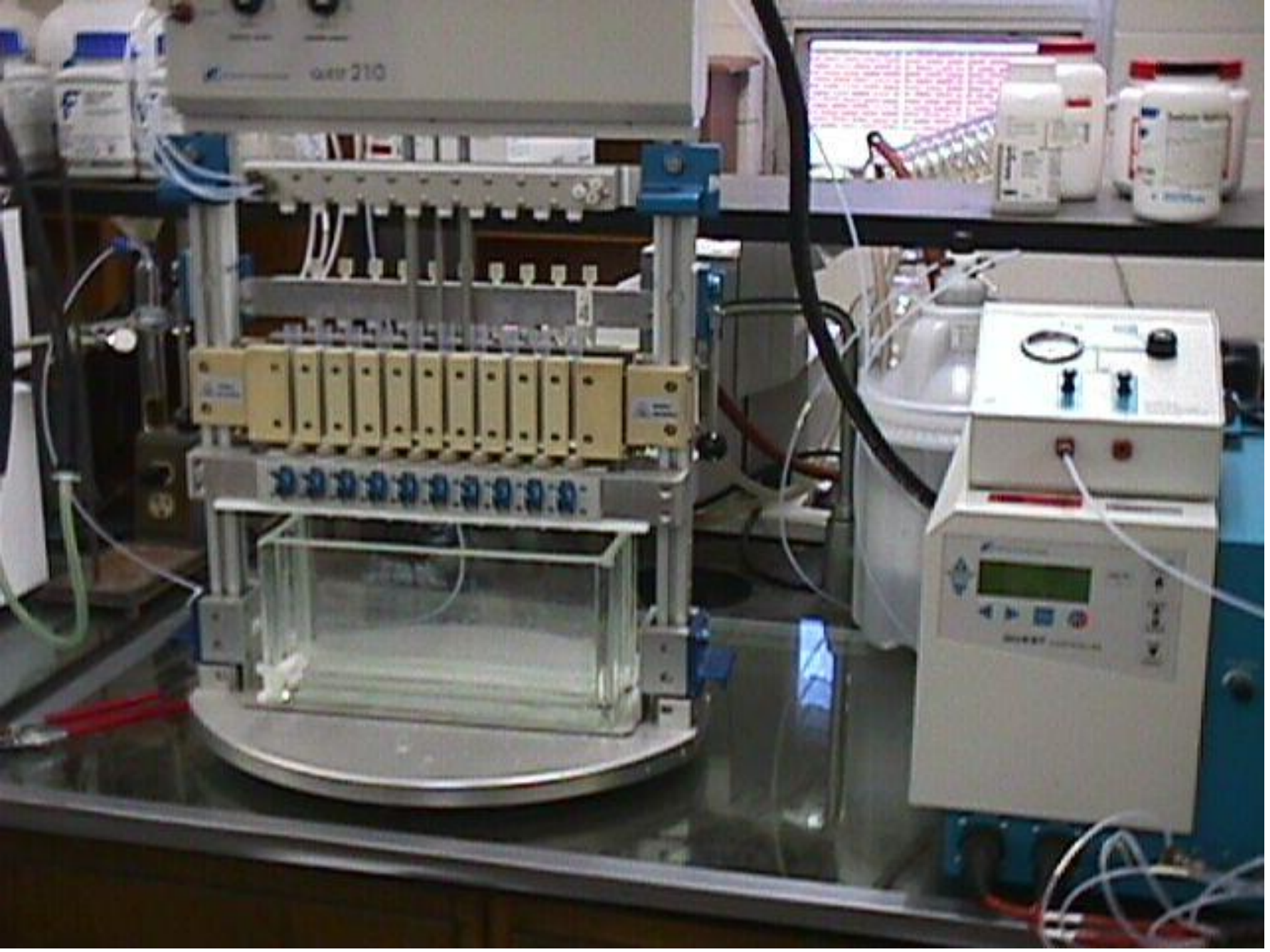


http://www.chemistry.adelaide.edu.au/external/soc-rel/content/spe.htm



http://www.pharmacy.olemiss.edu/medicinal_chemistry/Instrumentation.html

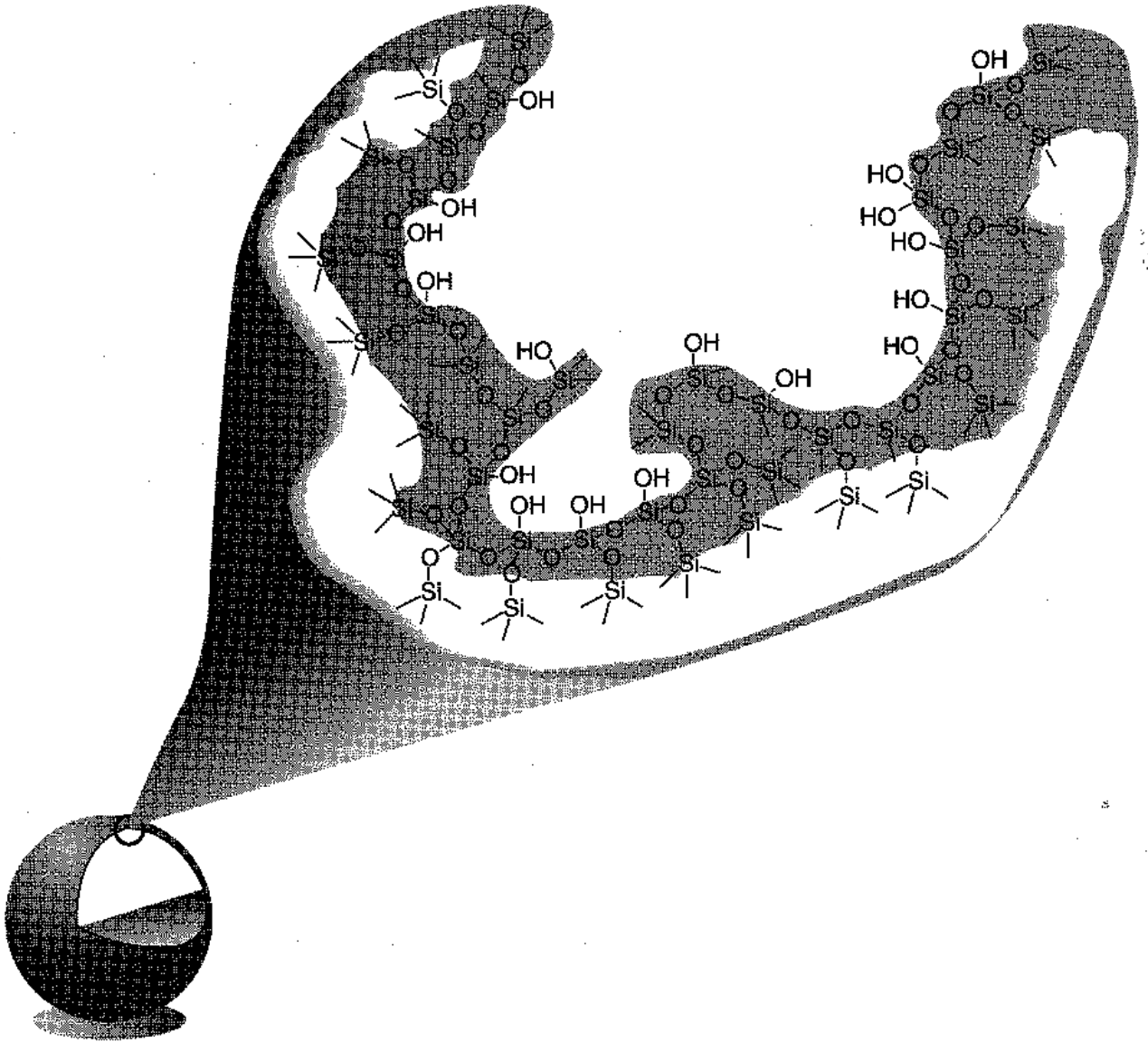
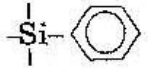


Figure 2.22. Representation of an unbonded silica particle. (Reprinted with permission from Ref. 84. Copyright © 2002 Waters Corporation.)

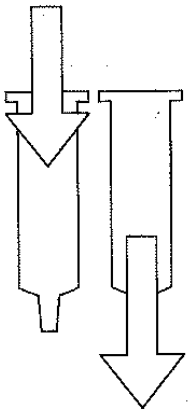
Mitra, S., Ed. (2003). Sample Preparation Techniques in Analytical Chemistry. Chemical Analysis. Hoboken, NJ, John Wiley and Sons.

TABLE 12.3

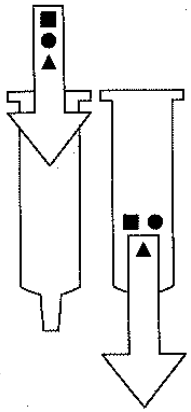
Structures of silica-based chemically bonded sorbents

Type	Functional group	Structure
C18	Octadecyl	$-Si-C_{18}H_{27}$
C8	Octyl	$-Si-C_8H_{17}$
C2	Ethyl	$-Si-C_2H_5$
CH	Cyclohexyl	
PH	Phenyl	
CN	Cyanopropyl	$-Si-CH_2CH_2CH_2CN$
NH_2	Aminopropyl	$-Si-CH_2CH_2CH_2NH_2$
DIOL	2,3-Dihydroxypropoxypropyl	$-Si-CH_2CH_2CH_2OCH_2CH(OH)-CH_2(OH)$
SAX	Trimethylaminopropyl (Quaternary amine)	$-Si-CH_2CH_2CH_2\overset{\oplus}{N}-(CH_3)_3Cl^{\ominus}$
CBA	Carboxypropyl	$-Si-CH_2CH_2CH_2COOH$
SCX	Benzenesulfonic acid	
PRS	Propylsulfonic acid	$-Si-CH_2CH_2CH_2SO_3^{\ominus}H^{\oplus}$

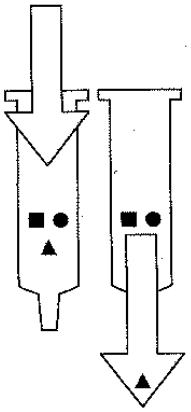
Pawliszyn, J., Ed. (2002). Sampling and Sample Preparation for Field and Laboratory: Fundamentals and New Directions in Sample Preparation. Comprehensive Analytical Chemistry. New York, Elsevier.



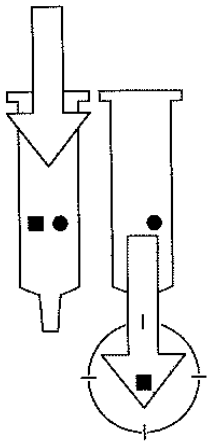
CONDITIONING
Conditioning the sorbent prior to sample application ensures reproducible retention of the compound of interest (the isolate).



RETENTION
■ Adsorbed isolate
● Undesired matrix constituents
▲ Other undesired matrix components



RINSE
▲ Rinse the columns to remove undesired matrix components



ELUTION
● Undesired components remain
■ Purified and concentrated isolate ready for analysis

Figure 2.42. Four basic steps for solid-phase extraction. (Reprinted with permission from Ref. 118. Copyright © 2002 Varian, Inc.)

Mitra, S., Ed. (2003). Sample Preparation Techniques in Analytical Chemistry. Chemical Analysis. Hoboken, NJ, John Wiley and Sons.

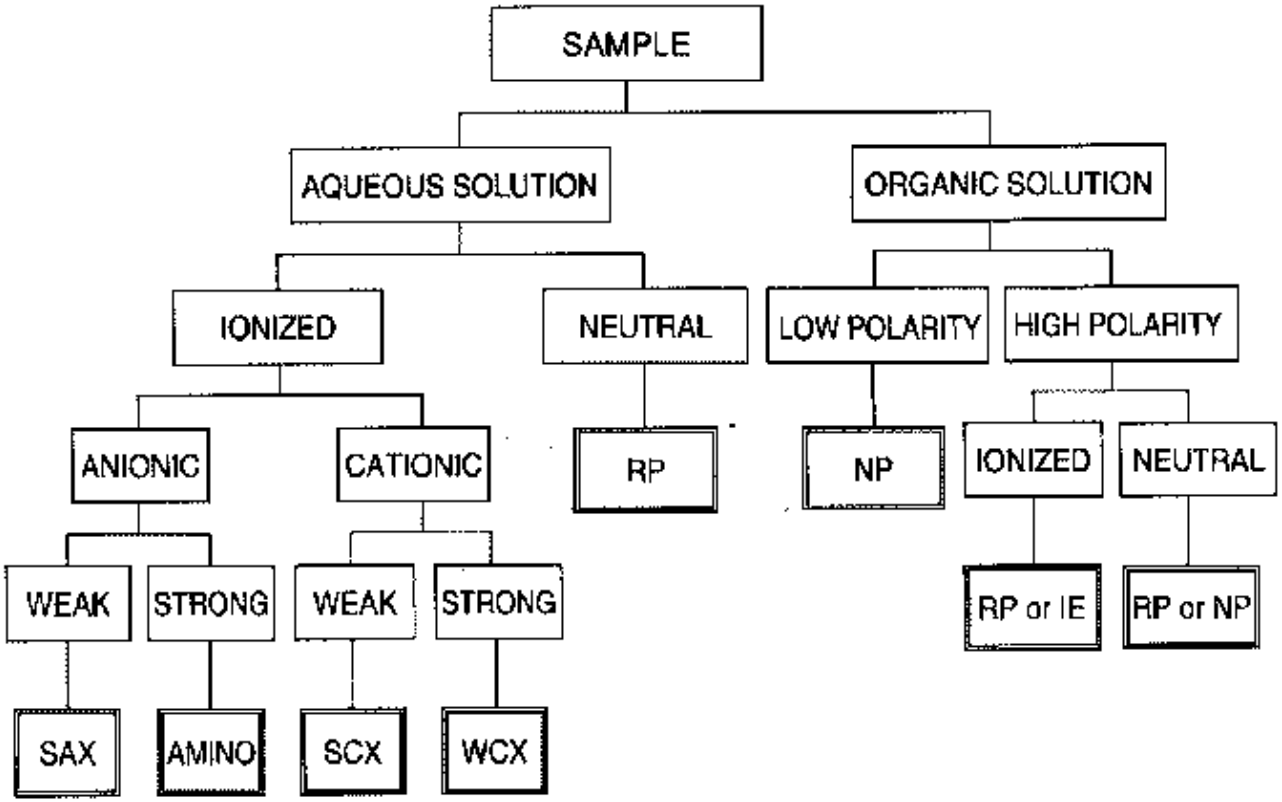


Figure 2.34. Method selection guide for the isolation of organic compounds from solution. SAX, strong anion exchanger; SCX, strong cation exchanger; WCX, weak cation exchanger; RP, reversed-phase sampling conditions; NP, normal-phase sampling conditions; IE, ion-exchange sampling conditions. (Reprinted with permission from Ref. 77. Copyright © 2000 Elsevier Science.)

Mitra, S., Ed. (2003). Sample Preparation Techniques in Analytical Chemistry. Chemical Analysis. Hoboken, NJ, John Wiley and Sons.

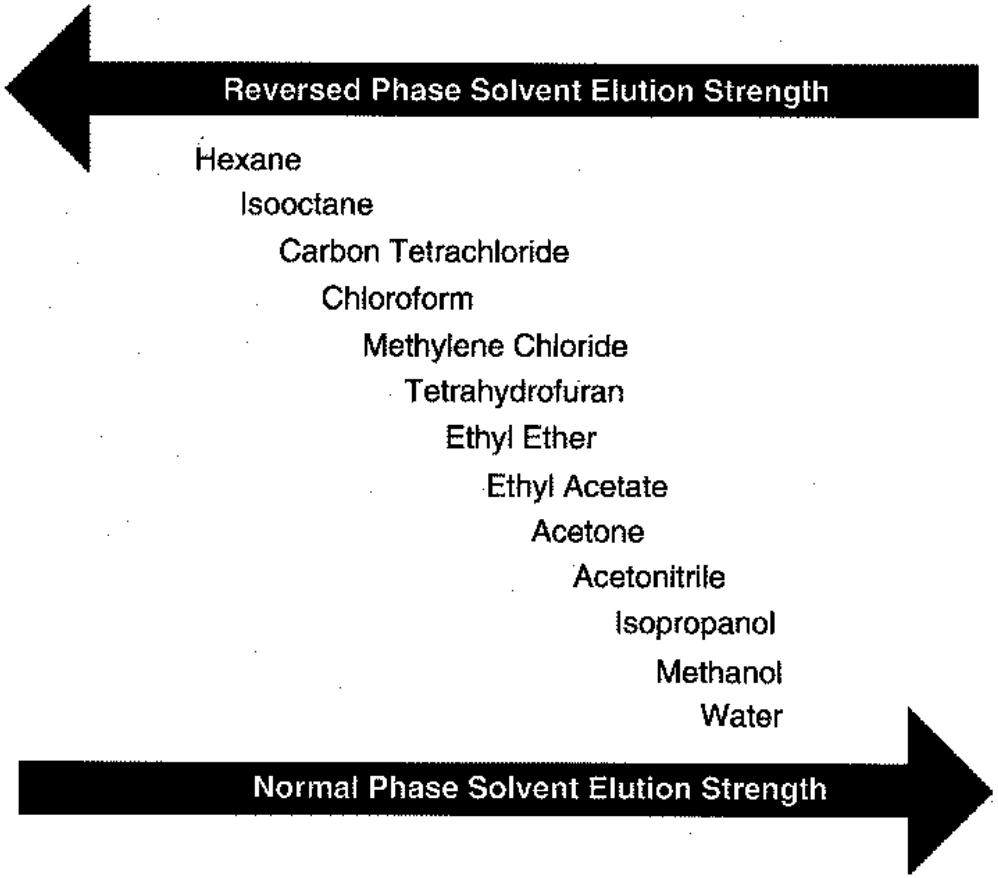


Figure 2.39. Solvent polarity chart indicates relative elution strength. (Reprinted with permission from Ref. 116. Copyright © 2002 Alltech Associates.)

Mitra, S., Ed. (2003). Sample Preparation Techniques in Analytical Chemistry. Chemical Analysis. Hoboken, NJ, John Wiley and Sons.

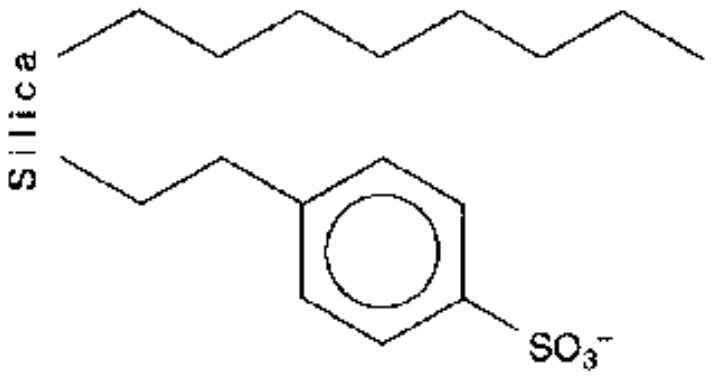


Figure 2.32. Example of a mixed-mode sorbent consisting of silica modified with octyl (C_8) alkyl chains and strong cation-exchange sites bonded on the same sorbent particle.

Mitra, S., Ed. (2003). Sample Preparation Techniques in Analytical Chemistry. Chemical Analysis. Hoboken, NJ, John Wiley and Sons.

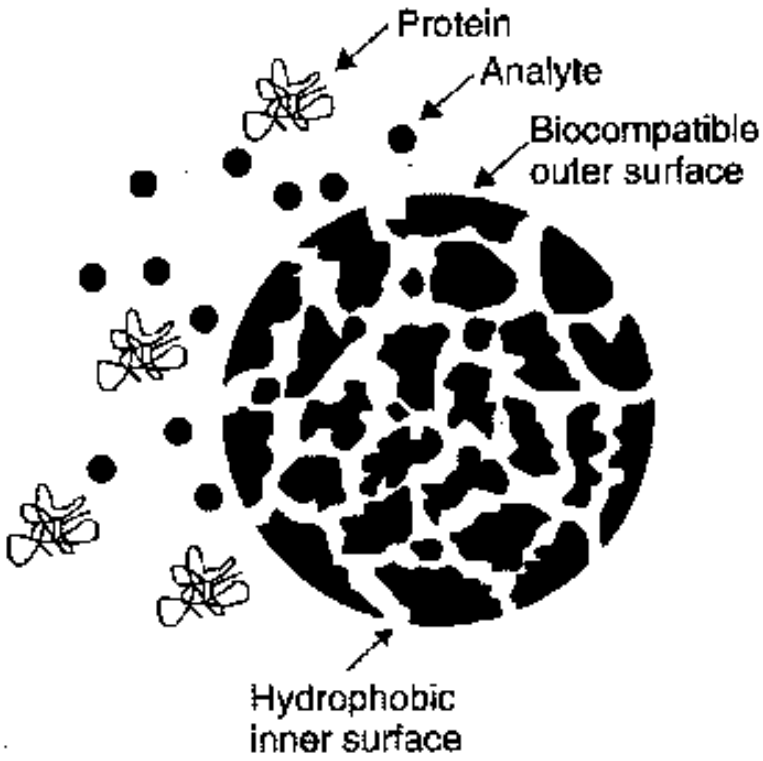


Figure 2.29. Schematic representation of a sorbent particle for restricted-access media chromatography. This medium allows proteins and macromolecules to be excluded and elute in the solvent front, while small analyte molecules enter the pores and are retained. (Reprinted with permission from Ref. 100. Copyright © 2000 Elsevier Science.)

Mitra, S., Ed. (2003). Sample Preparation Techniques in Analytical Chemistry. Chemical Analysis. Hoboken, NJ, John Wiley and Sons.

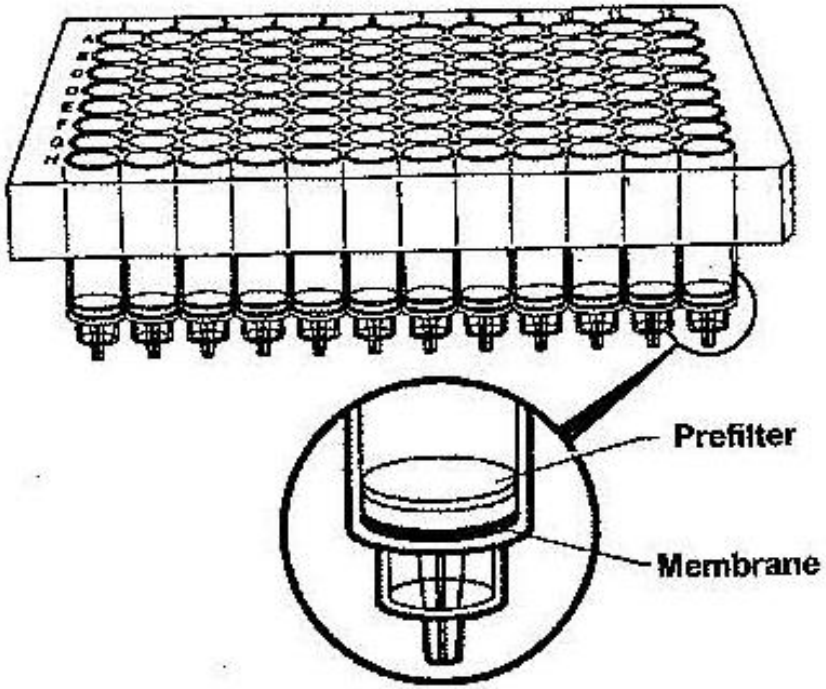
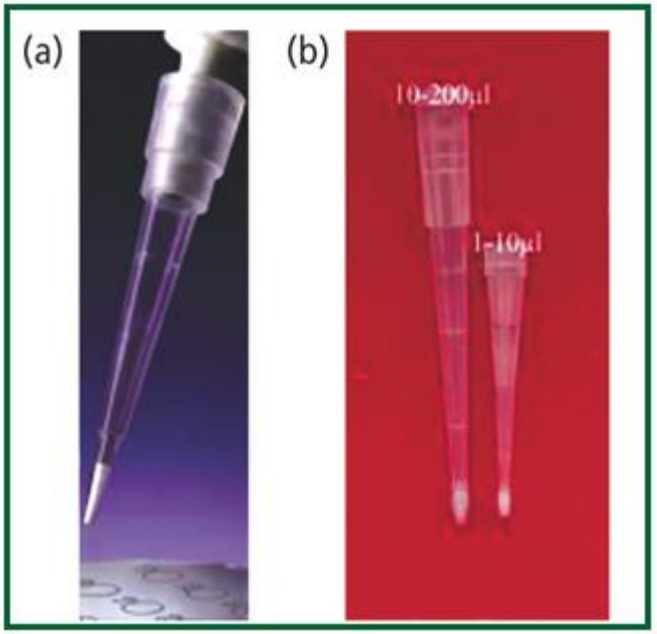
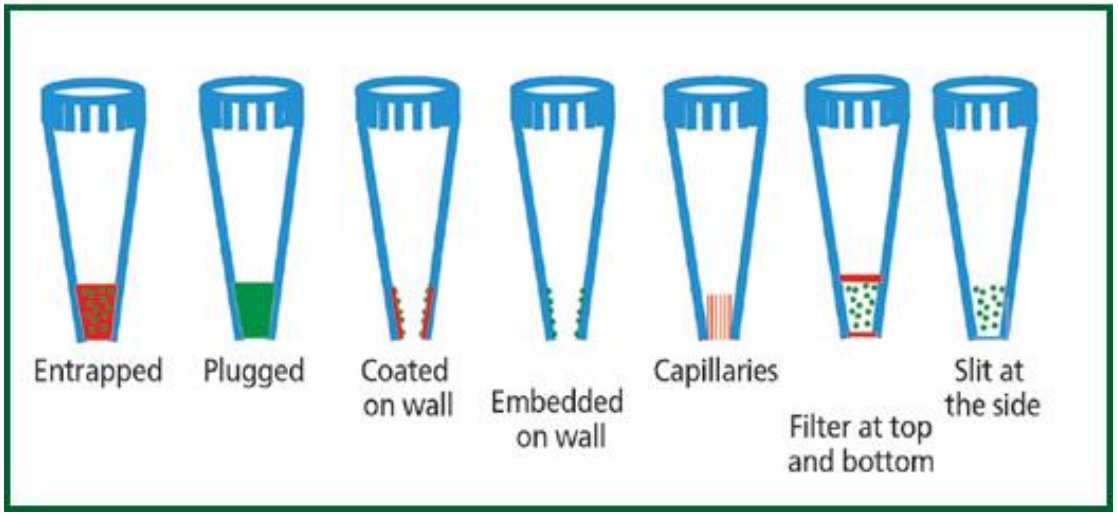


Fig. 12.3. Multiwell plate for automated SPE. (From Ref. [31]; copyright Elsevier).





Shukla, A. and R. E. Majors (2005). "Micropipette tip-based sample preparation for bioanalysis." LCGC North Am. 23(7): 646, 648, 654, 656, 658, 660.

Table 1: Typical commercial micropipette tip sample preparation products.

Product Name	Supplier	Type of Product	Mode(s)	Comments
BioTipPurifying	ChromBA	Capillaries	RPC, IEX, MC	Recommended for concentrating and purifying femto- to picomoles of biological samples; consists of 1 mm × 3 mm long multicapillary glass rod integrated into standard pipette nozzle; the tip contains over 4000 capillaries of 10 µm diameter with stationary phase chemically bonded to the capillary walls; C4, C18, metal chelate and strong cation exchange phases available.
Diachrom Tip	Glygen	Dialysis tubing containing media	Size exclusion/RPC	Chromatographic media inside of dialysis tubing with molecular weight cutoff filter (10000 Da), which operates by size separation and on inside of micropipette various sorbents (such as reversed-phase, ion exchange, carbon, IMAC, affinity) allow a dual mechanism clean-up of microlitre volume samples.
DPX-SPE	EST Analytical	Porous filter	RPC	Solid-phase sorbent is positioned inside of the pipette tip held in place by a screen and a filter; sample is drawn into the tip via pipette and vortex mixed inside a plastic test tube. Because of this mixing of the stationary phase with the sample, no conditioning step is required; sample is sorbed on stationary phase, excipients are washed away and adsorbed analytes are eluted by drawing up 0.1–0.3 mL of solvent, subjected to vortex mixing, and the solution is transferred to a vial.
Easy Tip	BioNano Technology	Wall coated	RPC, MC	C18 or IMAC material is immobilized onto a plastic pipette tip by photopolymerization; 25 fmol of tryptic haemoglobin digest loaded onto C18 had nearly 100% recovery; IMAC tips used for extracting tryptic phosphopepitdes of beta-casein at 10 pmol level.
Enzyme-in-a-Tip	Glygen	Embedded on wall	Enzyme	Micropipette tip with immobilized enzyme packing for sample preparation and protein degradation for peptide analysis and structure determination; 96-well plate versions available.

Shukla, A. and R. E. Majors (2005). "Micropipette tip-based sample preparation for bioanalysis." LCGC North Am. 23(7): 646, 648, 654, 656, 658, 660.

Glyphosate and Glufosinate by Solid-Phase Anion Exchange Extraction

Using 1g 6mL HyperSep Verify-AX Extraction Column *(Part Number: 60108-732)*

Sample Preparation

1L H_2O adjusted to pH 6

Condition HyperSep Verify-AX Extraction Column

1 x 5mL CH_3OH

1 x 10mL DI H_2O

Apply Sample

Load sample at 1 to 3mL/minute

Wash Column

1 x 10mL DI H_2O

Dry column (10 minutes at >10"Hg)

Elute

1 x 4mL of 1 mol/L HCl/CH_3OH (4/1)

Add eluant draw through at 1mL/minute

Consider the Thermo application note describing a SPE method for the analysis of glyphosate. For each step of the method, give a specific, chemically meaningful rationale for the conditions used.

Glyphosate and Glufosinate by Solid-Phase Anion Exchange Extraction

Using 1g 6mL HyperSep Verify-AX Extraction Column *(Part Number: 60108-732)*

Evaporate

Evaporate under a gentle stream of nitrogen in a water bath heated to 50°C

Analysis

Add 50µL of MTBSTFA* and 50µL of dimethylformamide for derivatization

Sonicate for 2 minutes

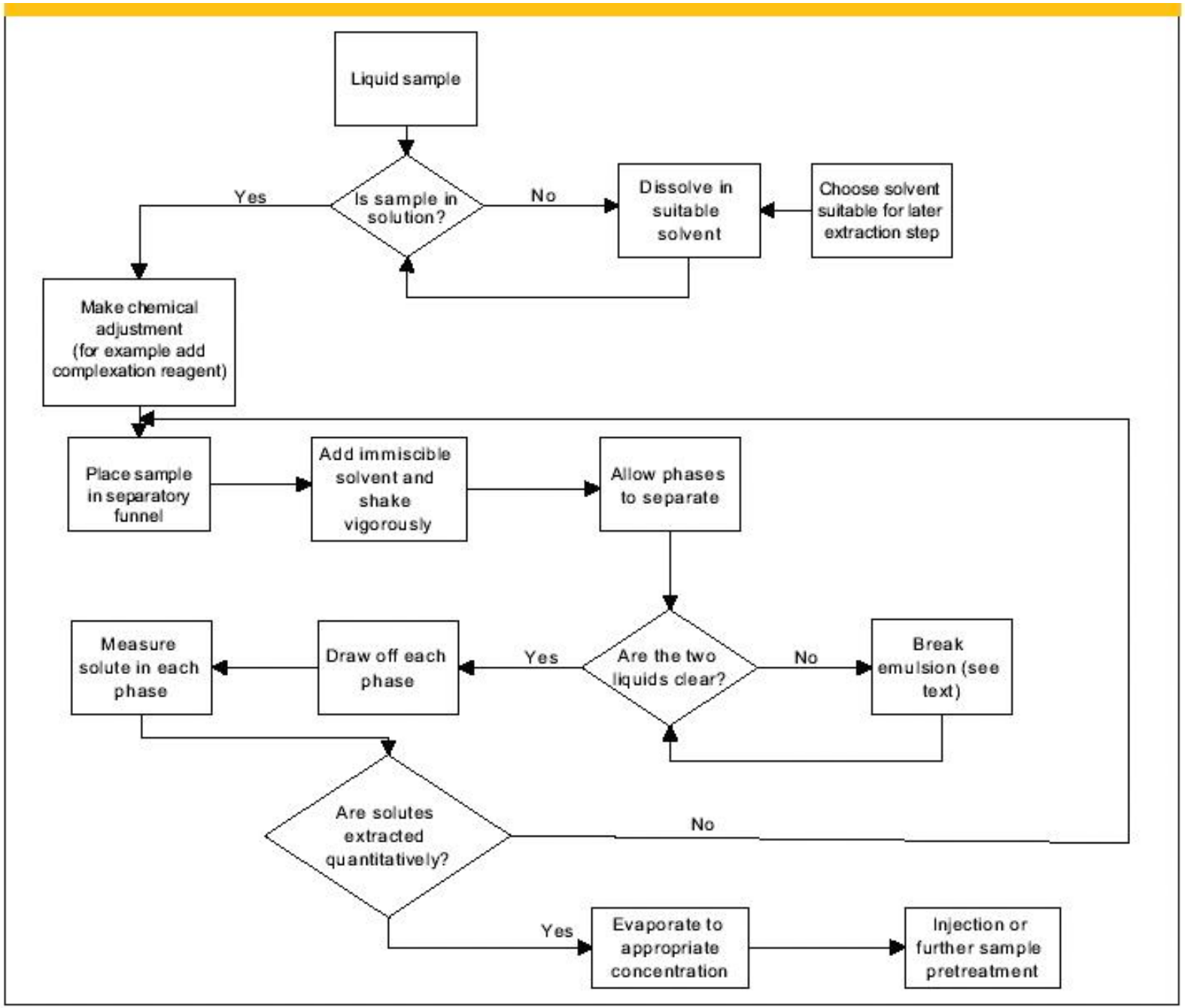
Heat to 80°C for 30 minutes

Cool to room temperature

Inject onto GC/MS

Suppose you want to extract pyridine from an aqueous sample using a SPE material. What functional group on the SPE material will you use? Draft a method similar to those in the Thermo application notebook that gives specific conditions for the extraction.

Part III – Liquid-Liquid Extraction (LLE)



Majors, R. E. (2009). "Practical aspects of solvent extraction." LCGC North Am.(Suppl.): 57-61.

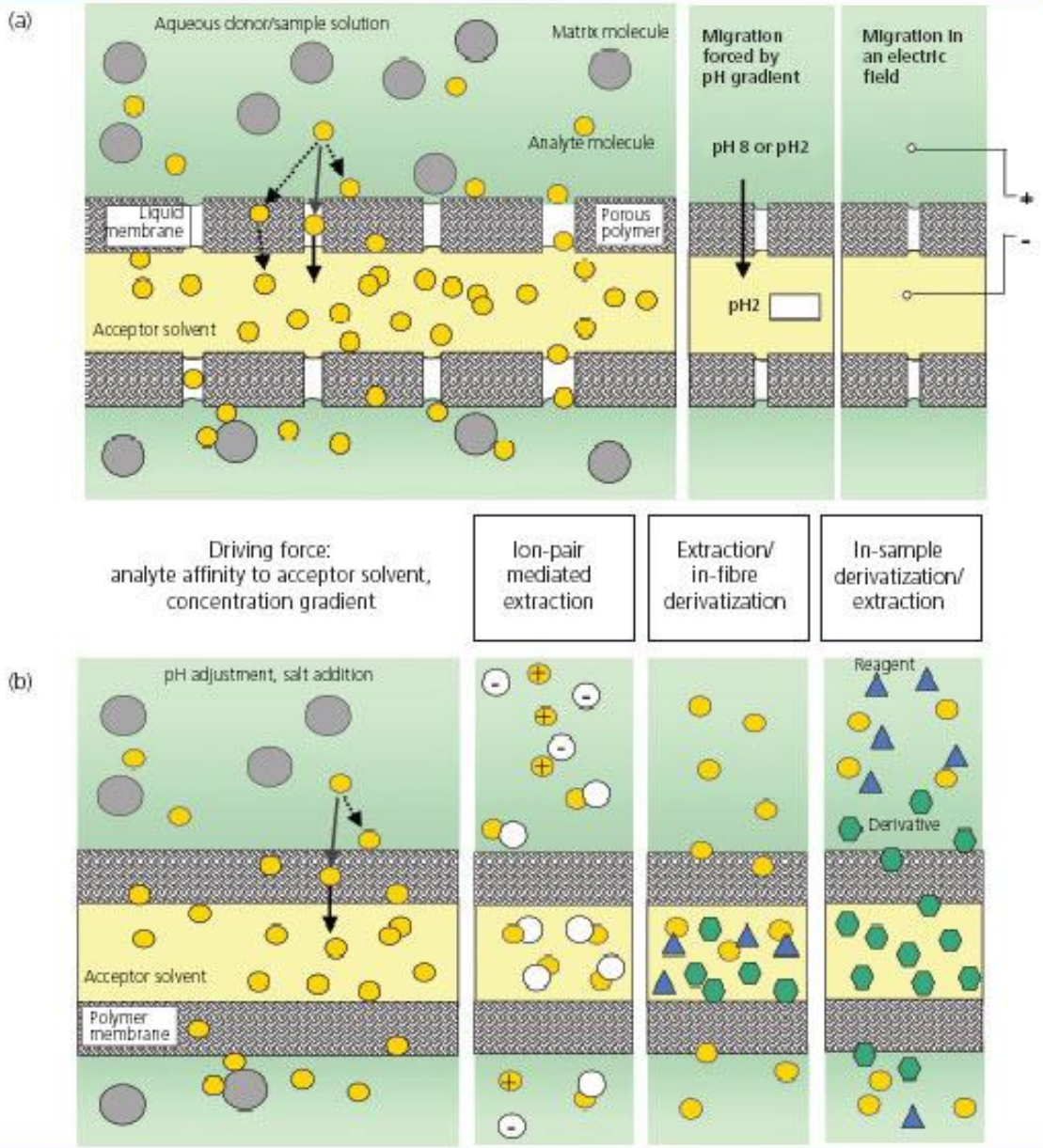
Table I: Extraction solvents for LLE(10)*

Aqueous Solvents	Water-Immiscible Organic Solvents	Water-Miscible Organic Solvents (Unsuitable for LLE)
Pure water	Aliphatic hydrocarbons (hexane, isooctane, petroleum ether, and so forth.)	Alcohols (low molecular weight)
Acidic solution	Diethyl ether or other ethers	Ketones (low molecular weight)
Basic solution	Methylene chloride	Aldehydes (low molecular weight)
High salt (salting out effect)	Chloroform	Carboxylic acids (low molecular weight)
Complexing agents (ion pairing, chelating, chiral, and so forth)	Ethyl acetate and other esters	Acetonitrile
Combination of two or more above	Aliphatic ketones (C_6 and above) Aliphatic alcohols (C_6 and above) Toluene, xylenes (UV absorbance) Combination of two or more above	Dimethyl sulfoxide Dioxane

* Any solvent from column 1 can be matched with any solvent from column 2; water-miscible organic solvents should not be used with aqueous solvents to perform LLE.

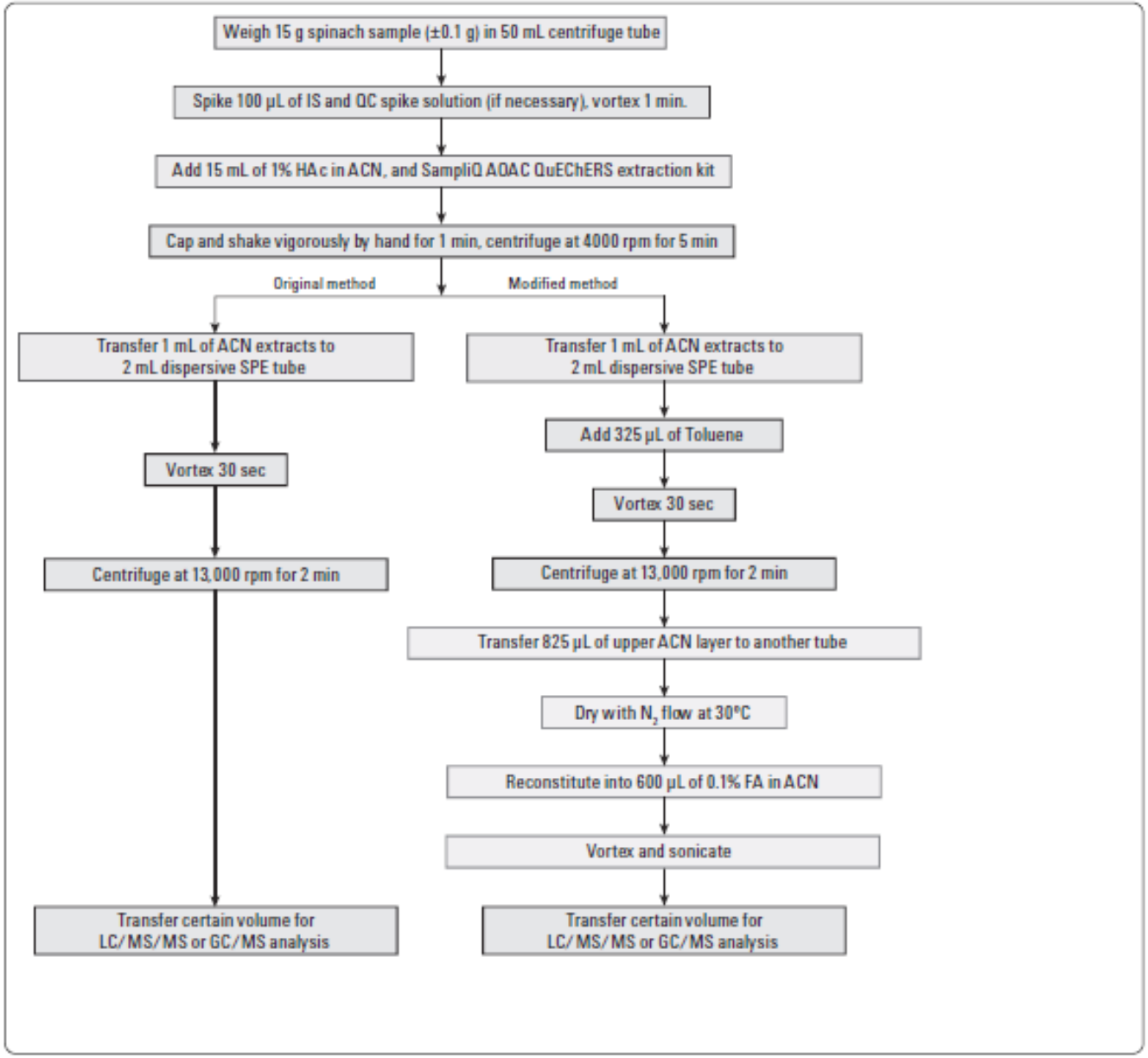
Majors, R. E. (2009). "Practical aspects of solvent extraction." LCGC North Am.(Suppl.): 57-61.

Figure 2: Basic principles of membrane-assisted extraction. (a) Liquid-phase microextraction LPME at microporous membranes (hollow fibre design) and (b) non–porous membranes for solvent extraction.



Moeder, M. and F. Lange (2007). "Membrane-assisted liquid-liquid extraction trace analysis of pharmaceutical compounds in aquatic environments." LC-GC Eur. 20(2): 97-98,100-103.

Part IV – QuECheRS, and other techniques


Weigh 15 g spinach sample (±0.1 g) in 50 mL centrifuge tube
Spike 100 µL of IS and QC spike solution (if necessary), vortex 1 min.
Add 15 mL of 1% HAc in ACN, and SampliQ AOAC QuEChERS extraction kit
Cap and shake vigorously by hand for 1 min, centrifuge at 4000 rpm for 5 min
Original method
Modified method
Transfer 1 mL of ACN extracts to 2 mL dispersive SPE tube
Vortex 30 sec
Centrifuge at 13,000 rpm for 2 min
Transfer certain volume for LC/MS/MS or GC/MS analysis
Transfer 1 mL of ACN extracts to 2 mL dispersive SPE tube
Add 325 µL of Toluene
Vortex 30 sec
Centrifuge at 13,000 rpm for 2 min
Transfer 825 µL of upper ACN layer to another tube
Dry with N_2 flow at 30°C
Reconstitute into 600 µL of 0.1% FA in ACN
Vortex and sonicate
Transfer certain volume for LC/MS/MS or GC/MS analysis

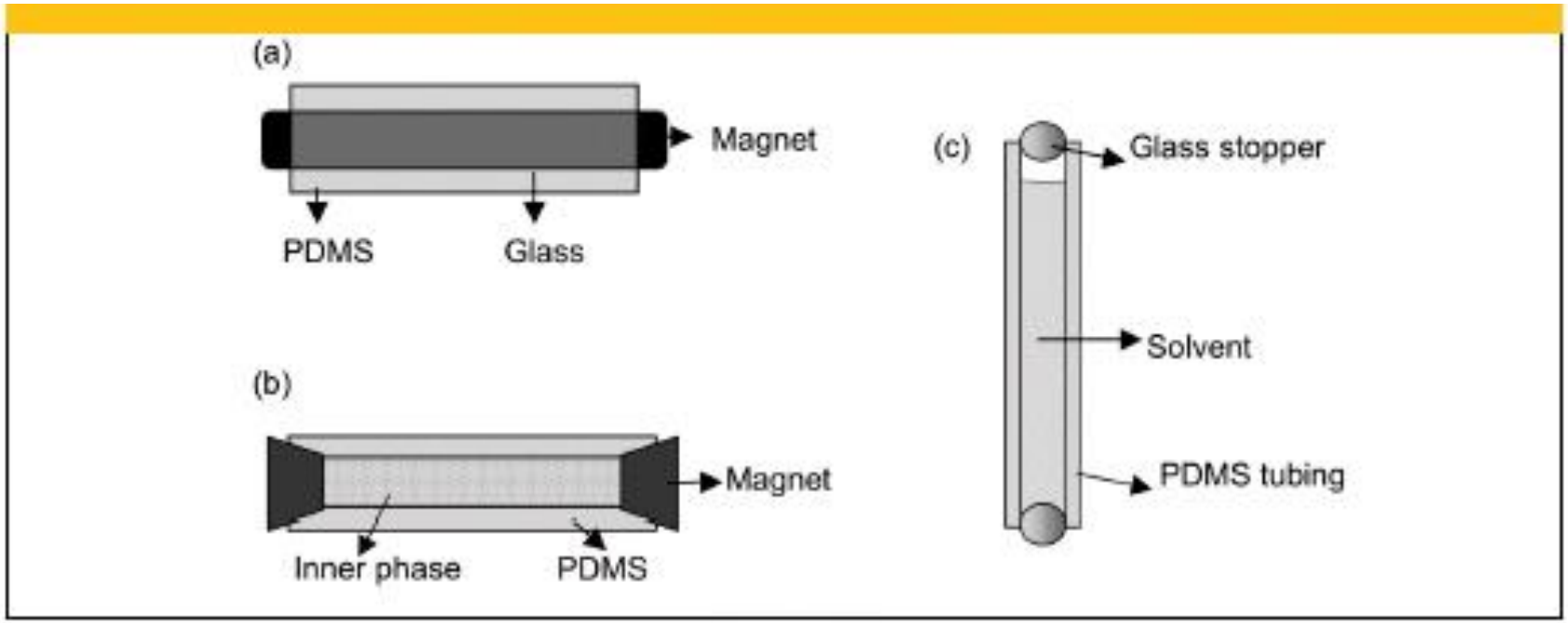


Figure 1: Schematic diagrams of (a) a conventional PDMS SBSE device, (b) a dual-phase SBSE device, and (c) a SMSE sampling device

Bicchi, C., E. Liberto, et al. (2009). "Stir-bar sorptive extraction and headspace sorptive extraction: an overview." LCGC North Am. 27(5): 376, 378, 380, 382, 384, 386, 388, 390.

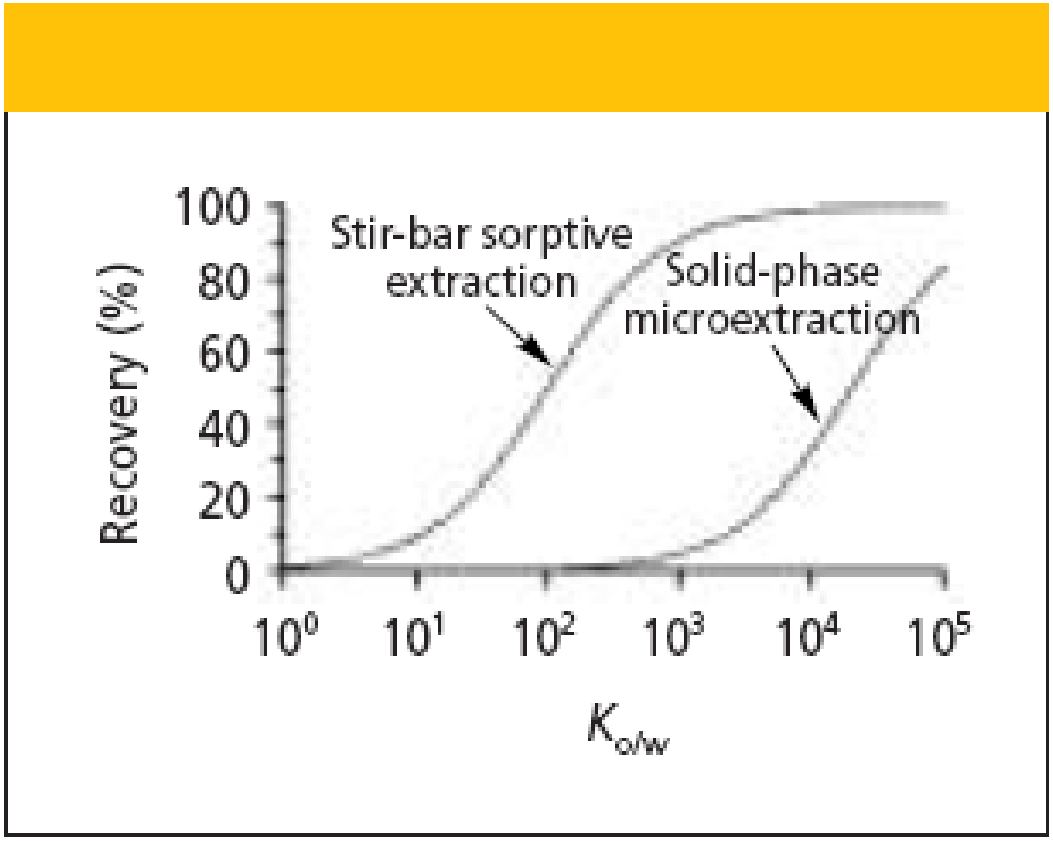


Figure 1: Recovery for solutes in function of the octanol–water partitioning coefficient $K_{o/w}$ for SPME (10-mL sample, 100-µm polydimethylsiloxane fiber) and for stir-bar sorptive extraction (10-mL sample, 10 mm × 0.5 mm polydimethylsiloxane-coated stir bar)

David, F., B. Tienpont, et al. (2003). "Stir-bar sorptive extraction of trace organic compounds from aqueous matrices." LCGC North Am. 21(2): 108, 111-112, 114, 116-118.

Part V – Solid Phase Microextraction (SPME)

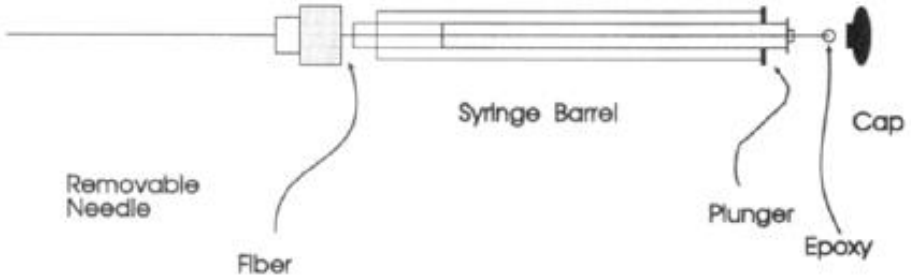


Figure 1. Construction of a SPME device.

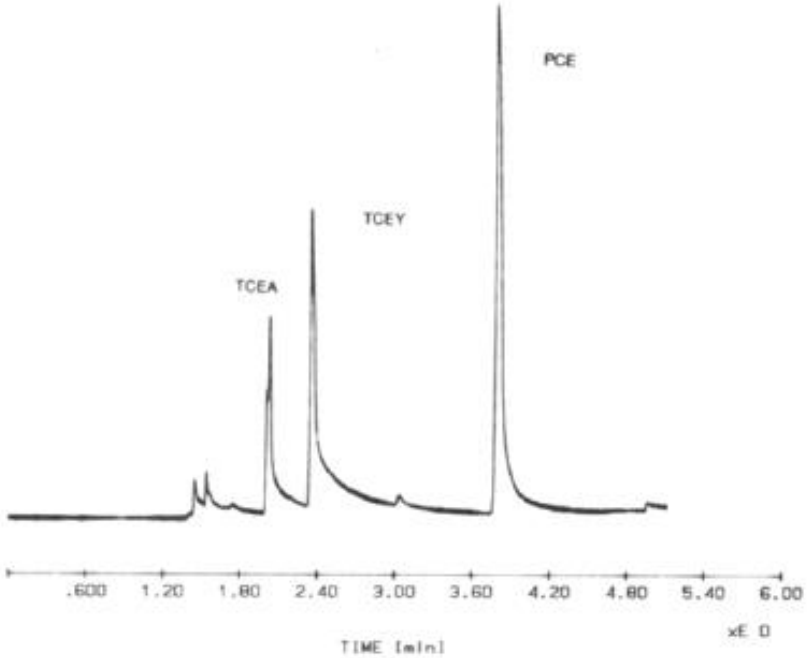


Figure 2. Separation of 1,1,1-trichloroethane (TCEA), trichloroethene (TCE), and perchloroethylene (PCE) after desorption from a fiber coated with polyimide. Concentrations were 0.69, 0.75, and 0.83 μg/L, respectively. Other conditions were as follows: He carrier, 29 cm/s; N_2 make-up, 24 mL/min; injector, 275 °C; oven, 60 °C isothermal; detector, 325 °C.

Arthur, C. L. and J. Pawliszyn (1990). "Solid phase microextraction with thermal desorption using fused silica optical fibers." Analytical Chemistry 62(19): 2145-2148.

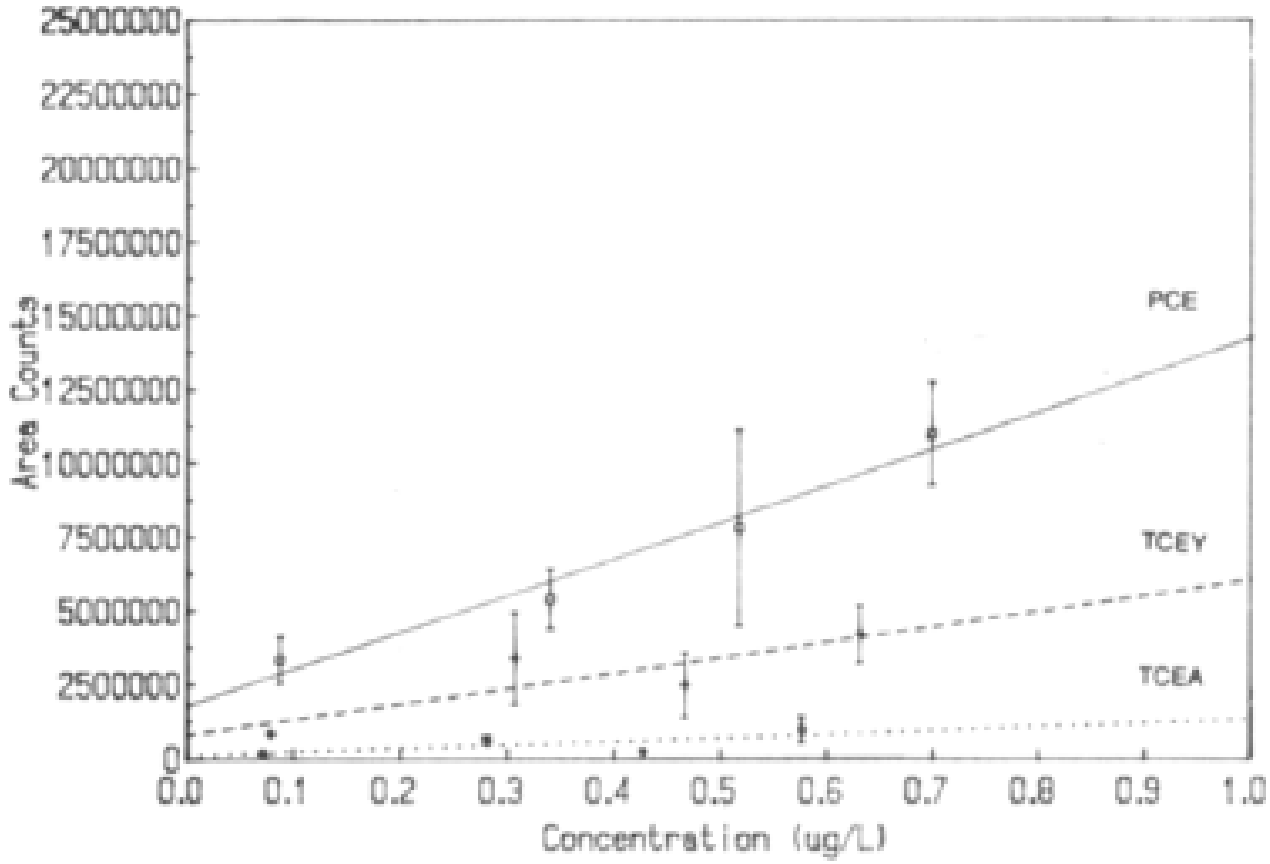


Figure 4. Linearity of fiber response for TCEA, TCEY, and perchloroethylene. Fibers were exposed for 2 min and points were obtained in duplicate.

Arthur, C. L. and J. Pawliszyn (1990). "Solid phase microextraction with thermal desorption using fused silica optical fibers." Analytical Chemistry 62(19): 2145-2148.

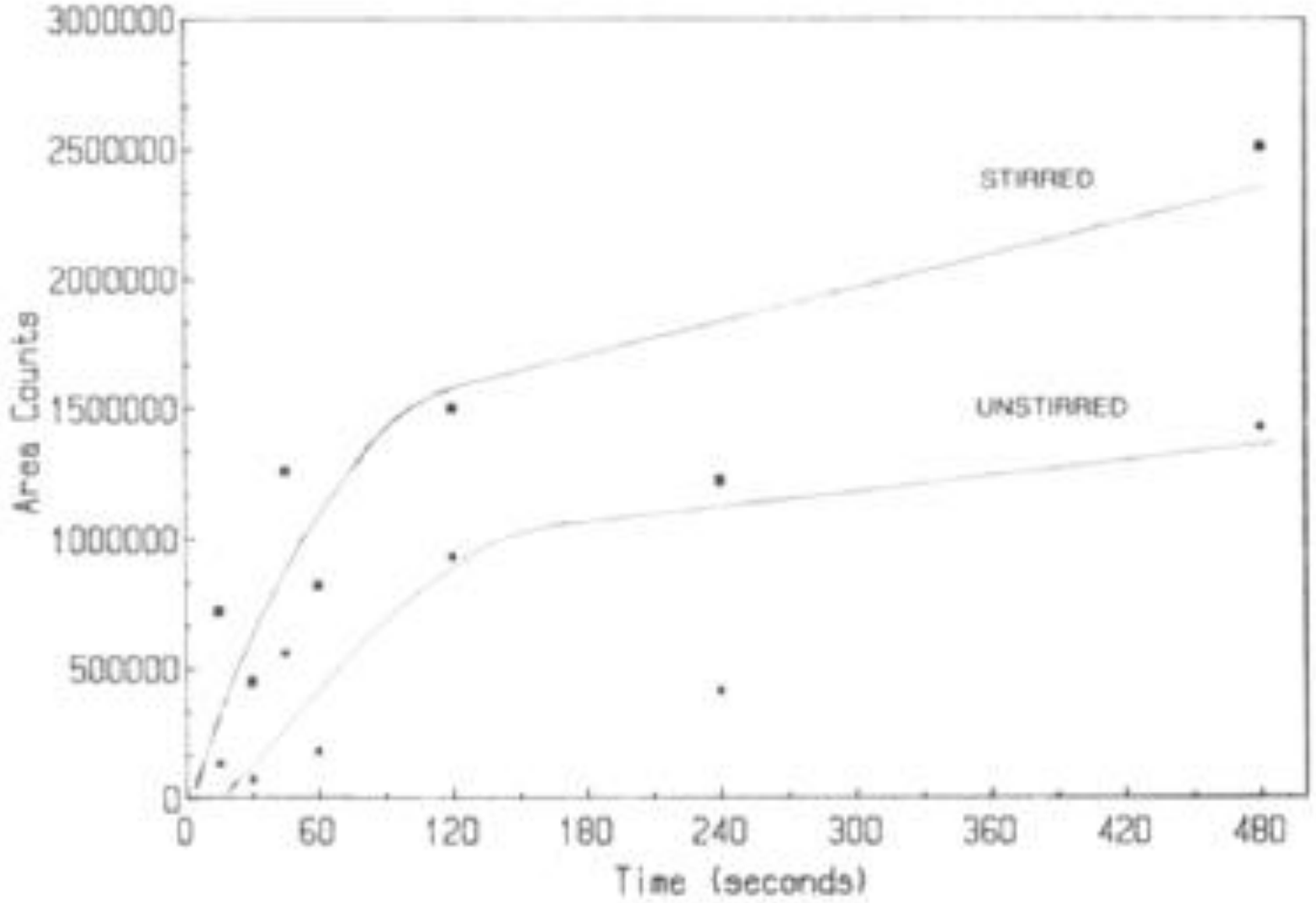


Figure 3. Adsorption–time profile for trichloroethene, both stirred and unstirred. Trichloroethene concentration was 0.75 μg/L.

Arthur, C. L. and J. Pawliszyn (1990). "Solid phase microextraction with thermal desorption using fused silica optical fibers." Analytical Chemistry 62(19): 2145-2148.

Table II. Effect of Salt Concentration on the Adsorption of Chlorinated Hydrocarbons from Aqueous Solutions

		concentration, μg/L		
		TCEA	TCEY	PCE
no salt	x	1.90	2.76	3.50
	s	0.33	0.40	1.10
1.98%	x	4.11	2.24	4.10
	s	1.88	0.40	0.33
3.87%	x	1.82	3.91	3.47
	s	0.13	0.28	0.33
10.19%	x	2.15	4.04	3.07
	s	na	na	na

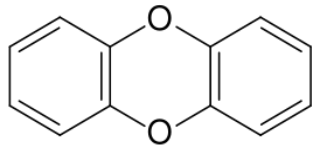
Arthur, C. L. and J. Pawliszyn (1990). "Solid phase microextraction with thermal desorption using fused silica optical fibers." Analytical Chemistry 62(19): 2145-2148.

Part VI – Applications and wrapup

Sample Treatment Examples – If required, what type?

1. Goal - Determination of the extinction coefficient (UV) of a novel antipsychotic drug that you have just synthesized

2. Goal – Analysis of dioxins at parts-per-trillion levels (ng/mL) in Minnesota River Water



3. Goal – Quantitative determination of formic acid in a 1.0 M solution in water, sold by Sigma-Aldrich

4. Goal – Analysis of Clara Cell Secretory protein in human serum at ng/mL levels