Intro to Sample Prep: SPE and SPME

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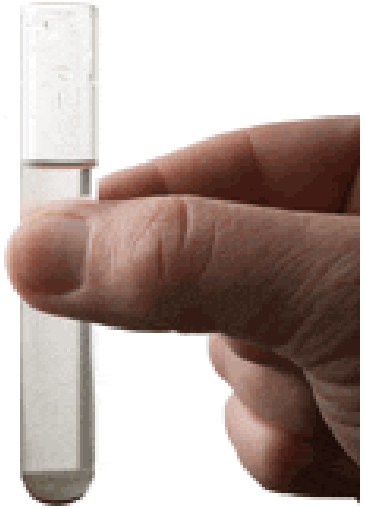
Why Sample Prep?

May require a unique sample prep solution…



..but the same technology workflow for analysis

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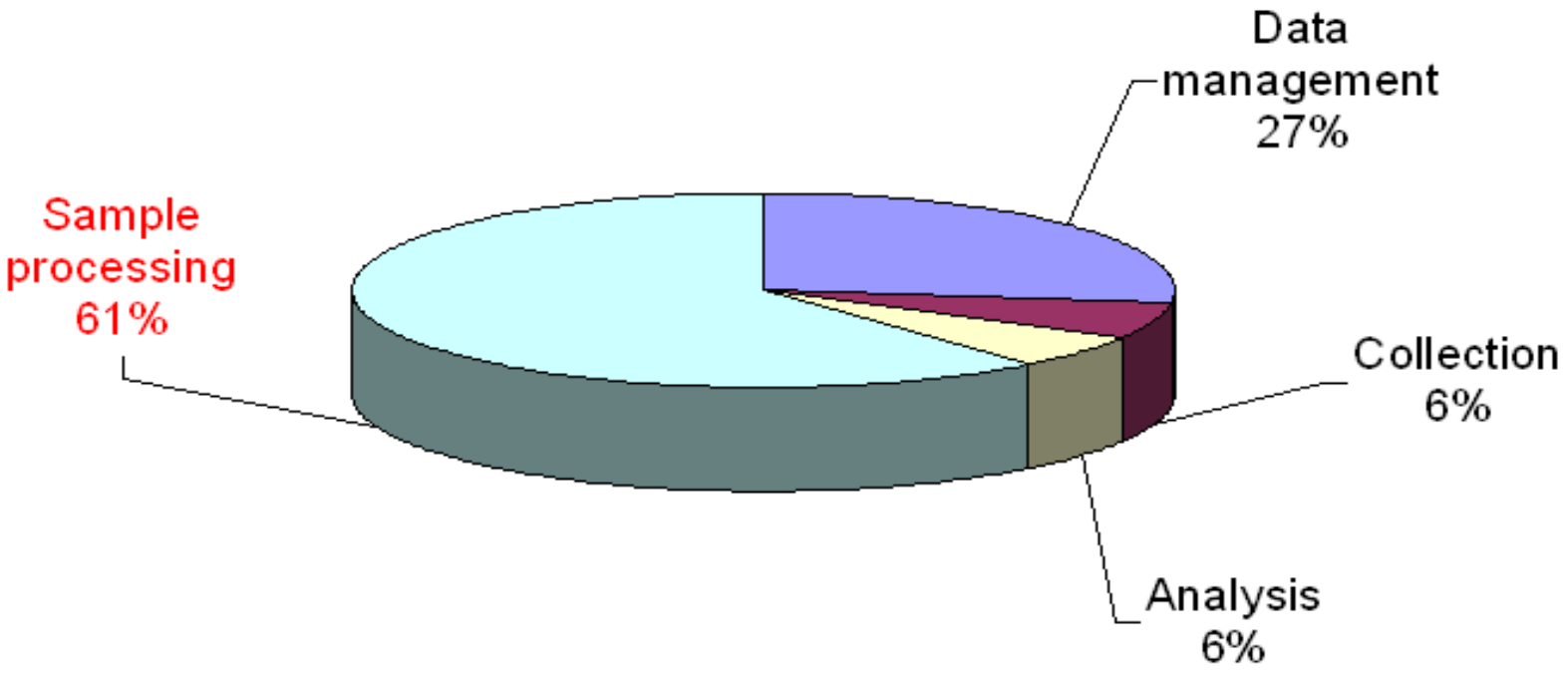
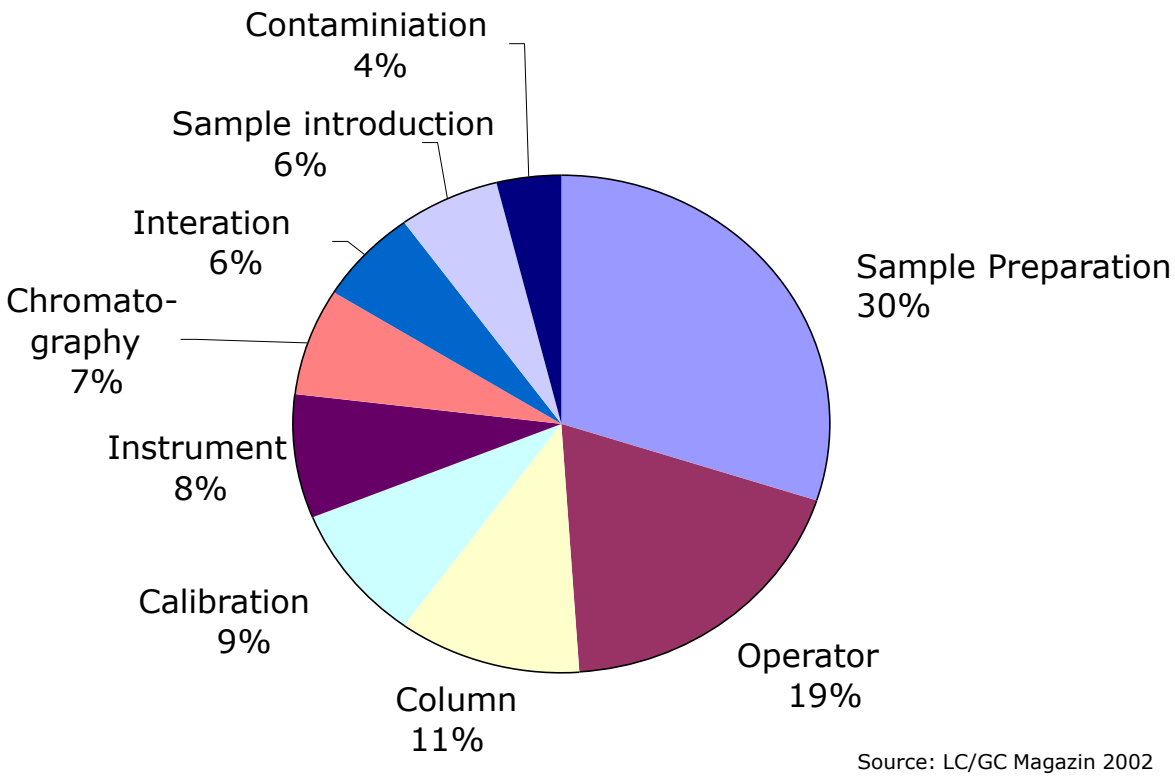
Time Spent on the Analytical Process



Sample prep
Sources of Chromatographic Errors



Source: LC/GC Magazin 2002

SPE

Solid Phase Extraction

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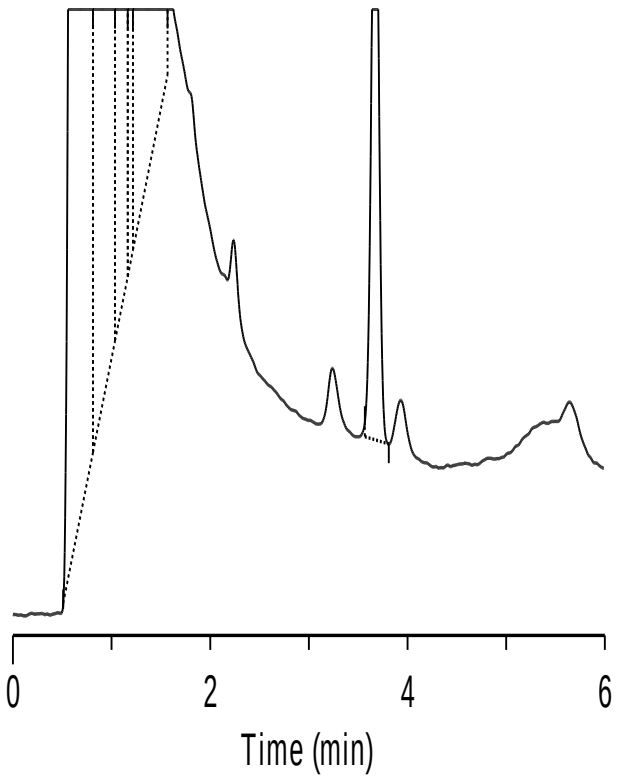
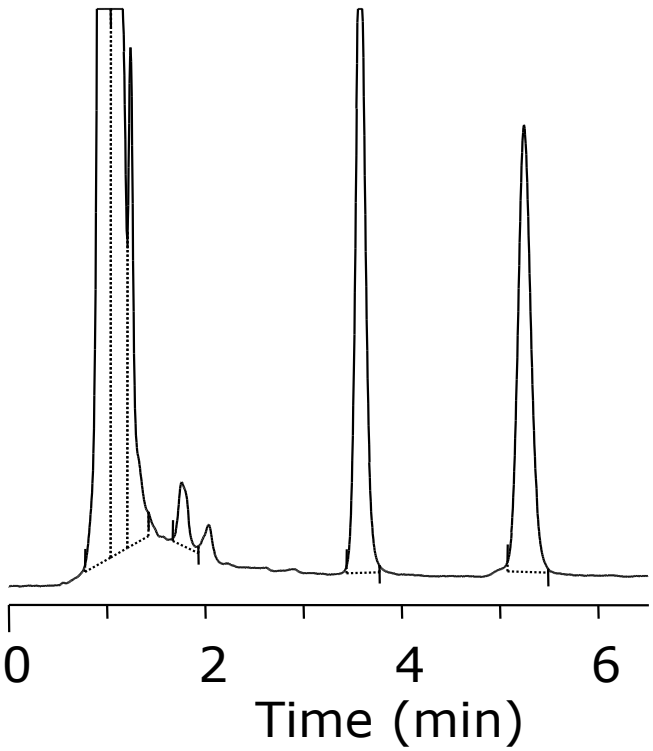
Real World & Real Samples

The Importance of Sample Preparation

Urine Sample without SPE



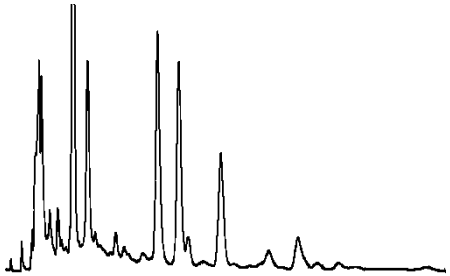
Urine Sample with SPE



Why is sample preparation required?

Collected Sample

GC, HPLC, or LC-MS/MS Analysis



Current Sample = Unsuitable for further analysis!!!… Why?

Too dirty- contains other sample matrix components that interfere with the analysis

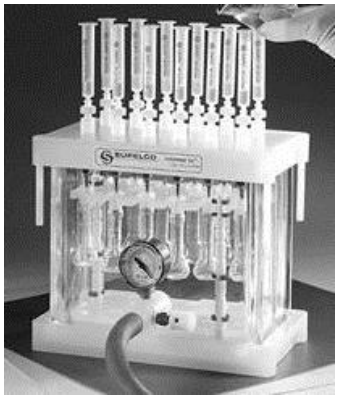
Too dilute- analyte(s) not concentrated enough for quantitative detection

Present **sample matrix not compatible** with or harmful to the chromatographic column/system

SPE Formats

Sorbent particles held securely in place
to withstand the force of the liquid flow.

Tubes



96-well plates

Disks

Loose/bulk sorbent (QuEChERS)

Online SPE

On the Inside: SPE Sorbents (Packing Materials)

The sorbent is the component of the tube responsible for the extraction. Most SPE sorbents are also used in HPLC applications, although with large particles in SPE. Some of the most common are:

Silica-based

- Reversed phase (C18, C8, cyano, phenyl)
- Normal phase (silica, diol, NH_2)
- Ion exchange (SAX, WCX, SCX)

Carbon-based

Polymer-based

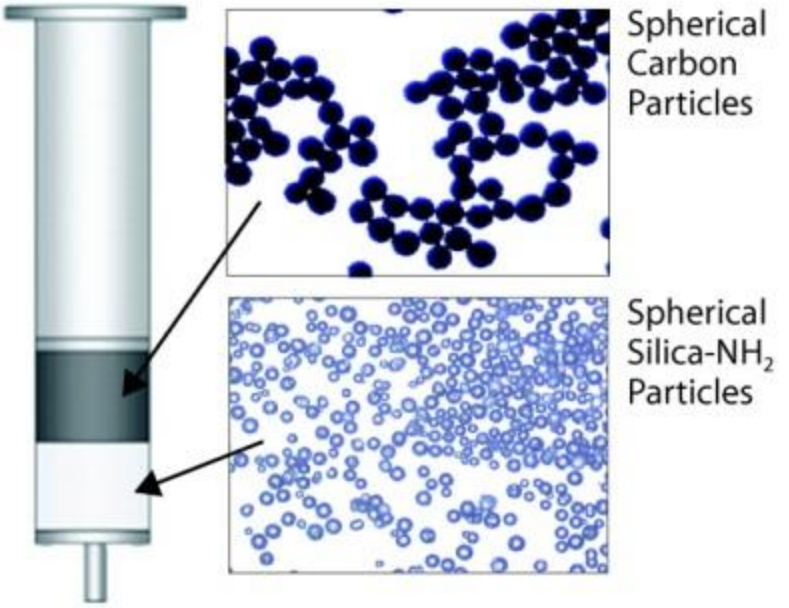
- Various compositions
- Different functionalities

Others

- Florisil® (magnesium silicate)
- Alumina

Mixed-bed

- Combinations of nearly any of the above are possible in sequential layers



Supel™ Sphere dual-layer

SPE Strategies

There are 2 different elution strategies in SPE. Which one to choose depends on the goal of the extraction.

1. Bind-Elute Strategy

- Most common
- Bind: Analytes bind to tube, unwanted matrix components are washed off
- Elute: Eluant changed to remove analytes from tube
  - Different eluents can be used to fractionate the analytes
- Analytes are concentrated via evaporation prior to HPLC or GC analysis
- Sorbent types employing this: DSC-C18, Supel™-Select HLB, SupelMIP®, ENVI™-Carb Plus, PS/DVB, DSC-MCAX, ENVI™-Chrom P

2. Interference Removal Strategy

- Bind all unwanted matrix components and allow analytes to pass through during the sample loading stage
- Like chemical filtration
- Sorbent types employing this: HybridSPE®, QuEChERS, PSA, ENVI™-Carb, Dual Layer

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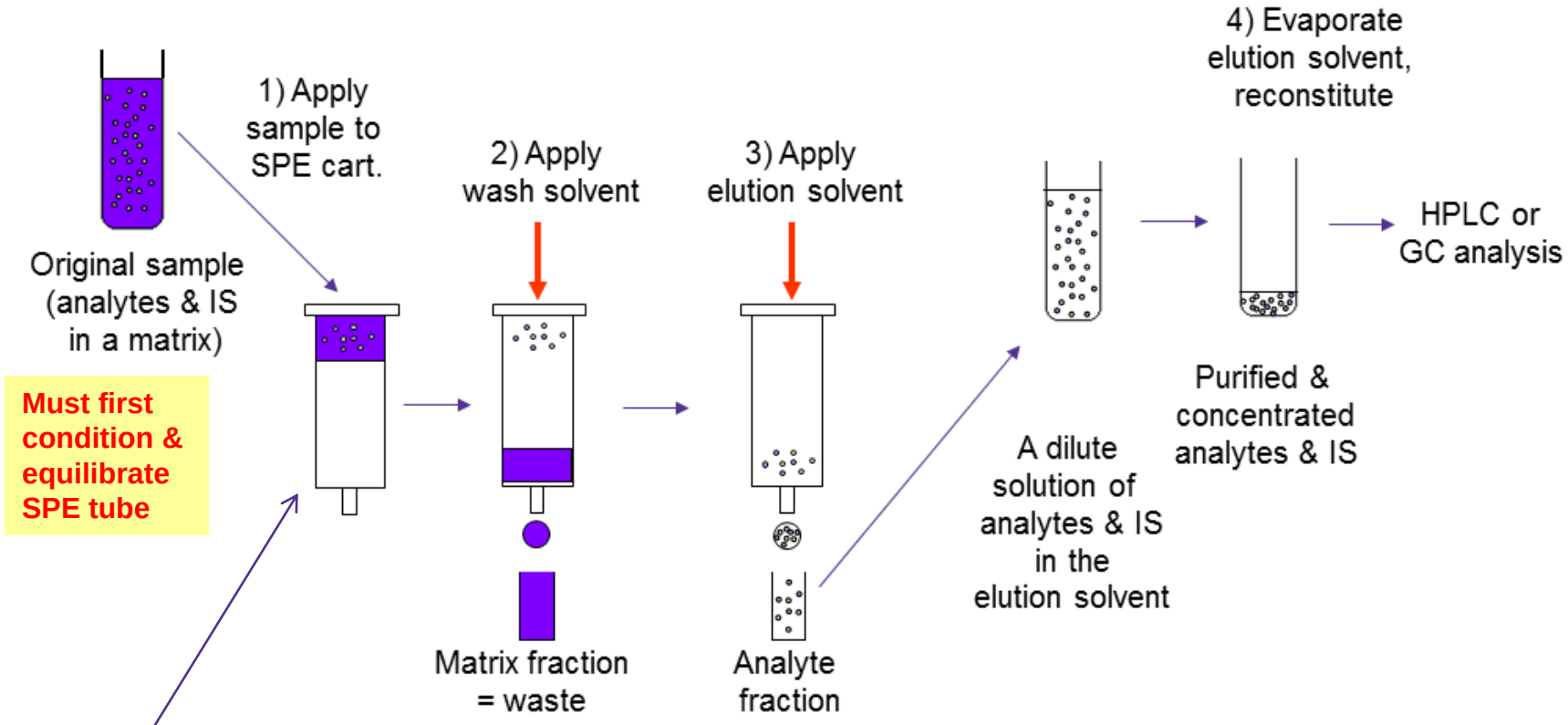
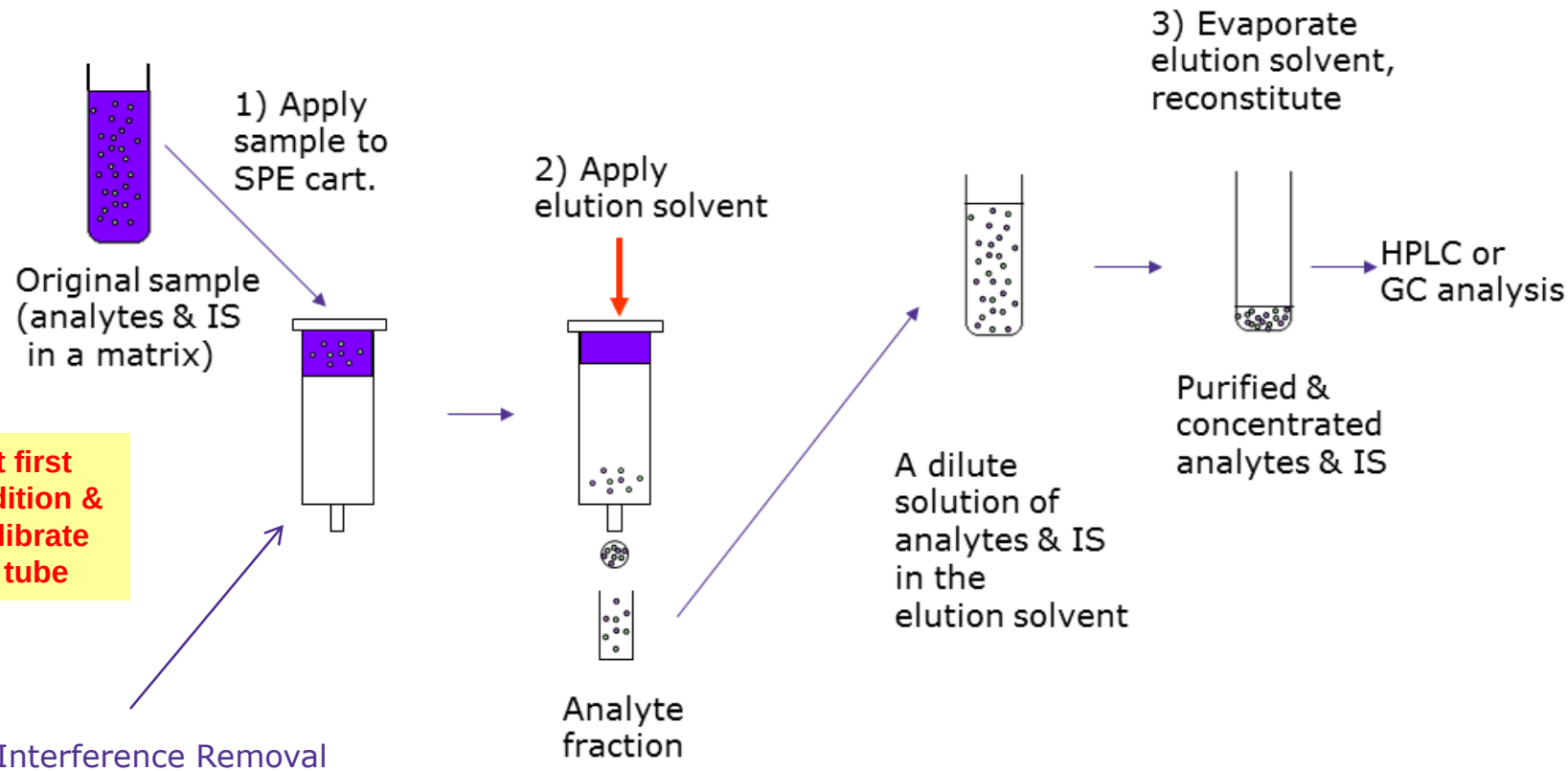
Bind-Elute Strategy



Bind-Elute sorbent types: DSC-C18, Supel™ -Select HLB, SupelMIP®, ENVI™-Carb Plus, PS/DVB, DSC-MCAX, ENVI™-Chrom P

Interference Removal Strategy
"Chemical Filtration"

Sample with Internal Standard in Matrix → Matrix adsorbed → Analytes & IS pass



Well-Established SPE Product Lines

Discovery®

Pharmaceutical focus

Tube and 96-well plates

Supelclean™ ENVI™

Environmental focus

ENVI™-Carb is a key product

Supel™-Select

Polymeric, “Universal SPE”

ENVI™-DSK™ disks

Porous glass fiber membranes embedded with sorbent particles

Sample Prep Star Products

Overcoated SPME

- Physically robust fiber for direct immersion that is less prone to chemical fouling.

Supelclean™ Ultra

- Dual layer cartridge for the cleanup of difficult matrices such as dry commodities (tea, spices, coffee, etc.)

Supel™MIP

- Molecularly imprinted polymers
- Highly selective for analytes in difficult matrices

Supel™ QuE

- QuEChERS tubes and supplies
- Pesticide Residue, PAH, PCB, PDBE analysis

Supel™ Tox

- Removes interferences associated with mycotoxin analysis

Supelclean™ EZ-POP NP

- Simple, effective extraction of lipophilic persistent organic pollutants (POPs) from oily samples

Supel™-Select Polymeric SPE

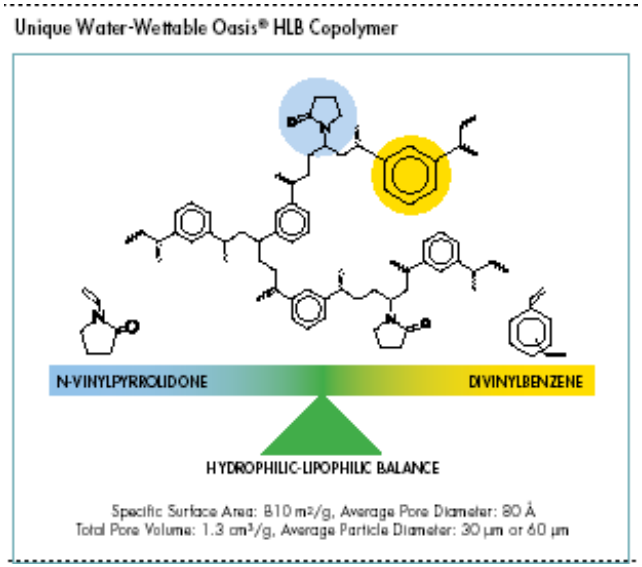
Supel-Select: What is Hydrophilic Polymer SPE?

Polymer chromatographic media designed for SPE

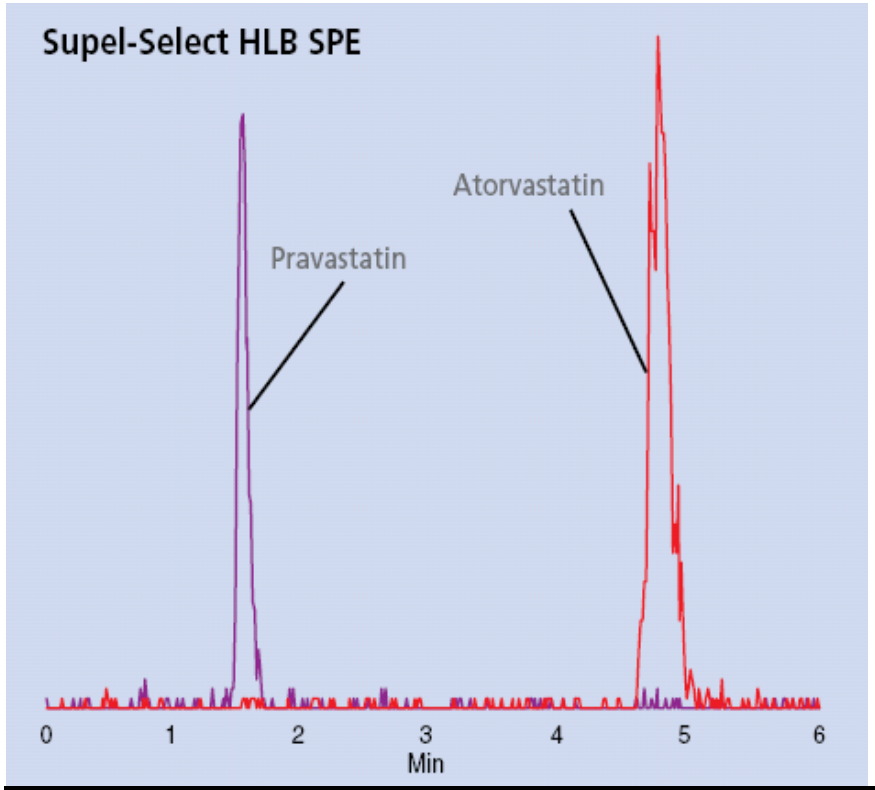
Comprises of a hydrophilic component and a hydrophobic component:

- Hydrophilic component examples:
  - N-vinyl pyrilidone, methacrylate, hydroxyl, vinylamidizol
- Hydrophobic component examples:
  - Polystyrene, divinyl benzene

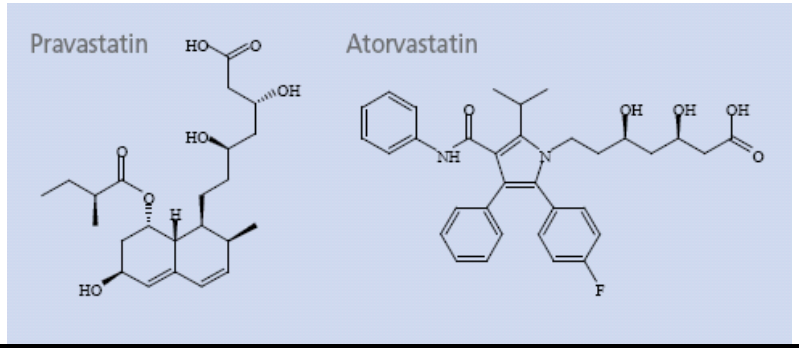
HLB Phase Chemistry:	Hydrophilic modified styrene polymer
SAX Phase Chemistry	Quaternary amine functionalized hydrophilic modified styrene polymer
SCX Phase Chemistry	Sulfonic acid functionalized hydrophilic modified styrene polymer
pH Compatibility:	0-14
Particle Size:	55-60 µm
MS Suitable:	Yes
Surface Area:	400-410 m^2/g
Pore Volume:	0.88 mL/g
Pore Size:	87 Å



Supel-Select HLB: Statins from Rat Plasma



Total Ion Chromatogram (MRM, 4 pairs: 557.3/397.2) Rat Plasma spiked with 5 ng/mL Statins



	Absolute Recovery ± RSD (n=3)			
	5 ng/mL spike		100 ng/mL spike	
	Pravastatin	Atorvastatin	Pravastatin	Atorvastatin
Supel-Select HLB	84 ± 8%	92 ± 5%	103 ± 4.2%	89 ± 3.9%
Competitor W	83 ± 17%	92 ± 2%	104 ± 2.2%	87 ± 1.1%
Competitor P	77 ± 5%	93 ± 2%	102 ± 3.0%	91 ± 1.3%

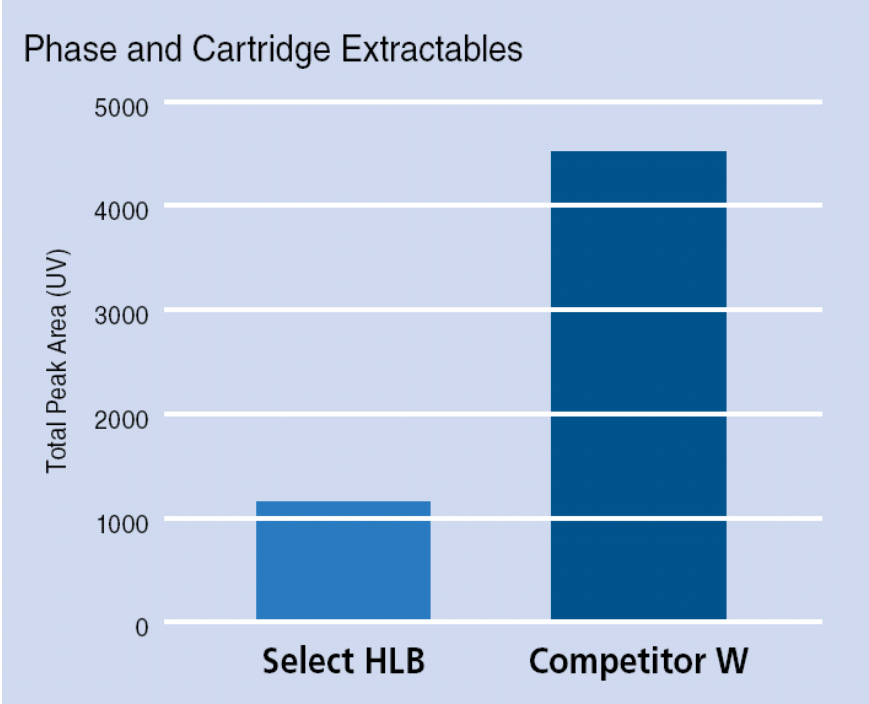
Supel-Select HLB: Minimum Extractables

Assays today require greater sensitivity

SPE phase chemistry and hardware should impart minimum extractables

Each lot is tested for:

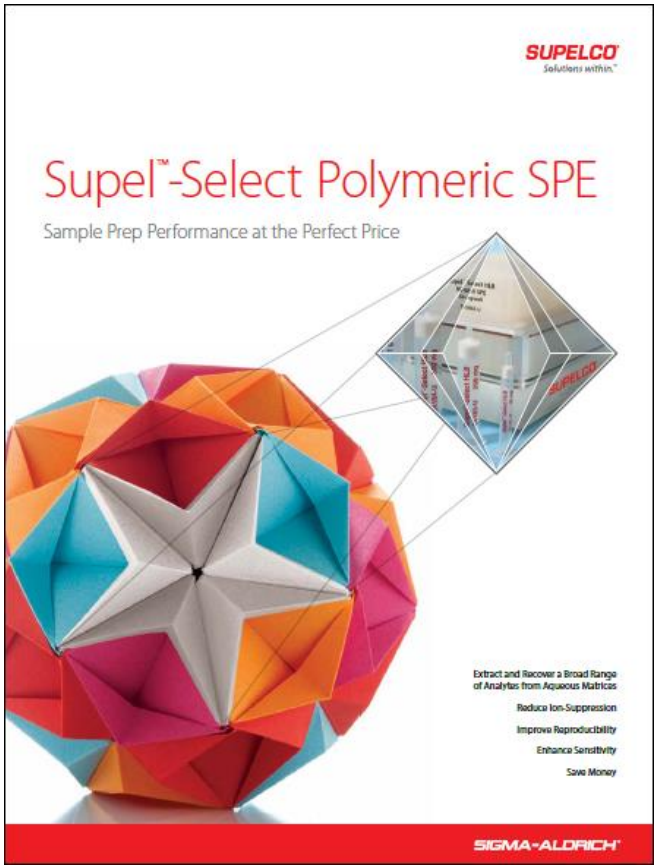
- Recovery
- LC-UV & LC-MS cleanliness
- Particle size
- Density
- Pore size
- Pore volume



Supel-Select Polymeric SPE

Why are they so popular in SPE?

- "Water – Wettable" – do not dry out => highly reproducible
- Amenable to generic methodology
- Often referred to as a universal SPE phase
- Can retain an extremely broad range of compounds (polar to non-polar; acidic – basic)
- Retained compounds easily eluted/ desorbed with MeOH or similar solvent
- Reduces ion suppression in LC/MS
- Low UV and MS extractables
- 1000s of references using this technology



KZQ

www.sigmaaldrich.com/supel-select

Current Supelclean™ Ultra 2400 Cartridge

- Cleanup difficult matrices prior to pesticide residue analysis by GC/MS/MS and LC/MS/MS
- Dry commodities (tea, spices, coffee, etc.), typically highly concentrated and with higher background than fresh samples
  - Pigments and oils
  - Not sufficiently cleaned by QuEChERS

- Dual layer SPE cartridge (1 mL and 3 mL) containing PSA, C18, specialty carbon, and Z-Sep
- Specialized Carbon
  - reduces pigmentation and allows for recovery of planar pesticides without toluene
- Z-Sep sorbent
  - Removes oils and some pigments, as was indicated in the cleanup of turmeric extracts for both GC and HPLC analysis

sigma-aldrich.com/supelcleanultra

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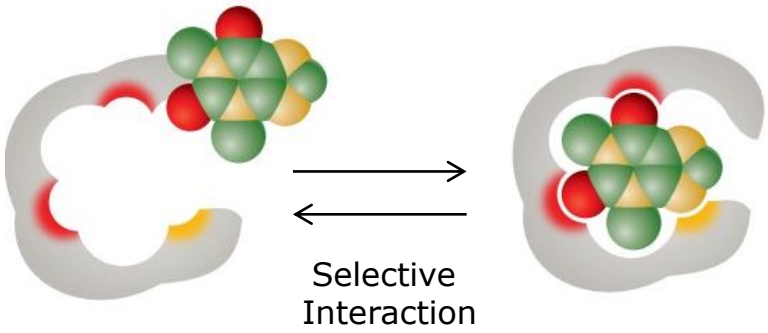
SupelMIP SPE – Molecularly Imprinted Polymer SPE

MIPs (molecularly imprinted polymers) are SPE products designed for the highly selective extraction of trace analytes from complex matrices

SupelMIP Phases and Methods Available for:

- PAHs in edible oil
- Non-steroidal anti-inflammatory drugs (NSAIDs) in wastewater and other sample matrices
- Nitroimidazoles in milk, eggs, and other food matrices
- Fluoroquinolones in bovine kidney, honey, and milk
- Chloramphenicol in milk, plasma, honey, urine and shrimp/prawns
- NNAL in urine
- TSNAs in urine and tobacco
- ß-agonists in tissue, urine and wastewater
- Clenbuterol in urine
- Riboflavin in milk
- Patulin in fruit matrices
- Aminoglycosides in animal tissue, cell culture, and honey
- Bisphenol A from broth or milk-based matrices



- Superior selectivity => reduced ion-suppression => achieve lower detection limits
- Robust & rapid methodology => Save time, money, & headache
- No method development req'd

sigma-aldrich.com/supelmip

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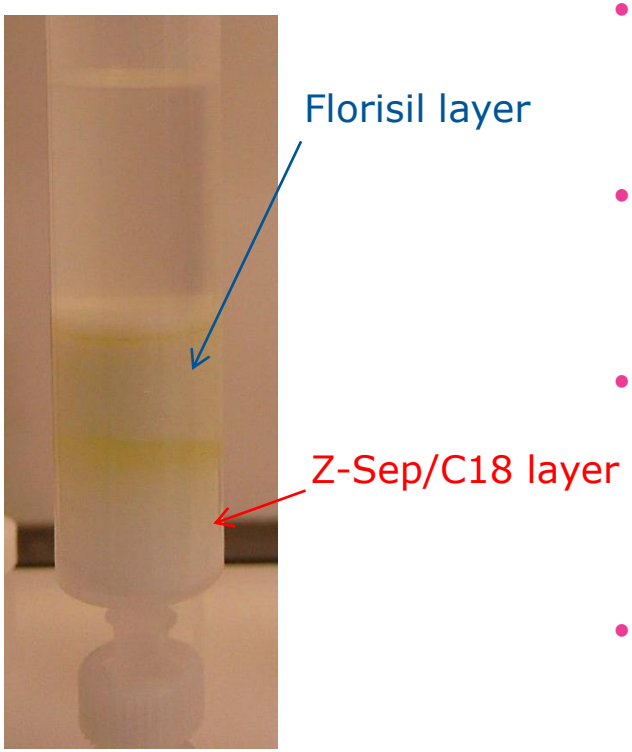
Supelclean™ EZ-POP NP:
For the extraction of non-polar compounds from edible oils



- Preferentially **retains fatty matrix interferences**
- **Non-polar compounds** are **washed through** and then analyzed
- **Compared to** other SPE methods:
  - Produces a **cleaner extract**
  - **Easier** and **more versatile methodology**
- The **final extracts** are in **acetonitrile**
  - Compatible with both **GC** and **HPLC**.

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SPME

Solid Phase Microextraction

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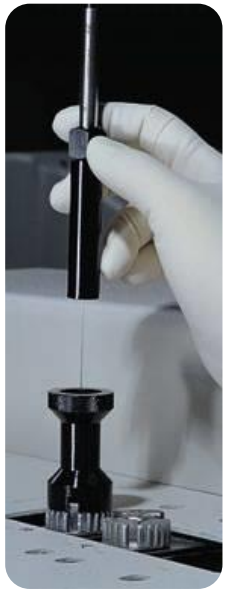
Solid Phase Microextraction (SPME)



- Economical enrichment technique mainly for trace analysis
  - Semivolatiles & volatile (GC)
- Coated fused silica or metal fibers (adsorbent/particle & absorbent/film coatings)
- Initially for GC analysis, now extended to LC

Features:

- Very limited or no use of solvents
- All types of samples & matrixes
- Direct immersion or headspace
- Designs for manual, auto samplers and robots

Benefits:

- One-step extraction that is easy to automate
- Quantitative and reproducible Extractions
- Portable (field use) and reusable

SigmaAldrich.com/spme

Official Methods / Applications applying SPME Europe

ISO 27108 (former DIN 38407-34/Germany)

- Determination of selected plant treatment agents and biocide products - Method using solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) – (Based on DIN 38407-F 34 from Germany)

ISO 17943 (former DIN 38407-41/Germany)

- German standard methods for the examination of water, waste water and sludge - Jointly determinable substances (group F) - Part 41: Determination of selected easily volatile organic compounds in water - Method using gas chromatography (GC-MS) after solid-phase micro extraction (SPME) (F 41)

OENORM A 1117, 2004-05-01

- Determination of volatile compounds in cellulose-based materials by Solid Phase Micro Extraction (SPME)

UNICIM 2237 / 2009 - Italian Method on SPME for air sampling

- Determinazione Delle Aldeidi Aerodisperse – Methodo per microestrazione su fase solida (SPME) ed analisi mediante gascromatografia accoppiata a spettrometria di massa (GC-MS)
- Determination of air borne aldehydes by SPME/GC-MS using derivatisation on fiber with PFBHA

Other official methods for SPME
US Methods

ASTM D 6438, 2005

- Standard Test Method for Acetone, Methyl Acetate, and Parachlorobenzotrifluoride Content of Paints, and Coatings by Solid Phase Microextraction-Gas Chromatography

ASTM D 6520, 2000

- Standard Practice for the Solid Phase Micro Extraction (SPME) of Water and its Headspace for the Analysis of Volatile and Semi-Volatile Organic Compounds

ASTM D 6889, 2003

- Standard Practice for Fast Screening for Volatile Organic Compounds in Water Using Solid Phase Microextraction (SPME)

ASTM E 2154, 2001

- Standard Practice for Separation and Concentration of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Solid Phase Microextraction (SPME)

EPA Method 8272 (Dec 2007)

- Parent and Alkyl Polycyclic Aromatics in Sediment Pore Water by SPME GC/MS

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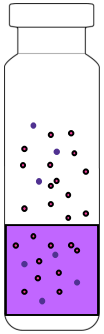
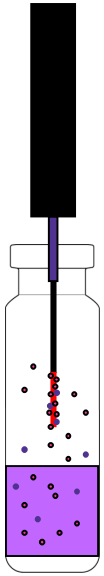
How SPME is used

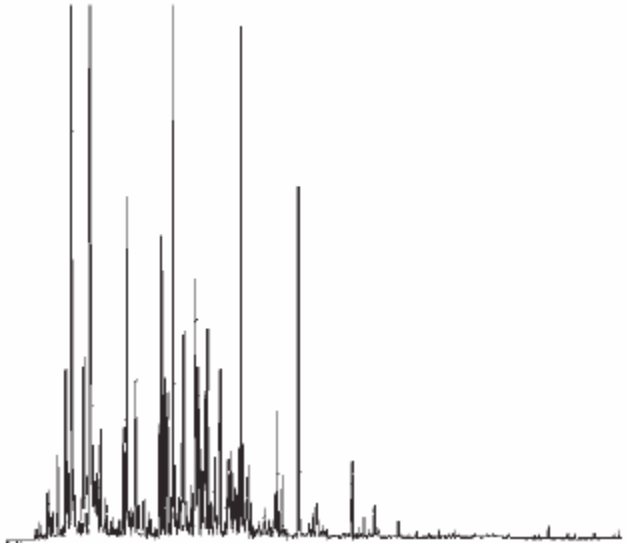
1 *Analyst has a **sample** they want to determine the content of*



If sample prep is required → **2** *They **extract** the compounds from the sample onto the SPME fiber*



3 *They **desorb** the fiber into the GC instrument which tells them what was in the sample*



GC chromatogram showing all the compounds that were extracted from the sample by the SPME fiber

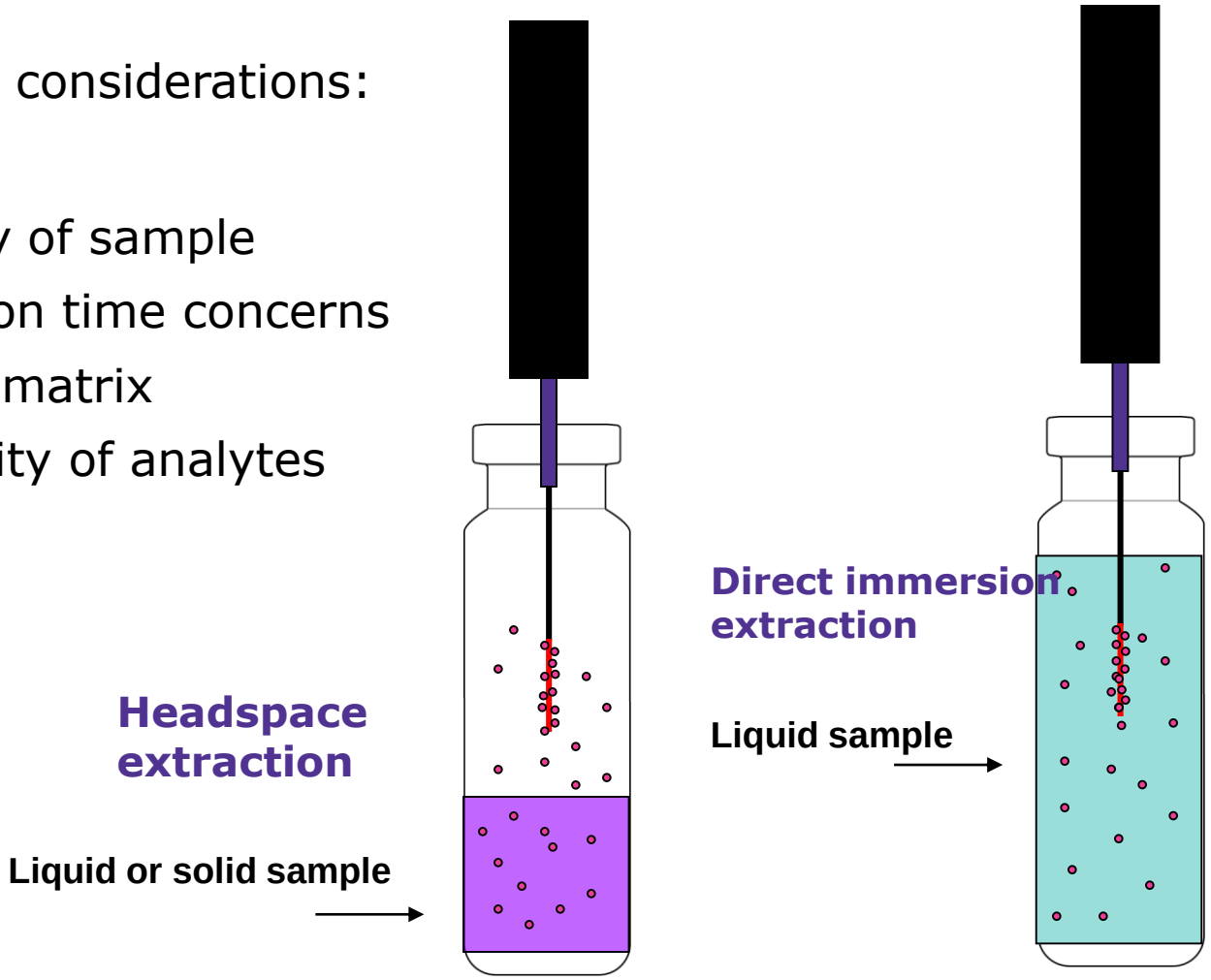
The SPME Concept

After extraction typically direct injection into GC injector port for thermal desorption

Sampling Technique: Headspace vs. Direct Immersion

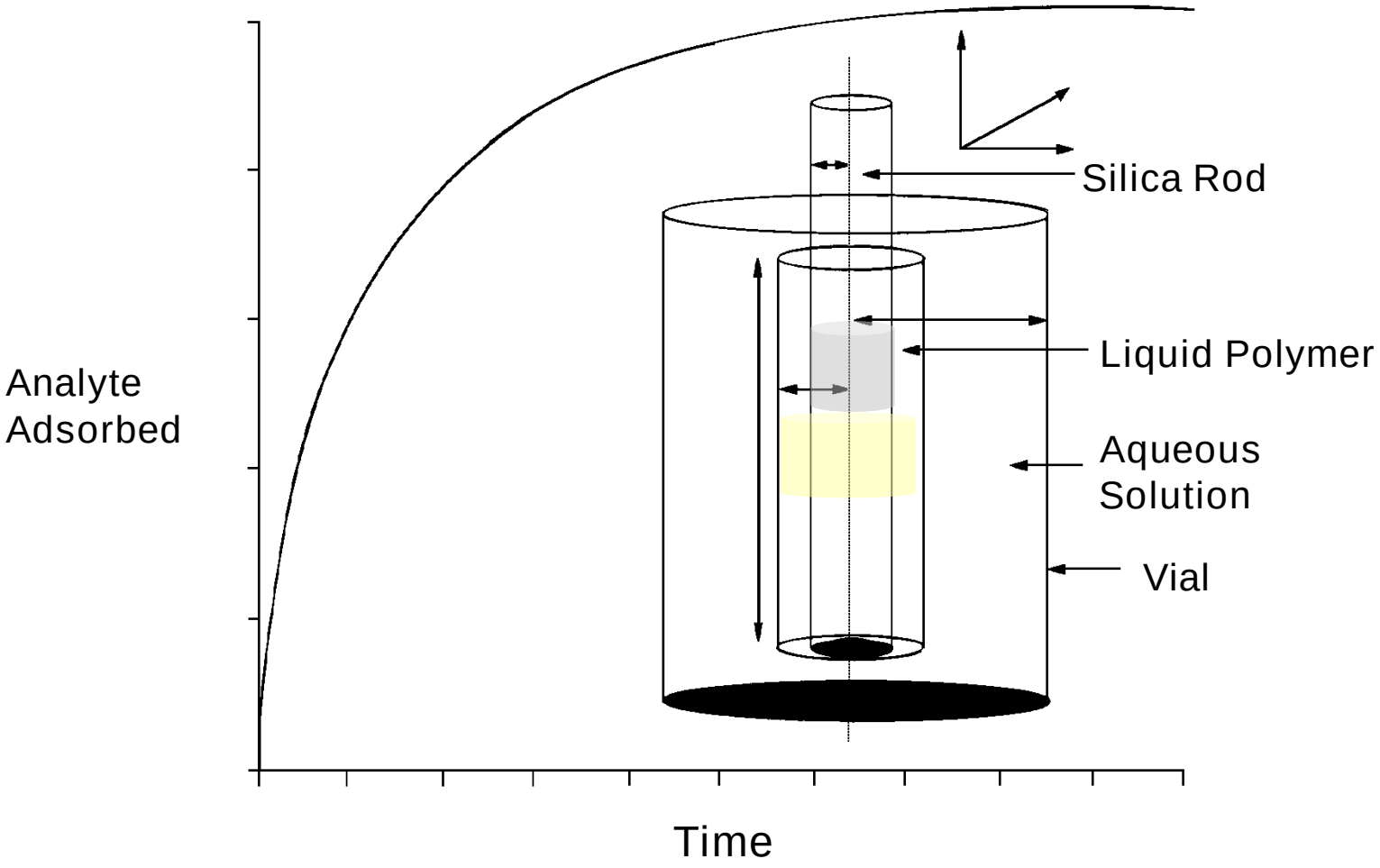
Analytical considerations:

- Volatility of sample
- Extraction time concerns
- Sample matrix
- Selectivity of analytes



Adsorption Mechanism for SPME

SPME is quantitative!!!

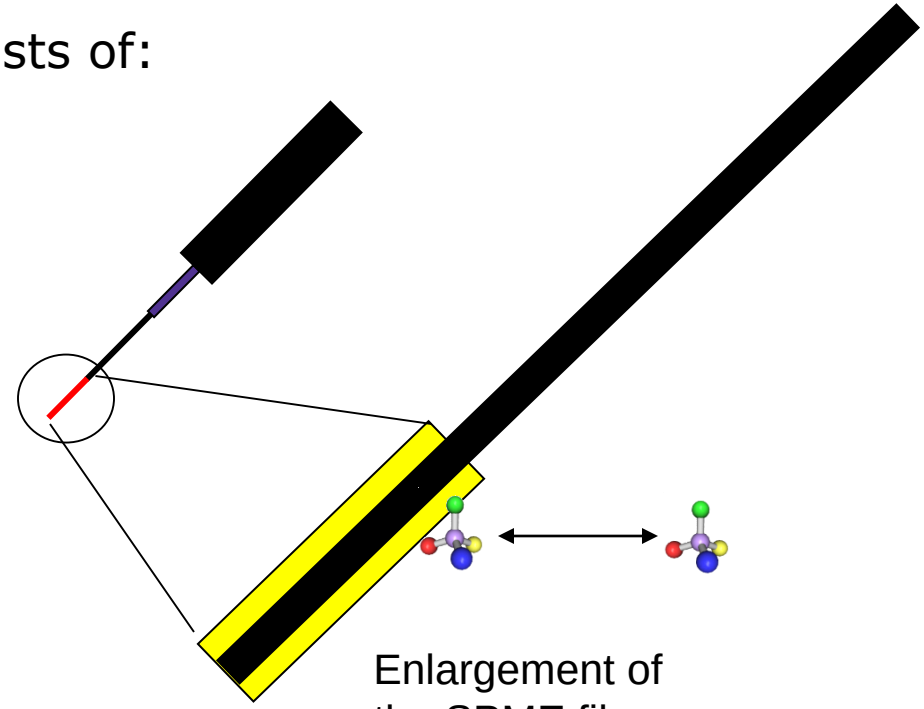


SPME Fiber Coating: The Business End

An equilibrium is set up between analytes dissolved in the sample (solution or gas phase) and in the liquid coating on the fiber.

The fiber coating consists of:

- GC-type phases
- Particles



Enlargement of the SPME fiber coating

Types of SPME Fiber Coatings

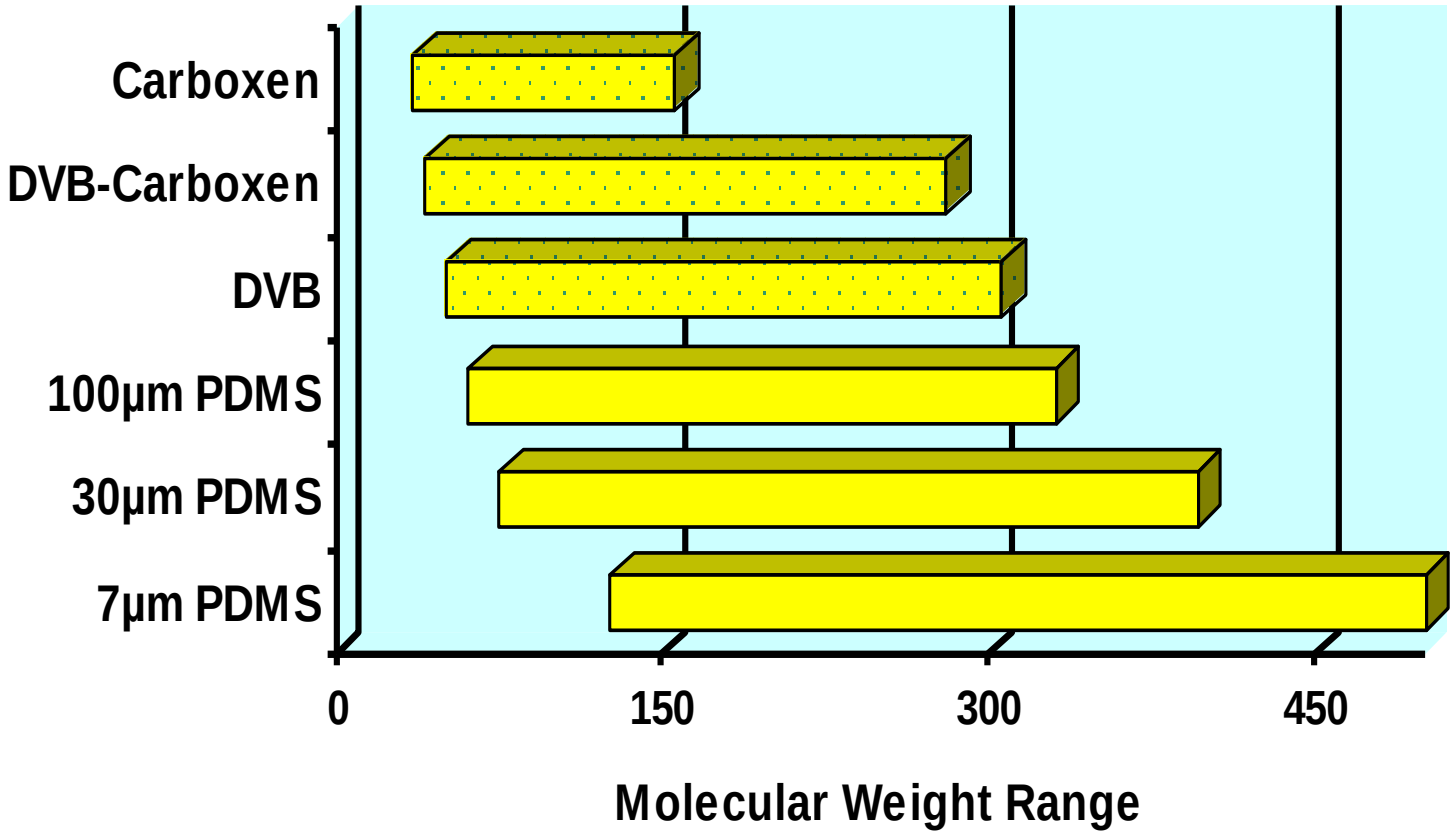
Films – Absorption:

Coating	Type	Polarity
7 µm Polydimethylsiloxane (PDMS)	Absorbent	Nonpolar
30 µm PDMS	Absorbent	Nonpolar
100 µm PDMS	Absorbent	Nonpolar
85 µm Polyacrylate (PA)	Absorbent	Polar
60 µm PEG (Carbowax)	Absorbent	Polar

Particles – Adsorption:

Coating	Type	Polarity
85 µm Carboxen-PDMS	Adsorbent	Bipolar
65 µm PDMS-DVB	Adsorbent	Bipolar
55 µm/30 µm DVB/Carboxen-PDMS	Adsorbent	Bipolar

Molecular Weight Range for SPME Fibers



Effects of Salt and pH

- Salt usually increases analyte uptake
  - Use 25-30% NaCl to salt-out samples
  - Not necessary for large non-polar analytes, such as PAHs and large hydrocarbons, and may reduce recovery

- Lower pH to extract acidic compounds
- Raise pH to extract basic compounds
- Beware of stability of analytes at different pH levels

Thank You For Your Kind Attention!