UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

Solid phase microextraction

Isoolation and separation methods

Solid phase extraction

Solid phase extraction (SPE)

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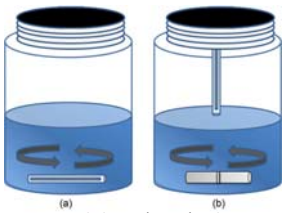
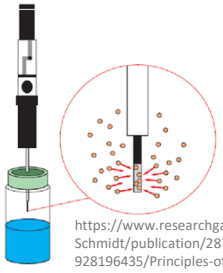
https://lh3.googleusercontent.com/proxy/z8-rguAvExBkv_yq7_1iHOjXjRUrkQP8DOn7flSYTXxyD_DzjquMxd8AdS2axDLGIVBlro62JPZHk7WWyQBzaI0weo_IeIkvKCKuyfubeyUw7GFv

Stir bar sorptive extraction (SBSE)



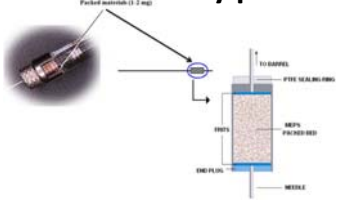
https://www.semanticscholar.org/paper/Stir-bar-sorptive-extraction%3A-A-view-on-method-and-Prieto-Basauri/96af601e27b8d4070f227ca5b86193dd262b6563/figure/2

Solid phase micro extraction (SPME(



https://www.researchgate.net/profile/Kamila-Schmidt/publication/287974185/figure/fig2/AS:372944040677377@1465928196435/Principles-of-extraction-by-headspace-solid-phase-microextraction-HS-SPME_Q640.jpg

Microextraction by packed sorbent (MEPS)



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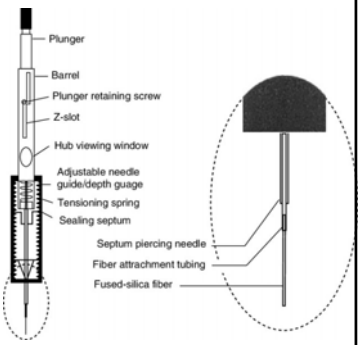
Solid phase microextraction (SPME)

- Solvent-free sample preparation technique
- Works on the principle of adsorption/absorption and desorption and uses a fiber coated with an extractive phase to concentrate analytes in a sample
- Combines the sampling, isolation, and enrichment of an analyte to one step

What is SPME?

- Sample preparation technique
- A fast, solvent-less sample extraction technique
- It is used for extracting organics from a matrix (solid, liquid, or gaseous) into or onto a stationary phase immobilized on a fiber
- In SPME, analytes establish equilibria among the sample matrix, the headspace above the sample, and a polymer-coated fused fiber
- Two types of extraction:
  - Headspace (most common)
  - Direct immersion (application specific) – SPME fiber is immersed into the aqueous sample



SPME history

- First described in Arthur, C.; Pawlisyzn, J.; *Solid phase microextraction with thermal desorption using fused silica optical fibers, Analytical Chemistry (1990) 62, 2145-2148.*
- Can be used for subsequent analysis by GC or HPLC, but most common with GC
- Typically, non-exhaustive type sampling (meaning only a portion of analyte in sample is trapped). Quantitation is based on keeping exposure to samples the same (easier with autosampler).
- While quantitation is often difficult, sensitivity is enhanced relative to SPE because whole trapped sample is injected
- The other advantage of SPME is the reduction in required labor

SPME application

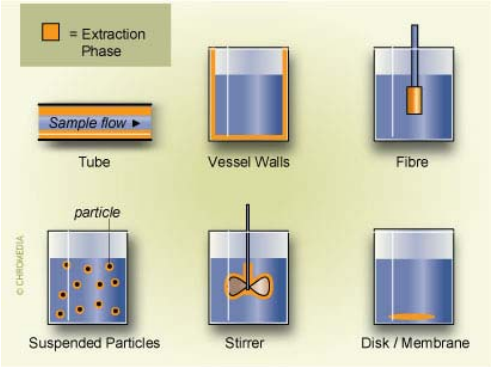
- Environmental analyses of water samples
- Headspace analysis of trace impurities in polymers and solid samples
- Odor analyses
- Flavor analyses of food products
- Forensic analyses of arson/explosives samples
- Toxicology analyses: blood alcohol or drugs in urine/serum
- Surfactants, other industrial applications

SPME

The most widely used technique of sampling with solid phase microextraction consists of exposing a small amount of extracting phase (coating) associated with a fibre to the sample, for a predetermined amount of time

There are different arrangements - sorption media

- fiber
- capillary
- stirrer
- suspended particles
- container wall
- disk / membrane
- other types are continuously tested



SPME principle

Silica fiber with chemically modified surface (stationary phases)
Length ≈ 1 cm
Diameter ≈ 0.05 – 1 mm

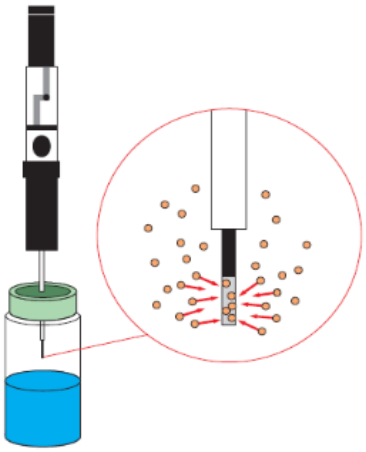


Figure 2: Principles of extraction by headspace-solid phase microextraction [HS-SPME].

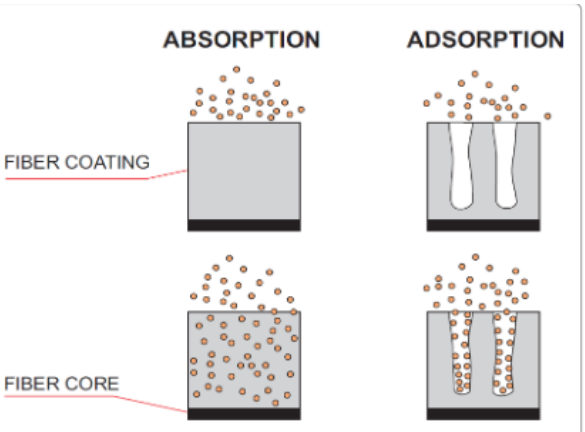


Figure 3: Extraction mechanisms for absorptive (liquid) and adsorptive (solid) fiber coatings.

SPME procedure

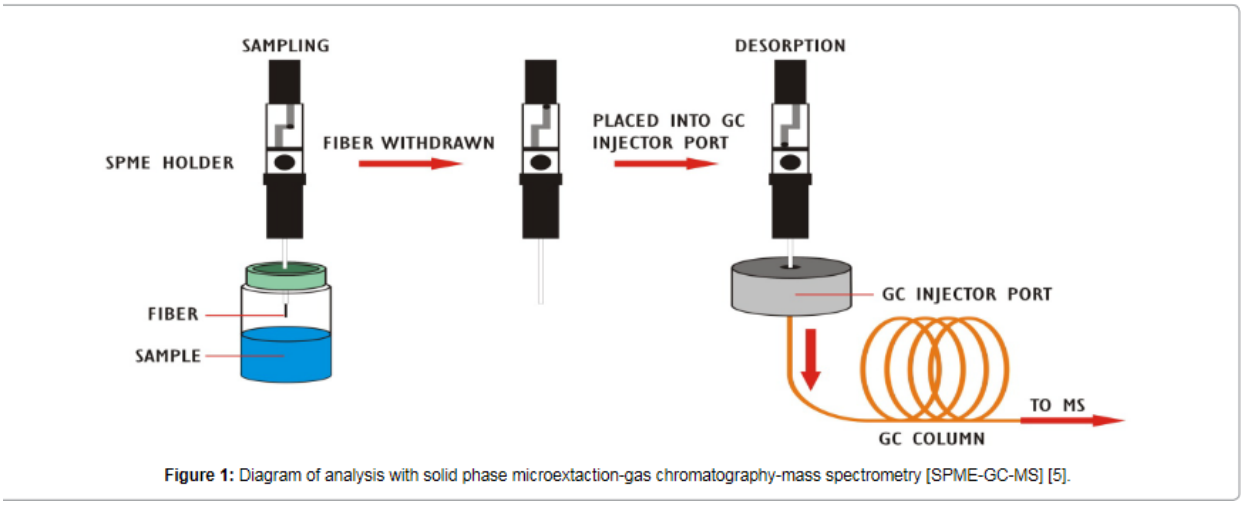
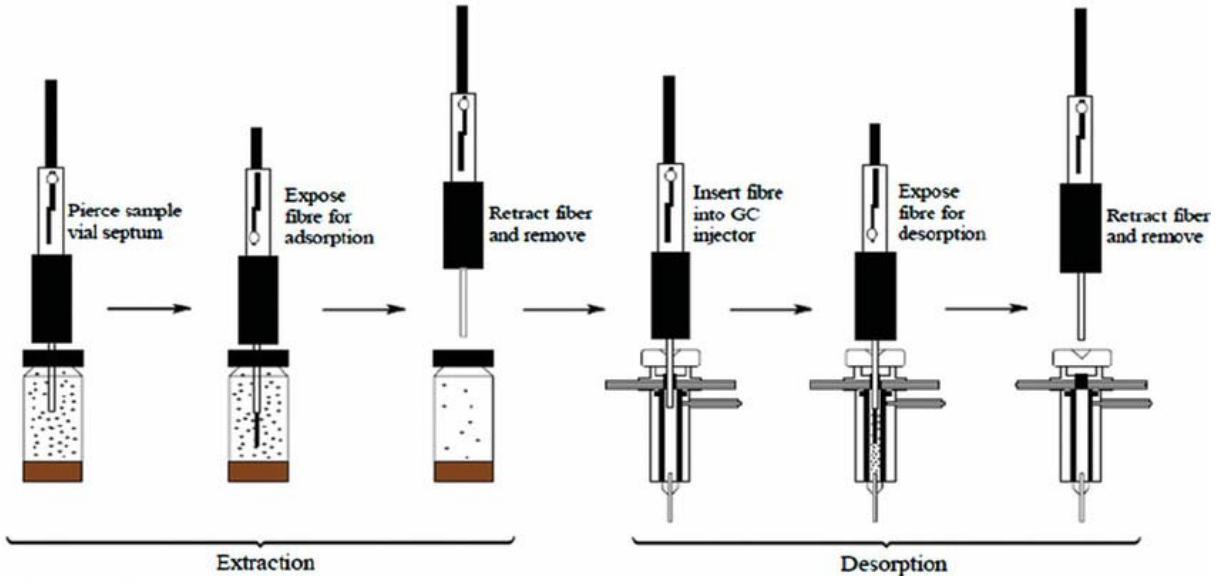


Figure 1: Diagram of analysis with solid phase microextaction-gas chromatography-mass spectrometry [SPME-GC-MS] [5].

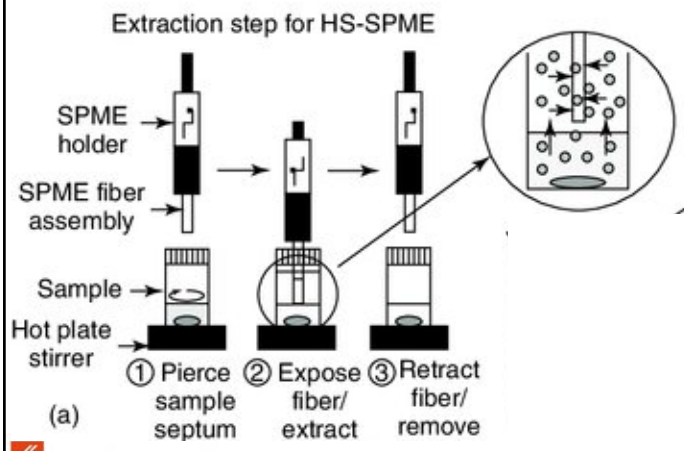
Schmidt K, Podmore I (2015) Solid Phase Microextraction (Spme) Method Development in Analysis of Volatile Organic Compounds (Vocs) as Potential Biomarkers of Cancer. J Mol Biomark Diagn 6: 253. doi:10.4172/2155-9929.1000253

SPME procedure

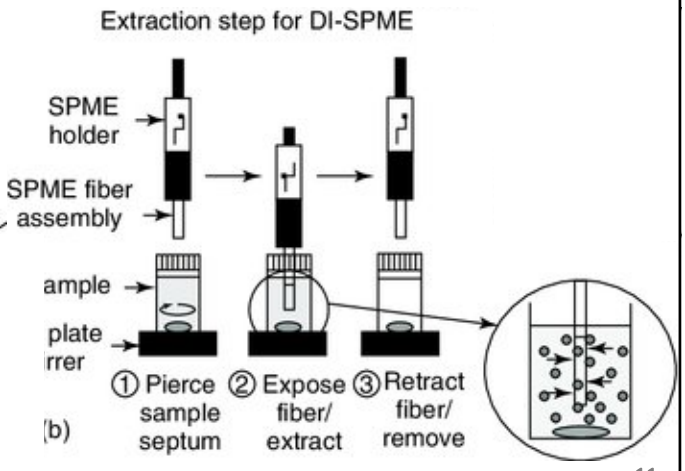


Typical laboratory sorption performance

Direct Immersion (DI) SPME
We sample the liquid phase



Head-space (HS) SPME
We sample the gas phase



SPME theory

Direct SPME

$c_o . V_s = c^{\infty}_f V_f + c^{\infty}_s . V_s$

$K_{fs} = c^{\infty}_f / c^{\infty}_s$

$$n = \frac{K_{fs} . V_f . c_0 . V_s}{K_{fs} . V_f + V_s}$$

If K_{fs} is small or V_s large,
i.e. $K_{fs} . V_f << V_s$, then

$n = K_{fs} . V_f . C_o$

Head-Space SPME

$c_o . V_s = c^{\infty}_f V_f + c^{\infty}_s . V_s + c^{\infty}_h . V_h$

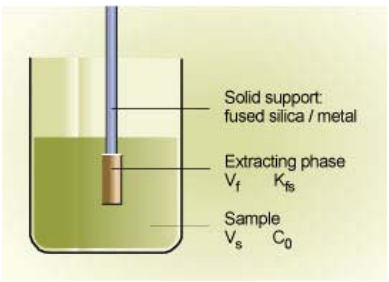
$K_{fh} = c^{\infty}_f / c^{\infty}_h$, $K_{hs} = c^{\infty}_h / c^{\infty}_s$

$$n = \frac{K_{fs} . V_f . c_0 . V_s}{K_{fs} . V_f + K_{hs} . V_h + V_s}$$

If $K_{fs\ is\ large}$: $K_{fs} . V_f >> V_s$

$n = V_s . c_o$

(quantitative extraction)



f ≈ fiber = stationary phase
s ≈ sample
h ≈ ***head-space***
c^{∞} ≈ equilibrium concentration

SPME

Sample Types (GC analysis)

- Liquid Samples
  - best for relatively clean samples at lower concentrations
  - best if analyte has polarity like coating and different than solvent (e.g. non-polar analyte and coating in water)
- Headspace Sampling
  - fiber is in space above liquid sample
  - good for “dirty” samples (e.g. flower components)
  - preferentially absorbs moderately volatile species
- Gas Samples
- In Fiber Derivatization (typically applied to polar organic compounds which often decompose on GC columns)

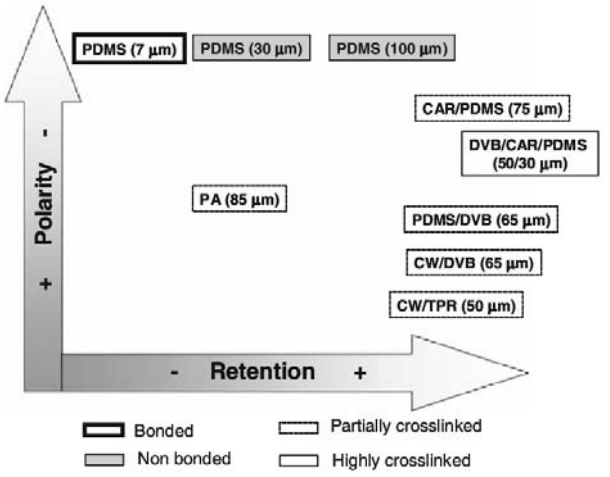
SPME parameters

- **Fiber:** polarity and thickness of the stationary phase
- **Sorption:** extraction mode, sample preparation, mixing
  - temperature, time, fiber position
  - (+ type of container and cap)
- **Desorption (GC):** fiber position, liner type, temperature, time

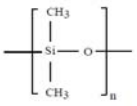
SPME

- Fiber "Variables"
  - One can select fibers of different polarities and film thicknesses
  - A polar fiber will selectively trap polar molecules
  - Thinner films used for faster sorption/desorption
  - A practical consideration is thermal stability

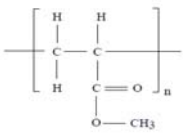
Overview of stationary phases of SPME fibers



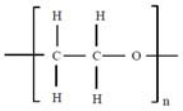
Polydimethylsiloxan (PDMS)



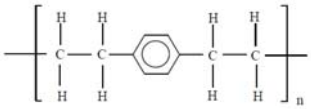
Polyakrylát (PA)



Carbowax (CW)

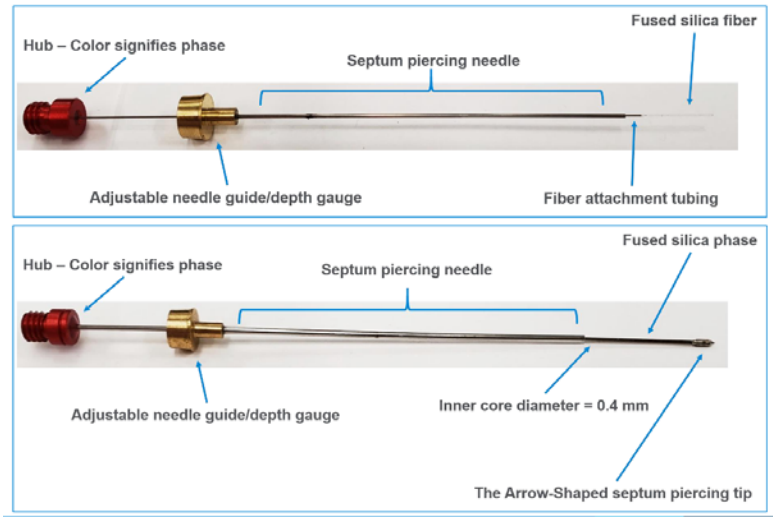


Divinylbenzen (DVB)



Parts of the SPME Fiber/Arrow

- SPME characteristics



Key Component – The Phase

- SPME fiber hub colors

Agilent

7 µm PDMS	Green
30 µm PDMS	Gold
100 µm PDMS	Red
85 µm Polyacrylate	Grey
95/10 µm CAR-WR/PDMS	Dark Blue
65 µm DVB/PDMS	Violet
80/10 µm DVB/CAR-WR/PDMS	Dark Grey

Acronym	Full Name
PDMS	Polydimethylsiloxane
PA	Polyacrylate (aka acrylate)
DVB	Divinylbenzene
C-WR	Carbon Wide Range

Thinner coatings
For higher MW compounds
Can accept higher conditioning temperature

7 µm PDMS
30 µm PDMS
100 µm PDMS
85 µm Polyacrylate
95/10 µm CAR-WR/PDMS
65 µm DVB/PDMS
80/10 µm DVB/CAR-WR/PDMS

Thicker coatings
Require longer extraction times
Use lower conditioning temperature

Absorbent

Adsorbent

SPME – sorption mechanism

Thickness SF

- ↑ thickness ⇒ ↑ sorbed amount
- ⇒ slower equilibrium (diffusion)

Small molecules (MR = 60 - 90)

- Sorption is influenced more by the type of sorption (AD x AB) and pore size Best experience with: CX, DVB / CX, PDMS / DVB

Medium size molecules (MR = 90 - 500)

- The shape (type of substituents) and the size of the molecule play an important role
- Polar SF suitable for polar analytes (MR> 90)

Mechanism of SPME sorption according to SF type on fiber

PARAMETER	ADSORBENT (DVB or CX)	ABSORBENT (PDMS or PA)
Phase character	Solid porous material Larga surface	Liquid polymer phase
Sorption mechanism	Physical interaction Capturing in pores Chemical bond	Migration of analytes to/from the phase, Retention corresponds to the phase thickness
Capacity	Limited (overload)	High (a thick layer)
Binding sites competition	Yes (esp. DVB – unified pores)	No
Linear range	Narrower (improvement: shorter extraction time Þ higher LOQ)	Wider
Limit of quantification	Low	Higher

Fiber/Arrow Conditioning

- Fibers/Arrows MUST be preconditioned prior to first use (or after sitting)
  - Done at a specified temperature in an inert gas phase environment (He or N2)
- Fibers/Arrows MUST be conditioned prior to and/or after desorption of sample – cleans the fiber
  - Done at a hotter temperature than the injection/inlet temperature

SPME - sample treatment

- Quantity (volume) - volume, weight
- Mechanical treatment - grinding, crushing (with auxiliary material)
- Grinding with sorbent - conversion to dry material
  - selective retention of analytes
- Matrix modification - pH adjustment, salting, dilution
  - - addition of a modifying solvent

SPME - sample treatment

- Influence of NaCl addition and pH adjustment
  - Positive effect: neutral molecules - attenuation of interactions
  - Negative effect: ionized analytes - increase in ionic strength

⇒ prefer a solution

- introduction of impurities (secondary contamination)

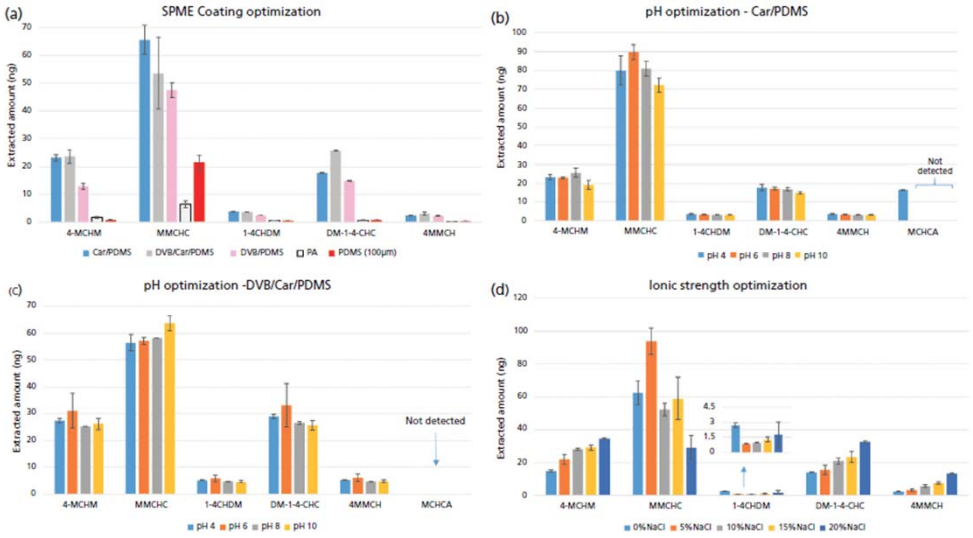
⇒ fiber damage may occur

Immersion sampling


Immersion

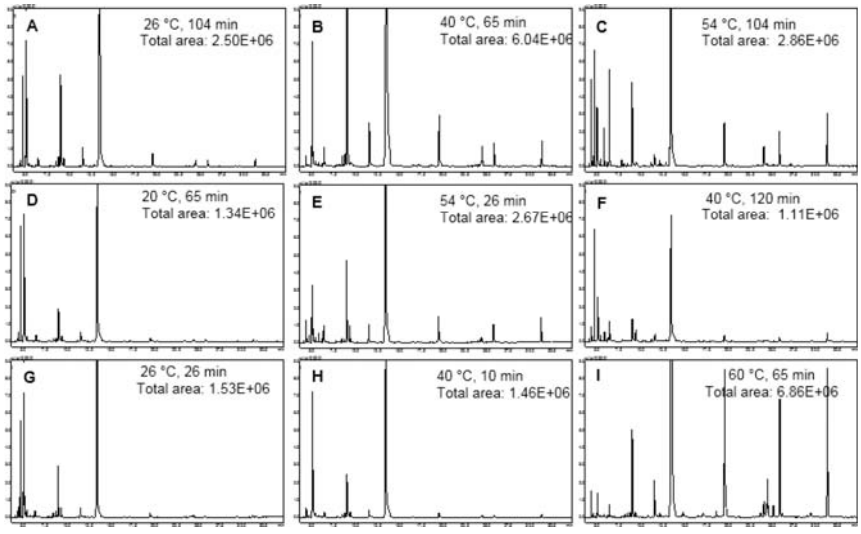
- Immersion sampling **sensitivity is improved** by filling the sampling vial to a minimum of 80%
- Immersion sampling works **best for low concentration water-based sample matrices**
- When using immersion for samples containing sugars, proteins, and particulate matter, rinse the fiber in water before desorption.
- This will extend fiber life and reduce injection port contamination.
- Position the fiber just below the sample surface for immersion sampling and maintain this position consistently for all extractions.

S txmq m-exmsr sjWTQ I$teveq ixiww mrgpyhmrk>$,e-$gsexmrk izepyexmsr sjjmzi gsq q ivgmepp} ezemqefpi jmfiw2tLstxmq m-exmsr ywmrk ,f-$Gev3THQ W$jmfiverh$g-$ HZF3Gev3THQ W$jmfiv2$erh$,h-$msrmg wxvirkxl stxmq m-exmsr { mxl Gev3THQ W$jmfiv2



https://www.chromatographyonline.com/view/exploring-efficiency-various-extraction-approaches-determination-crude-4-methylcyclohexylmethanol-mc

Chromatograms from CCRD experiment for optimization of extraction conditions of roasted beef volatile compounds by SPME using CAR/PDMS fibre coating



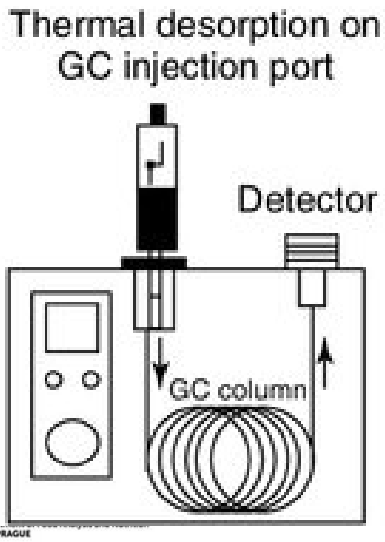
https://www.scielo.br/j/qn/a/cStcGxLFg5hSLRSs3WCx3hC/?lang=en#

SPME - injection

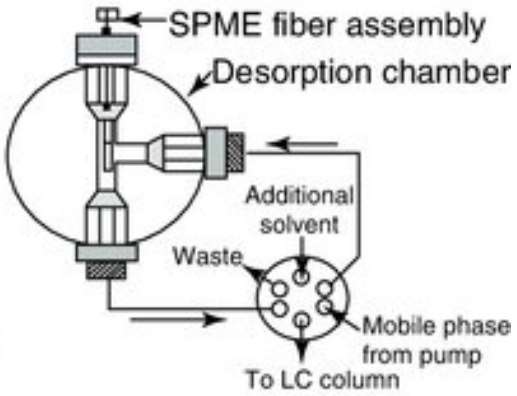
- In HPLC requires specialized injection valve
- Analytes desorbed from SPME fiber into solvent flowing around fiber (should be strong solvent)
- Use in HPLC is less developed
- This may take time, particularly for less volatile compounds in GC and for compounds with polarity more like stationary phase than solvent in HPLC
- Good peak shape usually requires “on column trapping”
- In GC, this is done by splitless injections with the column initially at low temperatures

Typical laboratory design of automatic desorption

Thermal



Solvent



(e) Solvent desorption using SPME interface

SPME applications

Authors	Matrix	Column	Analytes	Extraction conditions
Wada and Shibamoto [9]	Red wine	Ethylvinylbenzene-divinylbenzene copolymer	Flavour	*Solvent*: dichloromethane
López et al. [10]	Wine	Styrene-divinylbenzene copolymer	Flavour	*Sample volume*: 50 mL; *Solvent*: dichloromethane
Culleré and others [8]	Wine	Styrene-divinylbenzene copolymer	Flavour	*Sample volume*: 75 mL; *Solvents:* dichloromethane, pentane and pentane:dichloromethane (9:1)
Lukic et al. [13]	Grape distillate	Octadecylsilica	Flavour	*Sample volume*: 3 mL diluted to 25 mL; *Solvent:* dichloromethane
Dieguez et al. [15]	Spirits	Silica	Volatile organic acids	*Sample volume*: 50 mL; *Solvent:* dichloromethane
Genovese et al. [12]	Red wine	Ethylvinylbenzene-divinylbenzene copolymer	Flavour	*Sample volume:* 50 mL; *Solvents:* pentane:dichloromethane (20:1) and dichloromethane
Karagiannis et al. [16]	White wine	Silica	Terpenes	*Sample volume:* 25 mL; *Solvents:* dichloromethane and methanol
Piñeiro et al. [17]	White wine	Styrene-divinylbenzene copolymer	Terpenes	*Solvent:* dichloromethane
Ferreira et al. [19]	Wine	Styrene-divinylbenzene copolymer	Aliphatic lactones	*Sample volume:* 50 mL; *Solvent*: dichloromethane
Campo et al. [20]	Wine, whisky and brandy	Styrene-divinylbenzene copolymer	Flavour	*Sample volume:* 100 mL; *Solvent:* dichloromethane
Insa et al. [21]	Wine	Styrene-divinylbenzene copolymer	Anisoles	*Sample volume:* 50 mL; *Solvent:* dichloromethane
Dominguez et al. [23]	White wine	Styrene-divinylbenzene copolymer	Volatile phenols	*Sample volume:* 10 mL; *Solvent:* dichloromethane
Charles et al. [25]	Vinegar	Styrene-divinylbenzene copolymer	Flavour	–
Morales et al. [26]	Vinegar	Styrene-divinylbenzene copolymer	Flavour	*Solvent:* dichloromethane

SPME – advantages/limitation

Advantages:

- Listed as “Solvent-less” technique (at least great reduction in solvent injected into GC)
- Less interference from solvent peak
- Reduced injection of non-volatiles
- Less sample handling (+ ability to automate)
- Can chose fibers for good selectivity

Limitation:

- More difficult for quantitative results
- Limited lifetime of fibers
- Memory effects (slow desorption from fibers)

SBSE (Stir Bar Sorptive Extraction)

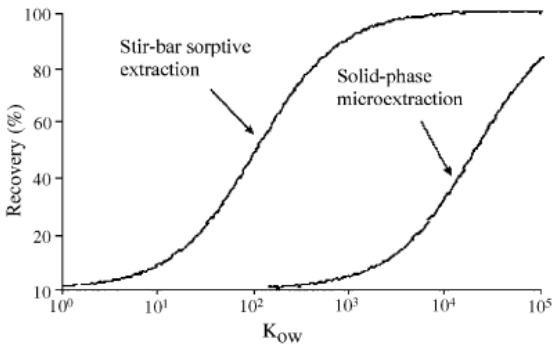
in SPME very low recoveries for compounds with log$K_{o/w}$ < 5
To overcome the limited extraction capacity of SPME fibres.

Idea: Extraction of aqueous samples with a PDMS coated stir bar

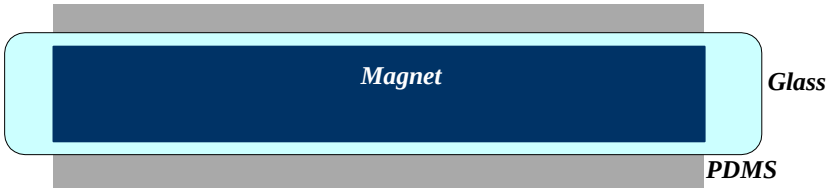
Sandra, Baltussen and David [1999]

SPME x SBSE

Extraction of analytes depends on the partitioning coefficient of solutes between the phases.
The octanol-water **distribution coefficient (*Kow)*** can be used as an indication as how well a given analyte will be extracted
Distribution equilibrium between the matrix sample and the sorbent



SBSE

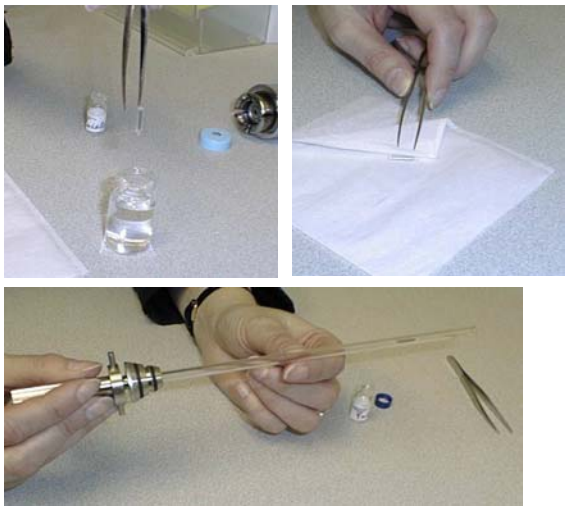


l = 10 mm, df = 0.5 mm	→	24 µl
df = 1.0 mm	→	63 µl
l = 20 mm, df = 0.5 mm	→	47 µl
df = 1.0 mm	→	126 µl

SPME : max. 0.5 µl

⇒ quantitative yield for compounds with $K_{ow} > 500$
⇒ suitable also for compounds with K_{ow} 10 – 500

SBSE - realization



Procedure

- Add stir bar to vial
- Stir
- Remove stir bar with tweezers.
- Rinse briefly in distilled water
- Dry with lint-free tissue
- Drop bar into thermal desorber
- Thermally extract (GC analysis)

SBSE – advantages/limitation

Advantages:

- 100 – 1000 x lower LODs (µg – ng/l) compare to SPME
- quantitative extraction – large linear range
- easy and fast realization
- automatic thermal desorption
- simultaneous sorption

Limitation:

- limited re-use - carry-over effect
- LODs higher for polar analytes
- high background of complex matrices
- (reverse solvent extraction)

A comparison of some characteristics of sample preparation techniques

Detection limit (MS)	Precision (RSD)	Expense	Time	Solvent used	Simplicity
Purge and Trap (ppb)	1–30	High	30 min	None	No
Stripping (ppt)	3–20	High	2 h	None	No
Headspace (ppm)		Low	30 min	None	Yes
Liquid–liquid extraction (ppt)	5–50	High	1 h	1000 mL	Yes
Solid-phase extraction (ppt)	7–15	Medium	30 min	To 100 mL	Yes
SPME (ppt)	<1–12	Low	5 min	None	Yes

Source: Reproduced with permission from Supelco Bulletin, (1998), **923**, 1–5. © Sigma-Aldrich Co.
Table provided by J. Pawliszyn, University of Waterloo, Ontario, Canada.

DOI: 10.1002/9780470027318.a9905

A comparison of some characteristics of sample preparation techniques

Table 3. A comparison of some characteristics of sample preparation techniques [17,93,94].

Feature	MEPS	FPSE	DLLME	SPE	SPME
Phase amount	0.5–4 mg	n.a.	n.a.	50–10,000 mg	150 mm thickness
Principle-separation	no emulsion	no emulsion	emulsion	no emulsion	no emulsion
Procedure time	1–2 min	5–30 min	5–15 min	10–15 min	10–40 min
Re-use	40–100 times	30–50 times	Single use	Single use	50–100 times
Recovery	+	+	+	+	−
Carryover	−	−	n.a.	+	+
Solvent consumption	−	+/−	+	+	solventless
Sensitivity	−	+	+	+	−
Easy-to-use	−	+	−	+	−
Sample quantity	−	+/−	+/−	+	+
Easily adaptable to	GC or HPLC	GC or HPLC	GC or HPLC	GC or HPLC	GC
Automatable	+	−	−	+	+
Target analytes	polar and charged analytes may be extracted	polar and charged analytes may be extracted	polar analytes difficult to extract	polar and charged analytes may be extracted	polar and charged analytes may be extracted
Cost	−	n.a.	+	+	+
Commercially available	+	−	+	+	+

n.a. not applicable; + high; − low; +/− high or low depend to the application field.

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