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

Solid phase extraction

Isoolation and separation methods

1

Solid phase extraction

Solid phase extraction (SPE)

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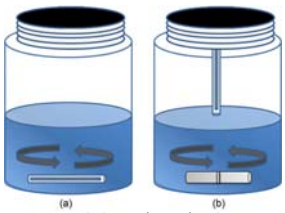
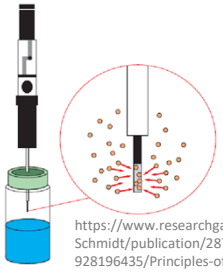
https://lh3.googleusercontent.com/proxy/z8-rguAvExBkv_yq7_1iHOjXjRUrkQP8DOn7flSYTXxyD_DzjquMxd8AdS2axDLGIVBlro62JPZHk7WWyQBzaI0weo_IeIkvKCKuyfubeyUw7GFv

Stir bar sorptive extraction (SBSE)



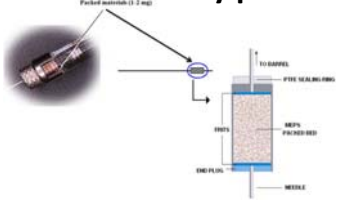
https://www.semanticscholar.org/paper/Stir-bar-sorptive-extraction%3A-A-view-on-method-and-Prieto-Basauri/96af601e27b8d4070f227ca5b86193dd262b6563/figure/2

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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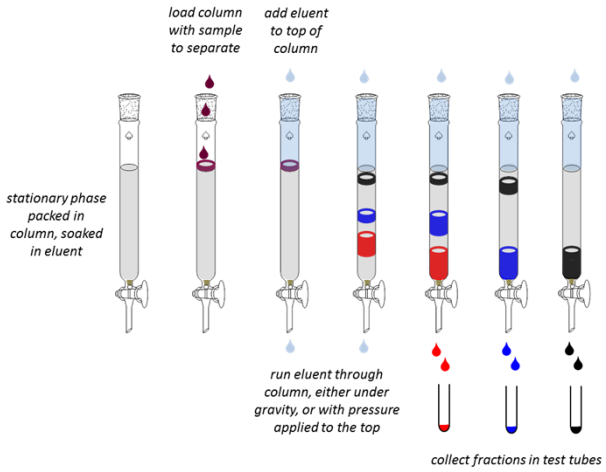
2

Adsorption chromatography (LSC)

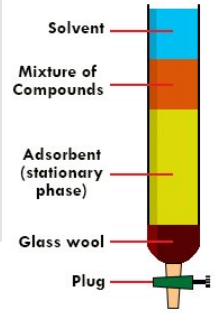
- **Separation of a mixture of substances** (primary extract) on a **column of sorbent** (e.g., silica gel, florisil) in a glass column with a frit (approx. 50 cm x 1 cm)
- It is usually a selective adsorption of undesirable substances (lipids, pigments) and subsequent elution of analytes with a suitable solvent
- The extract is applied in the solvent used to prepare the column



https://chembam.com/techniques/chromatography/adsorption-chromatography/

Adsorption chromatography

Alumina	Magnesium oxide	Activated carbon
• Separation of low polar substances (pigment capture), usually weakly alkaline (pH = 10) - can decompose esters by washing with HCl and H_2O to form neutral (pH = 7.5) - weaker sorption, acidic (pH = 4.5) - $AgNO_3$ impregnation	• Low affinity for compounds with double bonds mostly in mixed sorbents (with kieselguhr)	• Non-polar sorbent, often irreversible sorption, removal of pigments



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Solid Phase Extraction – SPE columns

- Interaction of 3 components: solid phase-analyte-solvent
- Different principle of SPE columns:
  - analyte retention, the matrix passes through
  - matrix retention, analyte passes
- SPE columns/cartridges
  - column body made of polypropylene or glass
  - inside the frit box, e.g., 20 mm made of polyethylene or steel
  - filled with sorbent, e.g., silica gel, particle size 40 mm, pore size 60 A
  - the volume of the column is different, e.g., 1, 3, 6 ml
  - amount of sorbent in the column: 100, 500 mg, 1, 2, 5, 10g
  - column capacity: 10-20 mg analyte / g charge (co-extracts = matrix must also be taken into account)
  - breakthrough point: sorbent saturation - analyte is no longer retained

SPE columns

- Possibilities of application of SPE columns (extraction methods)

Selective extraction
- capture of analytes and other components go through th a column
- elution of analytes

Selective elution
- capture of analytes and other components of the matrix
- elution of analytes only

Selective washing
- capture of analytes and other components of the matrix
- leaching of interfering substances (analytes sorbed)
- elution of analytes

Matrix removal
- Capture of interferences, analytes go thouth the columns

Solid Phase Extraction – SPE columns

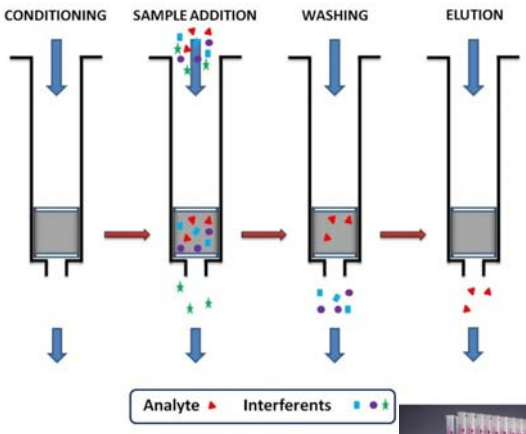
General SPE method

I. column preparation = solvent conditioning

II. sample application = capture of e.g. both analyte and matrix

III. sorbent wash = matrix elution

IV. sorbent wash = analyte elution



Implementation using: vacuum, overpressure, centrifugation

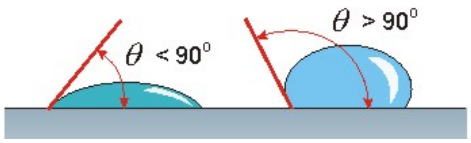
https://screeningdevices.com/wp-content/uploads/2018/05/spe-development-protocols.jpg

SPE steps

I. Conditioning

1 - 2 ml / 100 mg cartridge

- Elution of impurities (solvent strength same as sample Transfer to the sample solvent (less than or equal to the solvent strength as the sample)
- Wetting:



Well wetted surface

Insufficient surface wetting

SPE steps

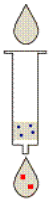
II. Sample application

- Adjustment (pH, ionic strength, dilution, reduction of analyte solubility in the sample)
- Volume (higher volume, removal of conditioning solvent, reduction of efficiency, yield)
- Flow rate (max. 5 ml/min, sufficient sample/phase contact)
- Volume affects:
  - column size and type
  - analyte concentration
  - amount of interference
  - retention of the analyte by sorbent

SPE steps

III. Washing

- 0.5 ml/100 mg cartridge
- Co-extract removal (same or greater strength)



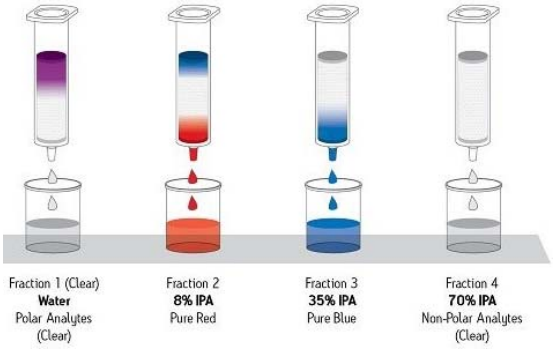
IV. Elution of analyte

- Type of solvent (similar dissolves similar, easily evaporable)
- Solvent volume (2 x dead volume, approx. 2 x 100 µl / 100 mg sorbent)

SPE columns application

Why we use SPE columns?

- Elimination of interfering compounds
- Preconcentration of analyte
- Fractionation of groups
- Change of sample (i.e. solvent)
- Desalination



increasing strength of the solvent

IPA = isopropanol

SPE – selection of solvents

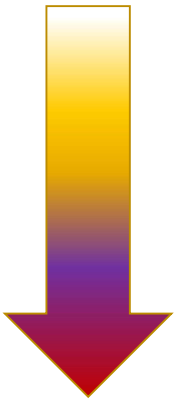
"Weak "solvent = sorption of analytes

"Strong" solvent = elution of analytes

Normal phase

Hexane
Isooctane
Toluene
Chloroform
Dichloromethane
Tetrahydrofuran
Diethyl ether
Ethyl acetate
Acetone
Acetonitrile
Isopropanol
Methanol
water

weak



strong

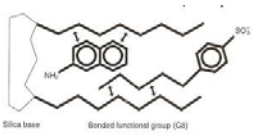
Reverse phase

Water
Methanol
Isopropanol
Acetonitrile
Acetone
Ethyl acetate
Diethyl ether
Tetrahydrofuran
Dichloromethane
Chloroform
Toluene
Isooctane
Hexane

SPE - Analyte-sorbent binding interactions

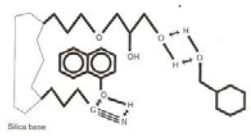
HYDROPHOBIC INTERACTIONS (non-polar phase)

- *Interactions between non-polar molecules due to the formation of induced dipoles:*
  - attractive and repulsive
  - weaker than hydrogen bonding or dipole-dipole interactions



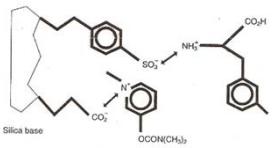
POLAR INTERACTION (polar phase)

- *Hydrogen bond - between molecules with hydrogen covalently bonded to a strongly electronegative element* (O, N, F)
  - Dipole-dipole - between polar molecules with a permanent dipole moment



ION (ELECTROSTATIC) INTERACTIONS

- *Interactions between oppositely charged groups of ion exchanger and analyte*



SPE – phases selection

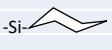
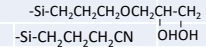
- Phase selection affects:
  - the nature of the analyte
  - sample solvent
  - type of interference
- Best analyte retention: analyte polarity similar to phase polarity
- Interference less polar than analyte - normal phase
- Interference more polar than analyte - reverse phase

SPE – sorbents (phases)

NORMAL PHASE (polar phase)	REVERSE PHASE (non-polar phase)	ION-EXCHANCHERS
• silica gel • alumina • florisil • polar modified silica gel (CN, diol, NH2)	• non-polar modified silica gel (C18, C8, C4, CH, PH, CN)	• ANEX - silica gel with chemically bound positively charged modifier (quaternary amine, secondary amine • KATEX - silica gel with a chemically bound negatively charged modifier (benzene or propylsulfonic acid.)

SPE – sorbents (phases)

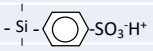
- non-polar sorbet - allows retention of substances from aqueous solutions

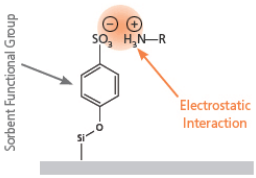
			captured substances / retention mechanism		
			Nonpolar	polar	ion exchange
C18 (EC)*	Octadecyl		1	3	3
C18	Octadecyl		1	2	2
MF C18	Octadecyl		1	2	2
C8 (EC)*	Octyl		1	3	3
C8	Octyl		1	2	2
C2 (EC)*	Ethyl		1	3	3
C2	Ethyl		1	2	2
CH (EC)*	Cyclohexyl		1	3	3
PH (EC)*	Phenyl		1	3	3
PH	Phenyl		1	2	2
CN (EC)*	kyanopropyl	-Si-$CH_2CH_2CH_2CN$	1	3	3
Silikagel	Silikagel	- Si – OH		1	3
NH_2	Aminopropyl	-Si-$CH_2CH_2CH_2NH_2$		1	2
DIOL	2,3-dihydroxypropoxypropyl			1	
CN	Kyanopropyl	-Si-$CH_2CH_2CH_2CN$		1	

1 – Primary interaction, secondary interaction, silanol cation exchange, 2 = strong, 3 = weak *EC = end capped*

SPE – sorbents (phases)

- non-polar sorbet - allows retention of substances from aqueous solutions

		captured substances / retention mechanism		
		nonpolar	polar	ion-exchange
Aminopropyl	-Si-$CH_2CH_2CH_2NH_2$	3		1
Trimethylaminopropyl (quartery amine)	-Si-$CH_2CH_2CH_2N^+(CH_3)_2Cl^-$	3		1
Carboxypropyl	-Si-$CH_2CH_2CH_2COOH$	3		1
Benzensulphonic acid		2		1
Propylsulphonic acid	-Si-$CH_2CH_2CH_2SO_3^-H^+$	3		1

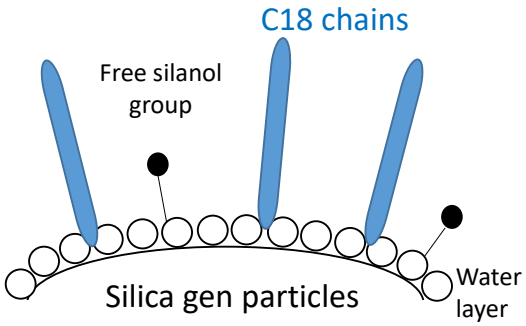


1 - Primary interaction, secondary interaction, silanol cation exchange, 2 = strong, 3 = weak

SPE - limitations of silica gel-based sorbents

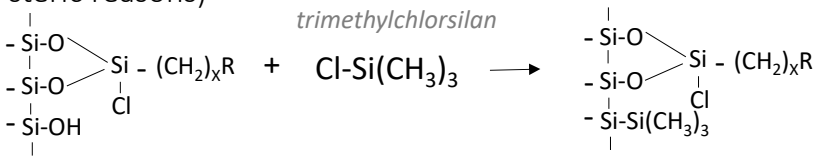
Problems:

- Necessary conditioning, must not dry out (x automation)
- Residual silanol groups (capture of basic compounds)
- Limited stability depending on pH (2-8)
  - ↓ pH → hydrolysis of the bound phase
  - ↑ pH → dissolution of silica gel
- Insufficient retention of more polar analytes
- Non-selective (purity of the extract!)
- Too strong (irreversible) sorption of nonpolar analytes



SPE - limitations of silica gel-based sorbents

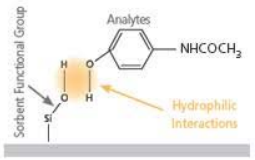
- The activity of residual silanol groups can be suppressed or promoted:
- **Suppression: -** "endcapped" phase (max. 70% of silanol groups can be unbound - steric reasons)

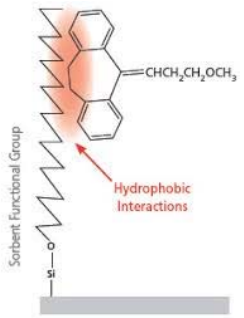


- pH adjustment (at pH 2 silanol is undissociated, pH <2 = dissociation - negative charge - electrostatic interactions)
- masking of silanols using a base (triethylamine)
- increase in ionic strength of the sample solvent = prevention of analyte binding
- **Support:**
  - pH adjustment (≥ 4 ionizations of silanols)
  - "non-endcapped" phases

SPE - limitations of silica gel-based sorbents

- **Silicagel** (normal phase):
  - Strong sorption of very polar compounds (glycerol) → use of modified silica gels (weaker retention)
- **C18** (reverse phase):
  - Non-selective, sometimes too strong sorption → use of phase with more polar modifiers (C8, C4) - some co-extracts pass without delay
- **Use of phases based on PS-DVB:**
  - polystyrene-divinylbenzene copolymer)
  - without silanols
  - Stable over a wide pH range
  - better retention of more polar analytes

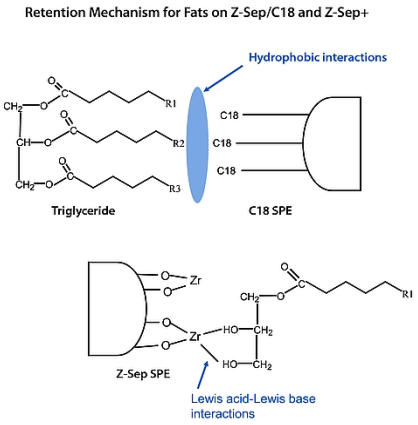




Trends in the development of sorbents for SPE

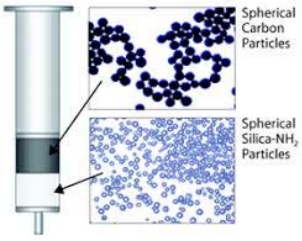
SupelQuE Z-Sep products enhance sample cleanup for complex matrices by effectively removing more fat and color from sample extracts than traditional phases for QuEChERS methods..

Z-Sep+ is recommended for cleanup of samples containing greater than 15% fat.
Z-Sep/C18 is recommended for cleanup of samples containing less than 15% fat.
Z-Sep is recommended for cleanup of samples prior to analysis of hydrophobic analytes.



Trends in the development of sorbents for SPE

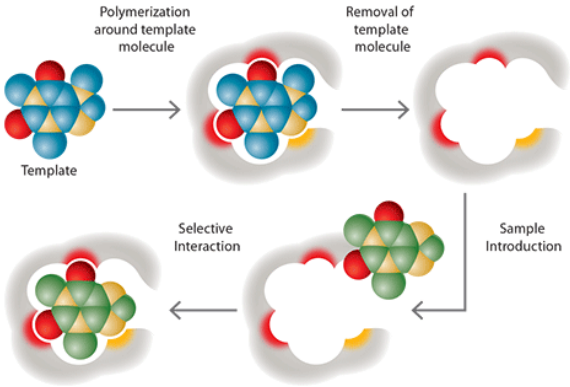
- Mixed sorbents
- Special products for pesticide analysis Supel Sphere Carbon / NH2
- Features and benefits:
  - SPE column filled with spherical fines
  - Higher flow characteristics and faster mobile phase flow
  - Carbon removes pigments and sterols that are often found in food and natural products
  - Aminopropyl (NH2) removes organic acids, polar pigments and sugars



Trends in the development of sorbents for SPE

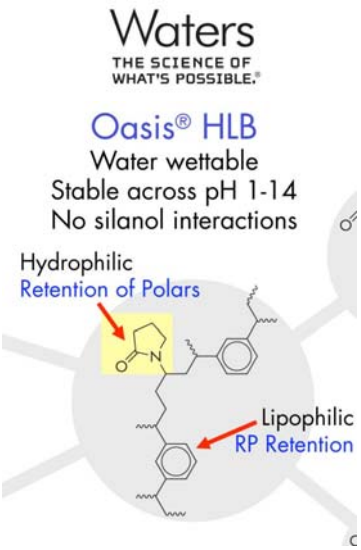
SupelMIP SPE – Molecularly Imprinted Polymers

- SupelMIP SPE offers tailor-made selectivity for the extraction of trace analytes in complex matrixes.
- Features and benefits:
  - Achieve lower detection limits through superior selectivity
  - Save time and reduce cost via robust and rapid methodology
  - Stable at broad pH ranges and high temperatures
  - Reduce ion suppression



Trends in the development of sorbents for SPE

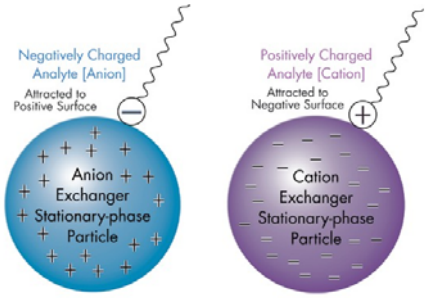
- Oasis HLB is an all purpose, strongly hydrophilic, reversed-phase, water-wettable polymer with a unique Hydrophilic-Lipophilic Balance.
- Oasis HLB maintains high retention and capacity even if it runs dry after conditioning. This sorbent is ideal for acidic, basic and neutral analytes as it's stable from pH 0-14.



SPE - Ion exchangers

- Ion-exchange media come in both **anionic and cationic forms** for the extraction of analytes with basic or acidic functional groups. Cation-exchange sorbents contain surface groups that are negatively charged, and the reverse is true for anion-exchange materials

Illustration of the Two Major Types of Phases—Anion and Cation Exchange—and How They Selectively Attract and Retain Molecules of Opposite Charge



Use the following table as a guideline to choose the appropriate SPE ion-exchange cartridge type for your particular analyte.

Ion-Exchange Guidelines

First, determine analyte type.
Then, follow corresponding arrows down for recommended particle and mobile phase pH.

Analyte Type	*Weak ACID*	*Strong ACID*	*Weak BASE*	*Strong BASE*
Charge State vs. pH*	No charge at pH < 3 / [anion] at pH > 7	[anion] Always Charged	[cation] at pH < 8 / No Charge at pH > 12	[cation] Always Charged
Stationary Phase Particle	*Strong Anion Exchanger*	*Weak Anion Exchanger*	*Strong Cation Exchanger*	*Weak Cation Exchanger*
Charge State vs. pH*	Always Charged	at pH < 8 / No Charge at pH > 12	Always Charged	No Charge at pH < 3 / at pH > 7
Mobile Phase pH Range				
to Retain analyte [capture]	pH > 7	pH < 8	pH < 8	pH > 7
to Release analyte [elute]	pH < 3	pH > 12	pH > 12	pH < 3

*Note: pH Ranges are approximate. They will depend upon specific analyte and particle characteristics.

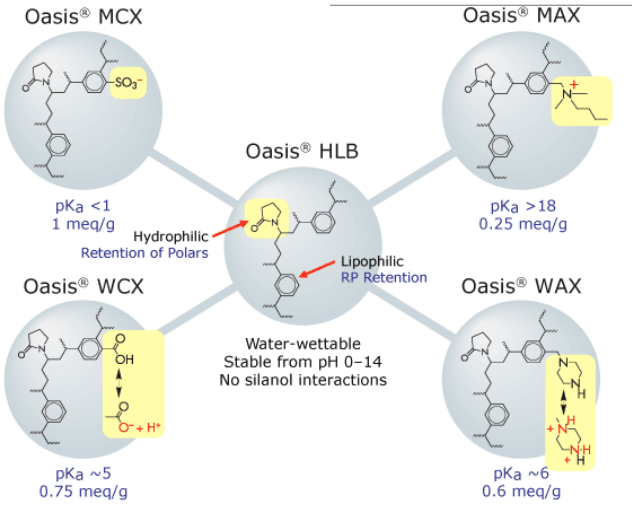
SPE - Ion exchangers

- pH - analytes must be ionized - according to the pK_a of the analyte, pH is adjusted 2 units above or below the pKa (acetic acid 4.75; cyclohexylamine 10.66)
- Counterion strength
  - CATEX: Li^{+}, H^{+}, Na^{+}, NH_4^{+}… easily replaceable
    Cu^{2+}, Ca^{2+}, Ba^{2+}… difficult to replace
  - ANNEX: OH^{-}, F^{-}… easily replaceable
    HSO_3^{-}, NO_3^{-}, CN^{-}, Cl^{-} … difficult to replace
- Ionic strength
  - total ion concentration in the sample
  - competition with an analyte for places on the ion exchanger

Trends in the development of sorbents

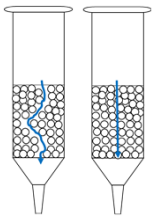
Waters
THE SCIENCE OF WHAT'S POSSIBLE.

- Further modifications of HLB sorbents
  - water-wettable polymers
  - stable in organic solvents
- **Oasis MCX** = Mixed-mode, strong Cation-eXchange, reversed-phase, **selective for bases**
- **Oasis MAX** = Mixed-mode, strong Anion-eXchange, reversed-phase, **acid selective**
- **Oasis WCX** = mixed-mode, Weak Cation-eXchange, reversed-phase, **to retain and release strong bases**
- **Oasis WAX =** mixed-mode, Weak Anion-eXchange, reversed-phase, **to retain and release strong acids**

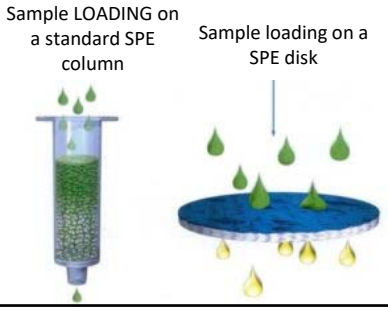


SPE - problems

- SPE columns
  - limited flow / small ratio of flow area and sorbent column)
  - formation of channels (inhomogeneity of sorbent, space between particles)
  - → **non-uniform flow → ↓ sorption capacity and reproducibility**



- SPE discs
  - Flat disks similar to membrane filters Thickness: ≤ 1 mm Diameter: 4 - 96 mm (↑ diameter → ↑ surface → ↑ flow
    - Rigid disks
    - Membane disks



SPE discs advantages

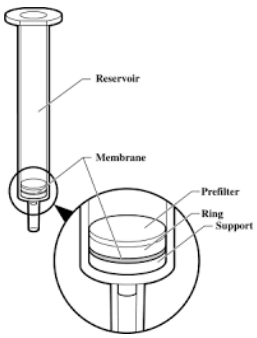
- Larger flow area - thin layer, small pressure difference → ↑ flows
- Lower amount of sorbents → ↓ dead volume → ↓ sample volume
  ↓ volume of elution solvent
  ↓ interference (smaller delay)
- No channel formation → ↑ retention efficiency, capacity and yield
- Raster drying
- Rime saving (1 l of water: 10 min 45 mm disc, 2 h box)

- *Limited number of phases*

Rigid discs

- Rigid glass fibers with anchored phase (silica gel, modified silica gel)
- Cheaper, faster flows compared to membrane disks, less risk of clogging

- SPEC microcolumns (Ansys)
  - body made of polypropylene, without frit
  - silica gel, NH_2, CN, PH, C2, C8, C18, cation exchange resin, annex
- ENVITM discs (Supleco, Inc.)
  - Diameter 47 and 90 mm
  - C8, C18



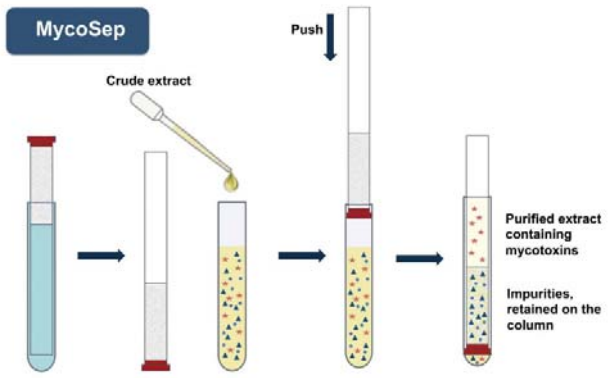
SPE membrane discs

- flexible PVC or PTFE network with chemically bonded stationary phase
- lower flow rates than rigid disks
- greater risk of clogging → pre-filtration

- EMPORETM disks (3M Corp. + Varian)
  - 10% PTFE + 90% silica gel phase
  - C8, C18, PS-DVB, anex, cation exchange resin
  - diameter: standard 25, 47, 90 mm
  - in boxes: reduced 4, 7, 11 mm

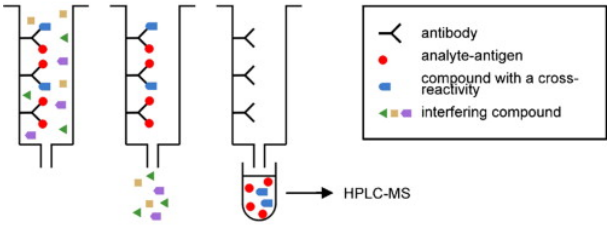
Mycosep

- Solid phase extraction (SPE) - "push-through" format used for mycotoxins (very fast, matrix components are captured on the sorbent)

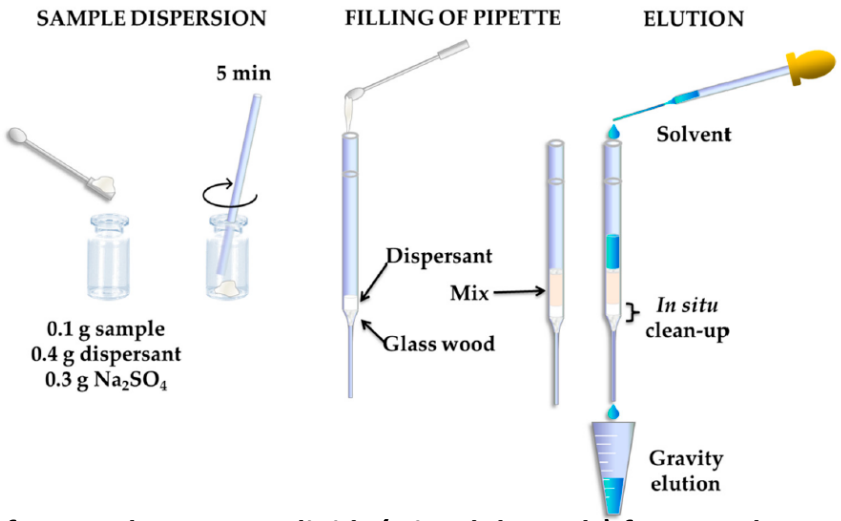


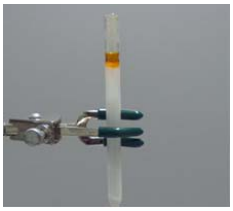
Immunoaffinity columns

- Same procedure as for normal SPE columns, the retention mechanism is based on a specific antibody-antigen interaction, i.e. specific antibodies are anchored to the sorbent column and target analytes function as antigens and are captured by antibodies and the matrix passes without retention. The analytes are then eluted from the column.
- Problem = so-called **cross-reactivity**
  - Substances similar to the target analyte can be captured by the antibody together with the target analyte
- Use: especially for the determination of mycotoxins, columns specific either directly for one of the mycotoxins or a group of similar mycotoxins.

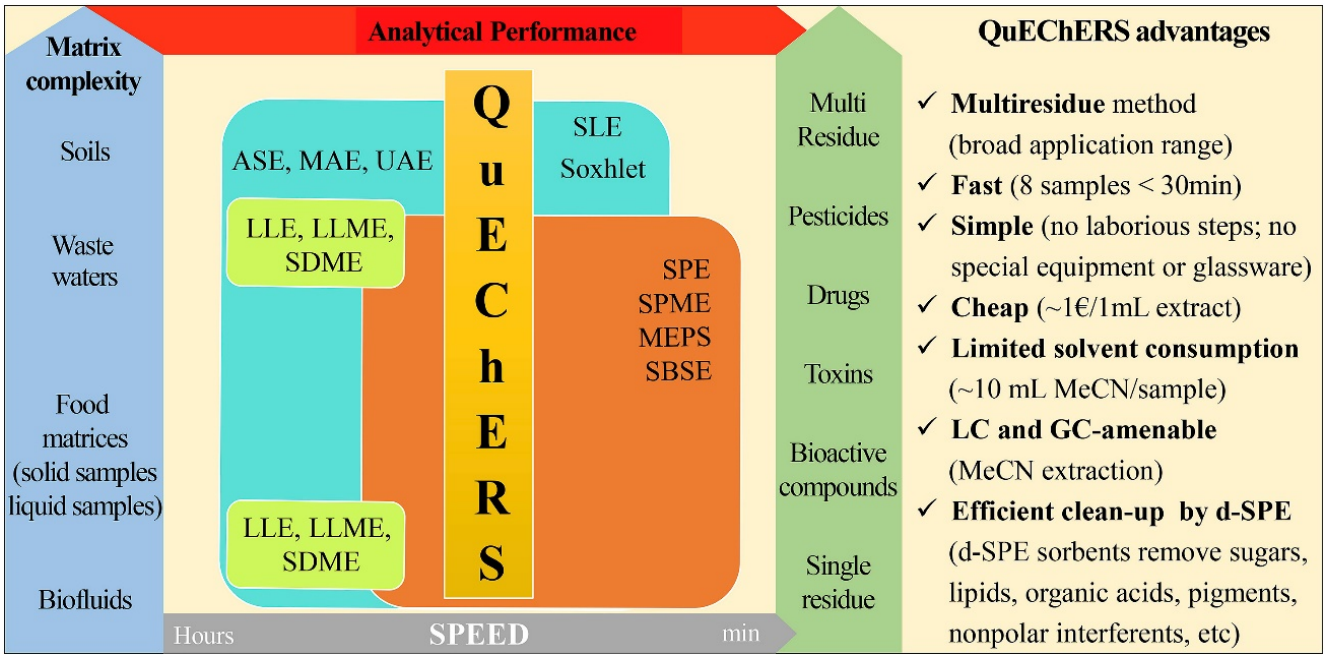


Pasteur pipettes filled with sorbent





Often used to remove lipids (triacylglycerols) from crude extract.



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QuEChERS

Classical Quechers for Fruits and Vegetables

Cryo-milling + water

Shake with Acetonitrile

Add $MgSO_4$ + NaCl

Centrifugation

Add PSA + $MgSO_4$ (DSPE)

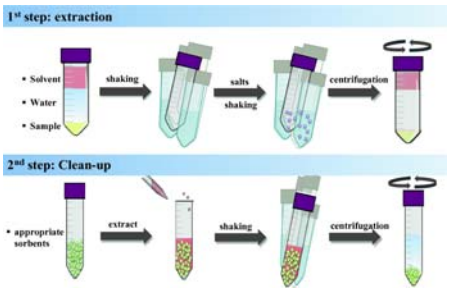
Centrifugation

MgSO4: to separate water from the organic solvent,

dSPE **primary secondary amine (PSA)**
to remove various polar organic acids, polar pigments, some sugars and fatty acids,

graphitised carbon black (GCB)
to remove sterols and pigments such as chlorophyll,

C18
to remove non-polar interfering substances like lipids.



Angelika Wilkowska, Marek Biziuk Food Chemistry 125 (2011) 803–812

Dispersion solid phase extraction (MSPD)

- grinding the sample with a suitable sorbent (C18)
- filling the mixture into the column (syringe body)
- (addition of another sorbent - e.g. Florisil)
- compression
- elution of analytes with solvent

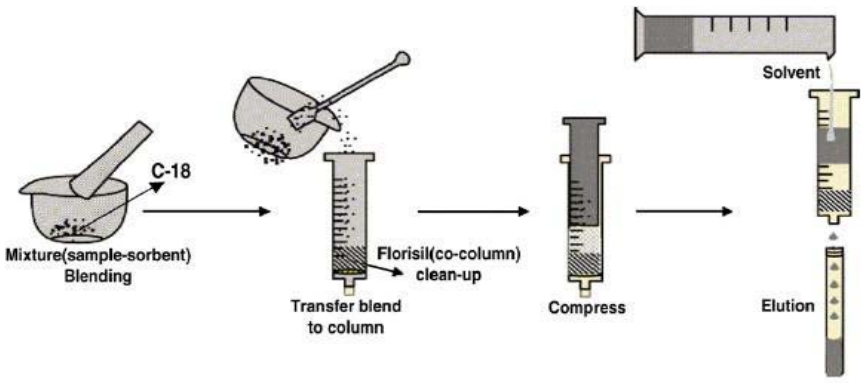
Advantages:

- isolation and purification in 1 step
- saving time and solvents
- operationally undemanding

Matrix Solid Phase Dispersion (MSPD)

For solid samples

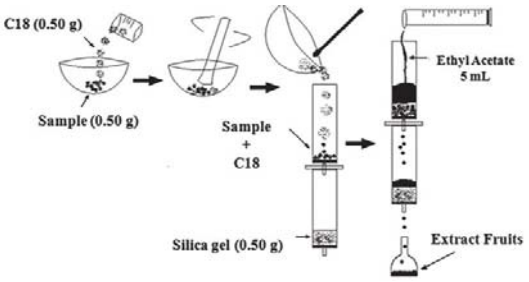
sample is mixed with a matrix (C_{18} bonded silica, Florisil, Na_2CO_3, Celite,…), washing and elution with a small volume of solvent.



S.A. Barker, A.R. Long, C.R. Short, J. Chromatogr. 475 (1989) 353.

MSPD

- Spreading the sample with sorbent (sample: C18 = 1: 4)
  - Damage to membranes by mechanical and hydrophobic forces (release of lipids, etc.)
  - The solid phase dissolves and disperses the components of the sample (non-polar components to C18, polar to -OH silica gel)
  - Large contact area
  - The matrix itself becomes a new sorption phase influencing the process (analytes elute in fractions not corresponding only to the system analyte - pure solid phase - selected solvent)



MSPD – advantages/limitations

Advantages:

Less solvent than liquid–liquid extraction Extraction in one step

Large scale of matrix able to use Relatively low cost per analysis

Simple equipment

Can be used under in situ conditions

Limitations:

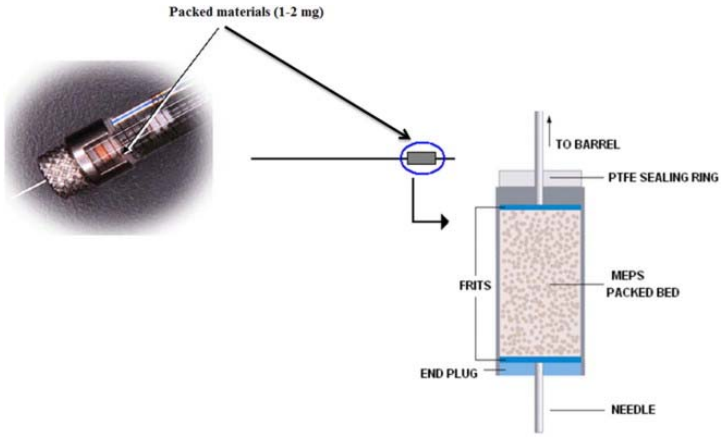
Restricted analytical range,

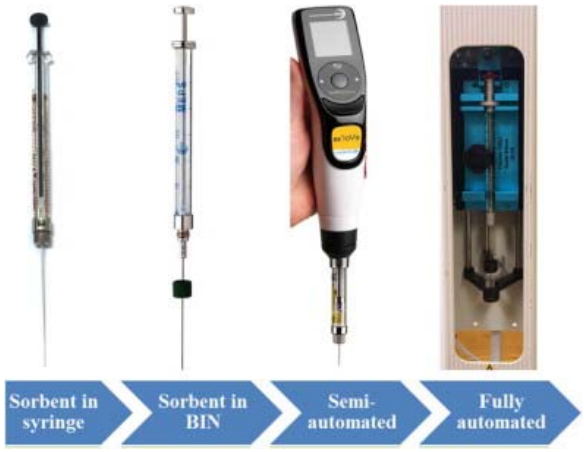
Not very suitable for dry samples or samples with high lipids content, relatively high adsorbent consumption

Micro Extraction by Packed Sorbent (MEPS)

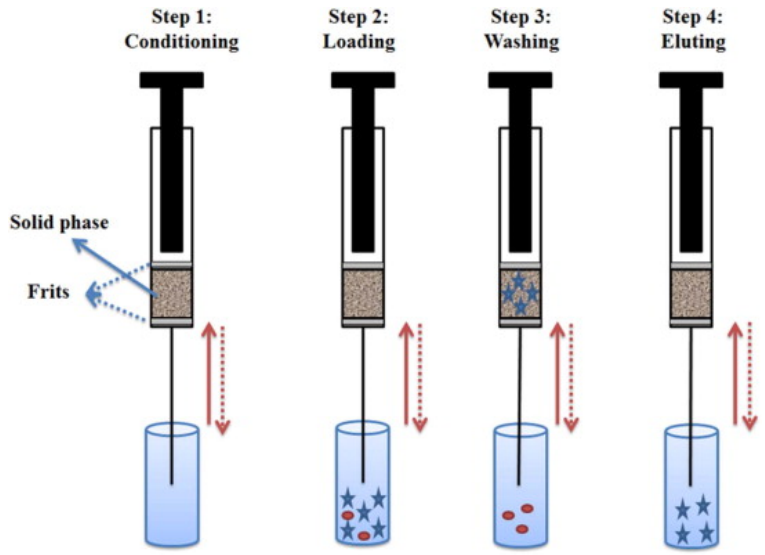
- MEPS performs the same function as SPE, but with some significant differences.
- MEPS works with much smaller sample amount (as small as 10 µL) than full scale SPE
- MEPS can be fully automated – the sample processing, extraction and injection steps are performed on-line using the same syringe
- MEPS is applicable to GC and LC
- Significantly reduces the volume of solvents and sample needed

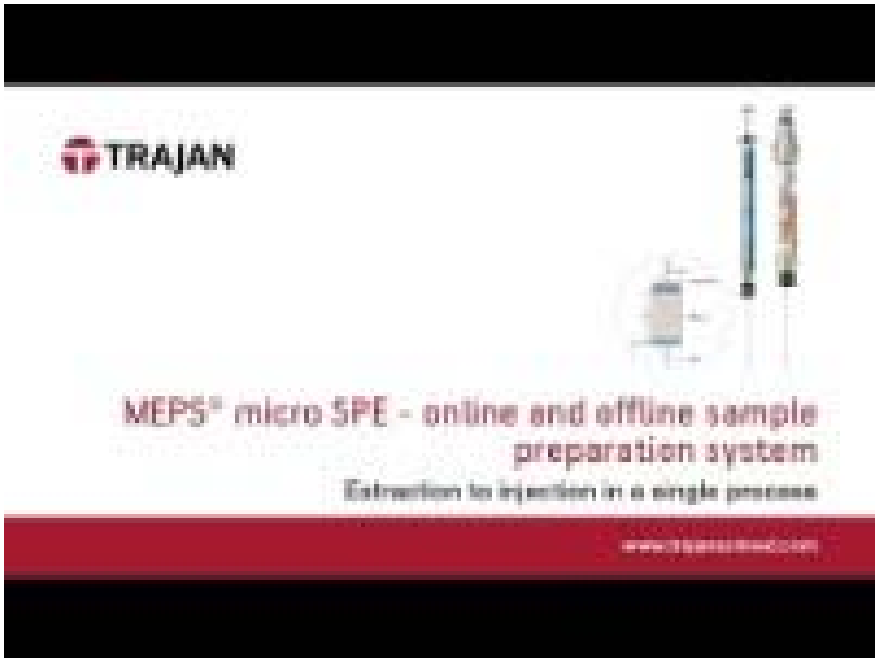
MEPS





MEPS


Step 1:
Conditioning
Step 2:
Loading
Step 3:
Washing
Step 4:
Eluting
Solid phase
Frits


TRAJAN
MEPS® micro SPE - online and offline sample preparation system

MEPS Phases

Phase	Particle size (µm)	Pore Size (A)
Silica	45	60
C2	45	60
C8	45	60
C8+SCX*	45	60
C18	45	60

Table 1. Some recent MEPS applications (2012–2017, not previously reviewed) [39,43] in different fields, and the performances obtained when applying this device.

Field	Analyte	MEPS	Matrix	Sample Volume	LOQ (LOD)	Reference
Biological	NSAIDs	C18	Plasma Urine	100 µL	0.10 µg/mL (0.03 µg/mL)	[44]
	Fluoroquinolones	C18	Sputum	200 µL	0.05 µg/mL (0.017 µg/mL)	[45]
	NSAIDs and Fluoroquinolones	C18	Plasma Urine	200 µL	0.10 µg/mL (0.03 µg/mL)	[46]
	Imidazoles and Triazoles	C18	Plasma Urine	200 µL	0.02 µg/mL (0.007 µg/mL)	[47]
	New psychoactive substances	mixed-mode C8/SCX	Oral fluid	300 µL	0.5 ng/mL (n.r.)	[48]
	Trans,trans-muconic acid	MIP-MEPS	Urine	100 µL	0.05 µg/mL (0.015 µg/mL)	[49]
	Statins	C18	Plasma	100 µL	10–20 ng/mL (n.r.)	[50]
	Drugs of abuse	C8/SCX	Plasma	300 µL	0.01 µg/mL (0.005 µg/mL)	[51]
	Cocaine and metabolites	Mixed mode M1	Urine	200 µL	25 ng/mL (n.r.)	[52]
Food and Food Supplements	Melatonin and other antioxidants	C8	Foodstuffs	100 µL	0.05 ng/mL (0.02 ng/mL)	[53]
Environmental	Brominated diphenyl ethers	C18	Sewage sludge	15 mL reduced to 1 mL	n.r. (3 pg/mL)	[54]
	Chlorophenols	C18	Soil samples	1 mL	0.353 µg/kg (0.118 µg/kg)	[55]
	Sulfonamides	C8	Wastewater	n.r.	5 ng/mL (n.r.)	[56]
	Phtalate esters	graphene and CNT/CNF–G nanostructures	Water	10 mL reduced to dry	0.02 ng/mL (0.004 ng/mL)	[57]
	Parabens	graphene supported on aminopropyl silica	Water	1 mL	0.2 µg/mL (n.r.)	[58]

n.r. not reported.