Intro to Sample Prep: SPE and SPME

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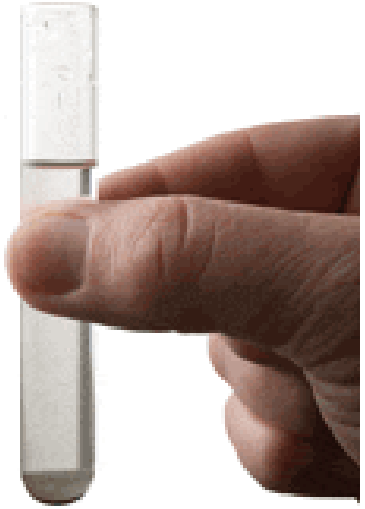
Why Sample Prep?

May require a unique sample prep solution…



..but the same technology workflow for analysis

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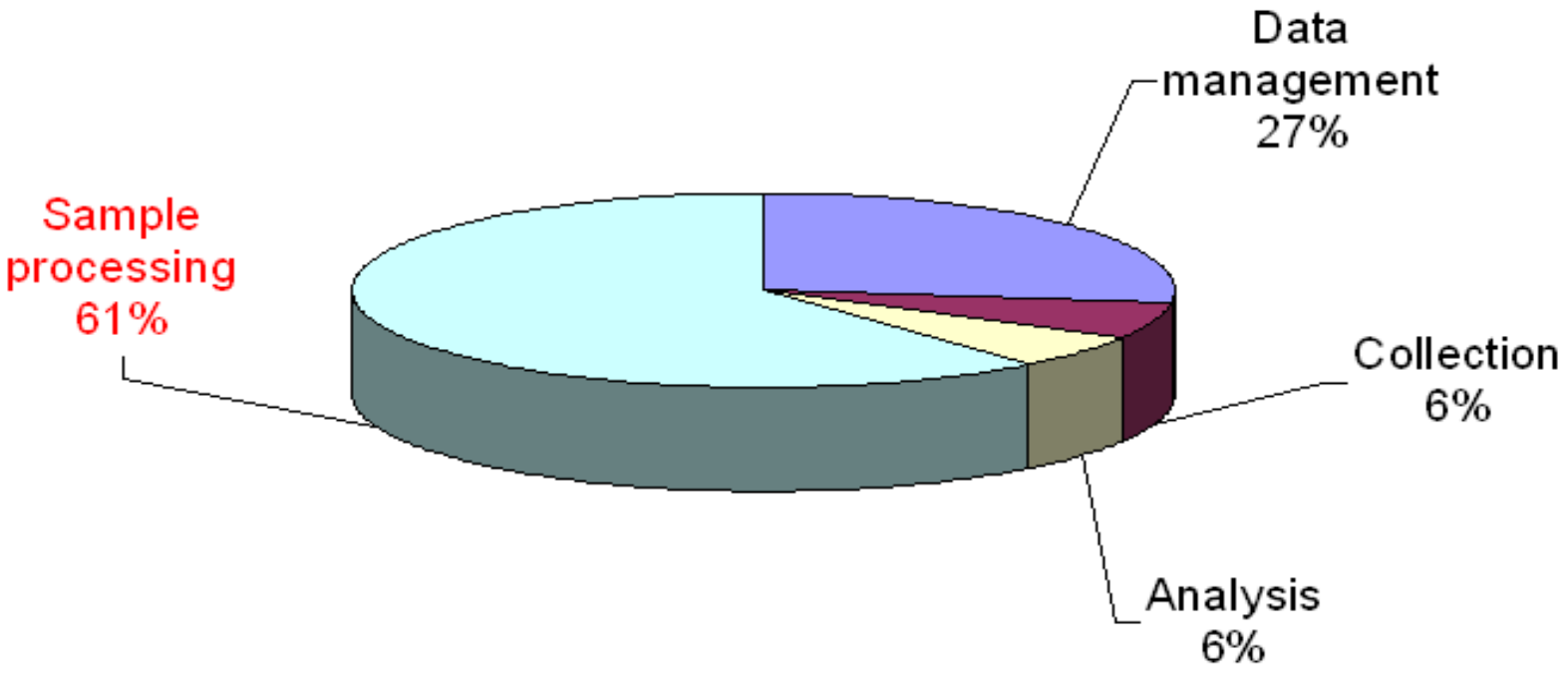
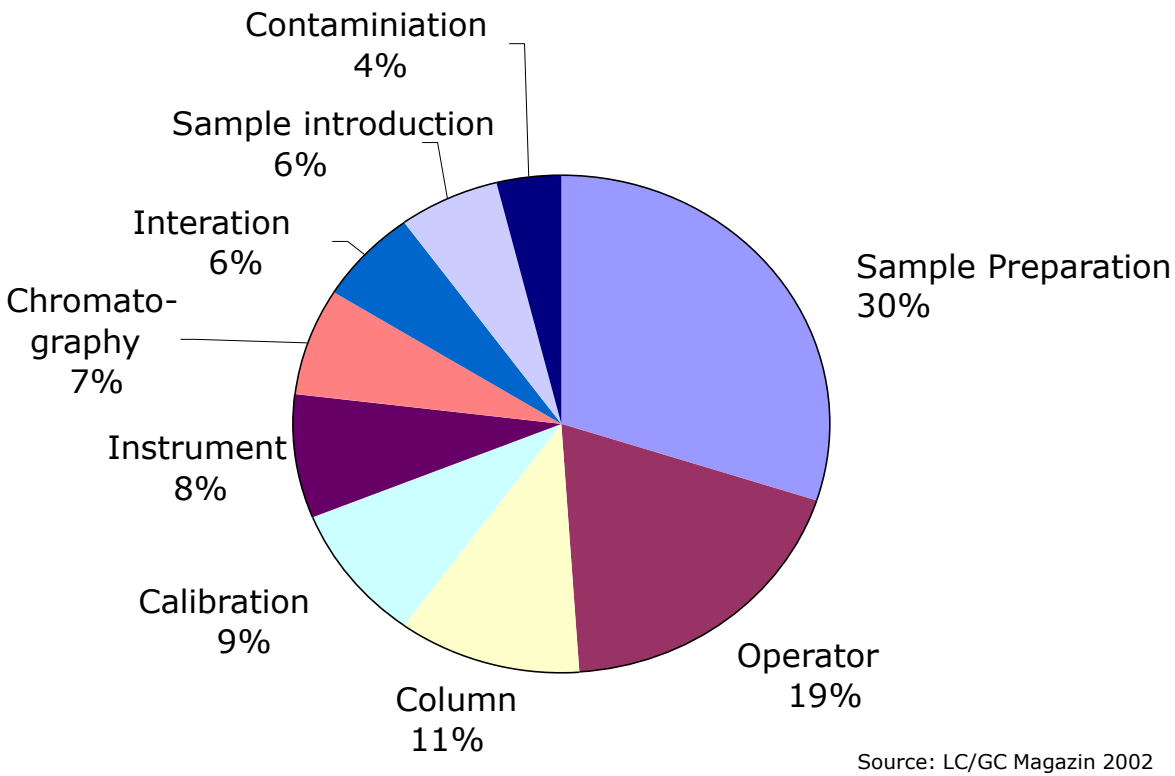
Time Spent on the Analytical Process



Sample prep
Sources of Chromatographic Errors



Source: LC/GC Magazin 2002

SPE

Solid Phase Extraction

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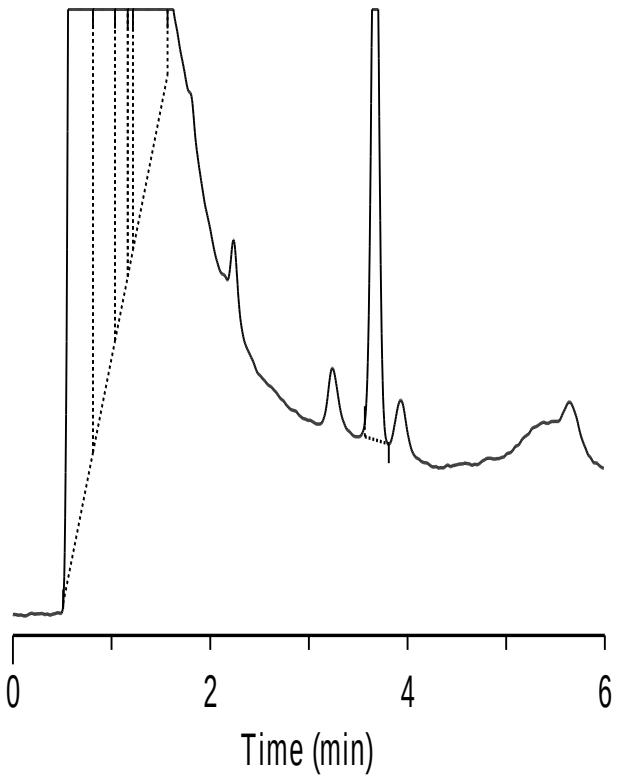
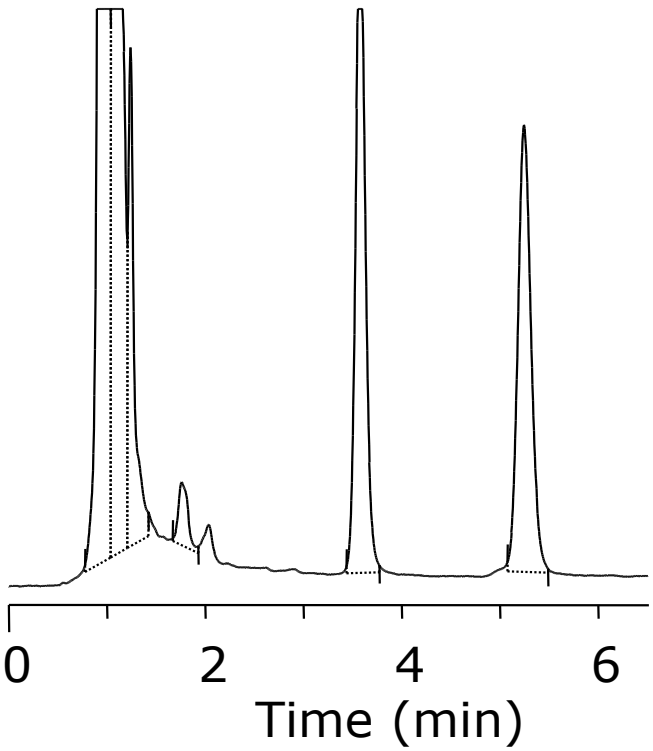
Real World & Real Samples

The Importance of Sample Preparation

Urine Sample without SPE



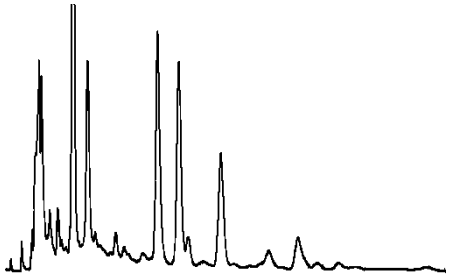
Urine Sample with SPE



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

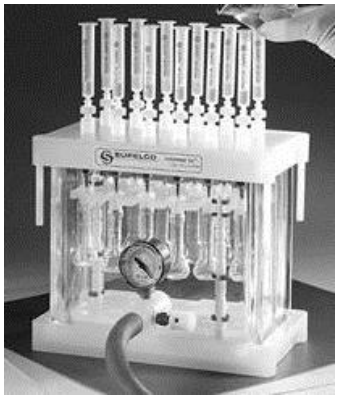
Too dilute- analyte(s) not concentrated enough for quantitative detection

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

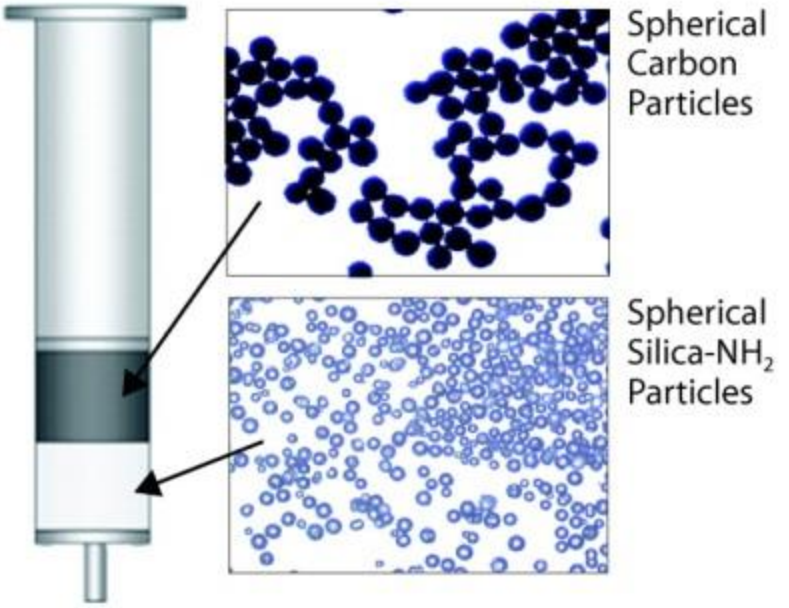
- Various compositions
- Different functionalities

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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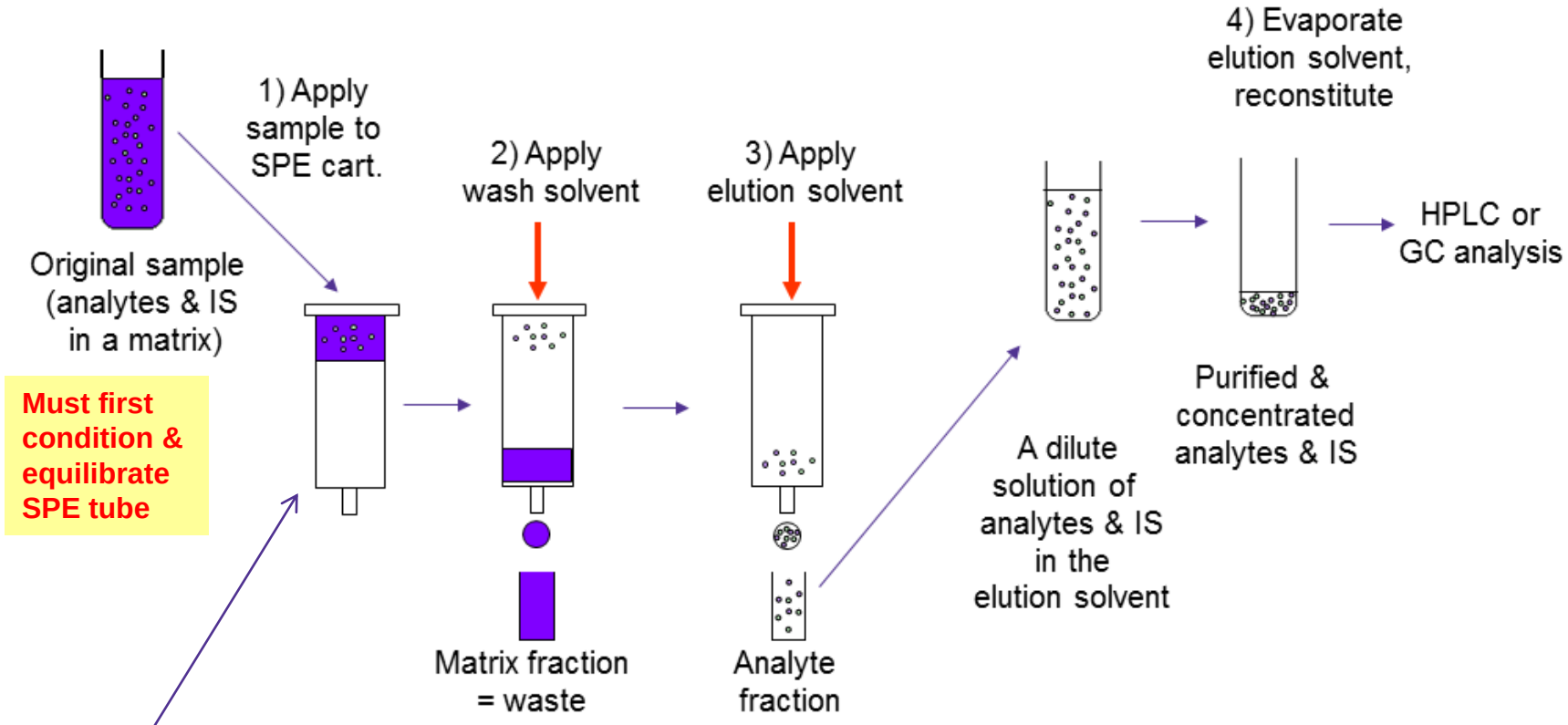
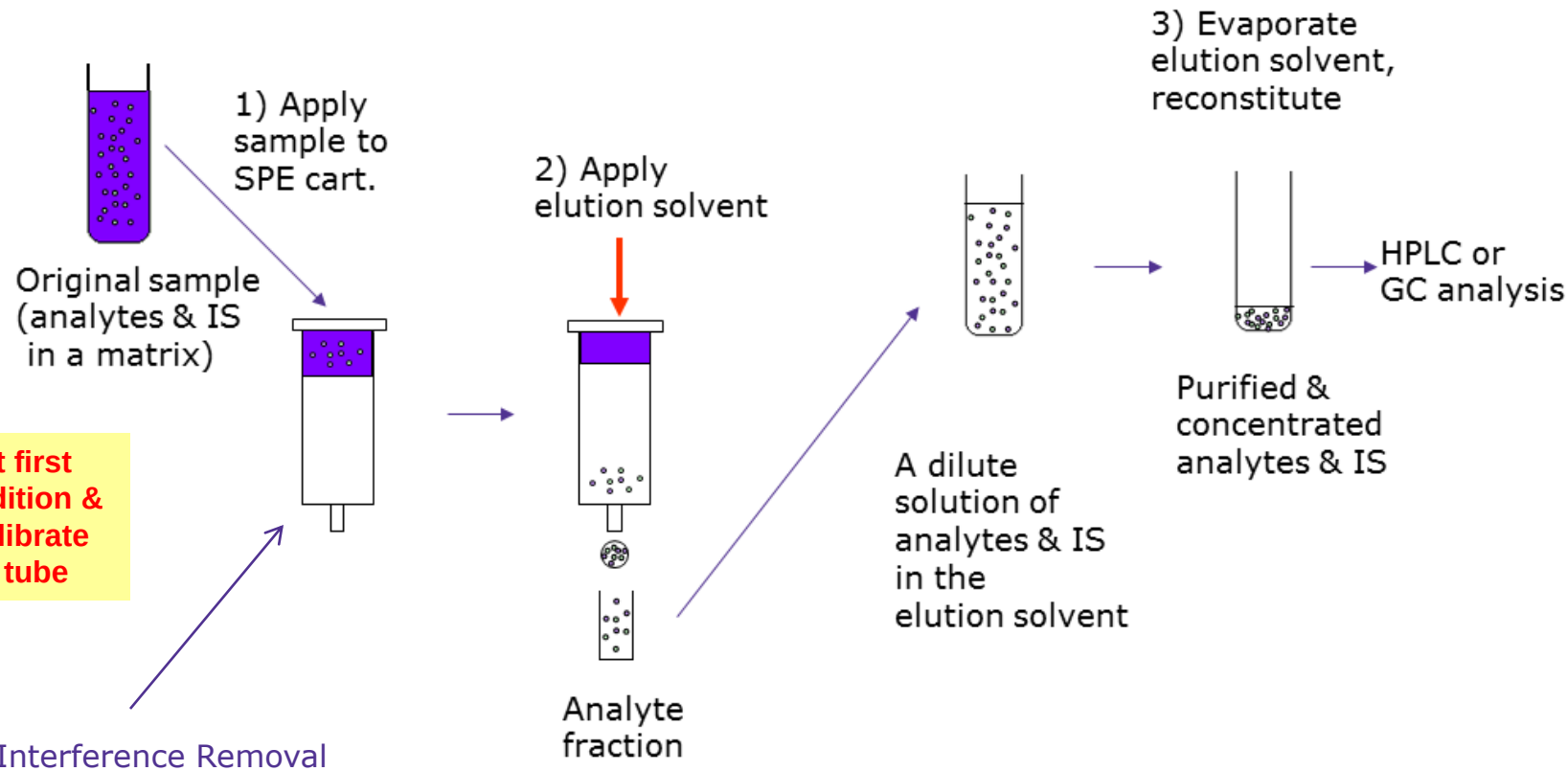
Bind-Elute Strategy



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 Key Products for **Food Analysis**

Overcoated SPME

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

Supel™ QuE

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

Supelclean™ Ultra

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

Supel™ Tox

- Removes interferences associated with mycotoxin analysis

Supel™MIP

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

Supelclean™ EZ-POP NP

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

Supel™-Select Polymeric SPE

- Yes, this can be used for food too!

Supel™Genie On-line SPE

- Eliminates human error and reduces labor cost

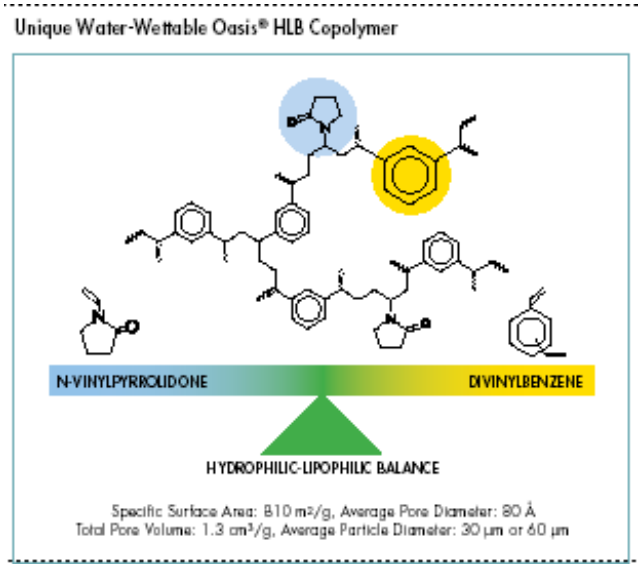
Supel-Select: What is Hydrophilic Polymer SPE?

Polymer chromatographic media designed for SPE

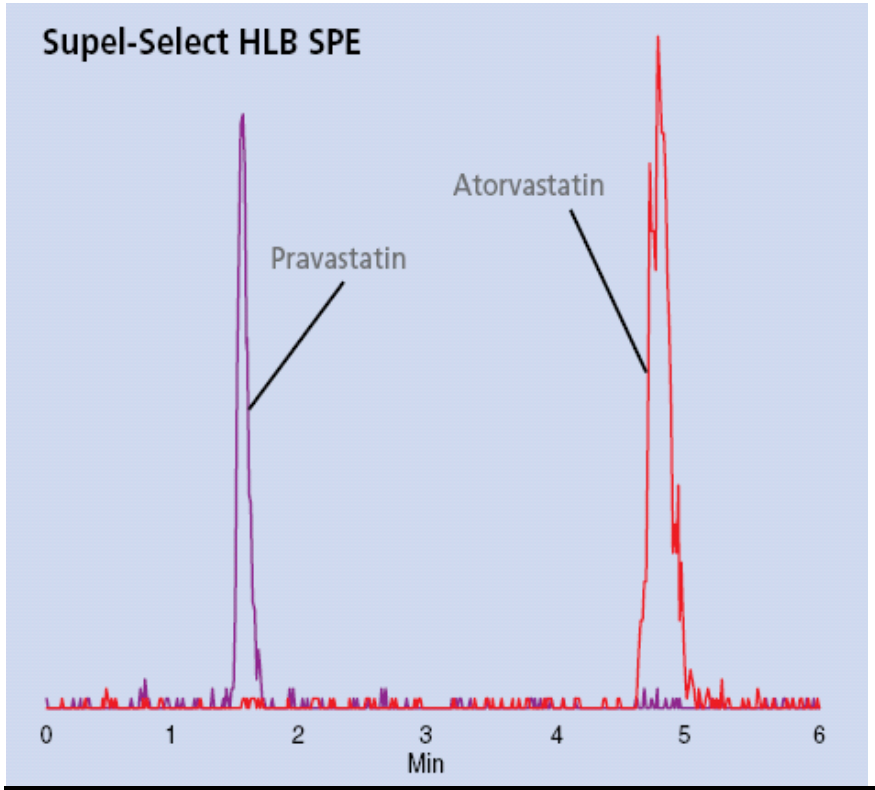
Comprises of a hydrophilic component and a hydrophobic component:

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

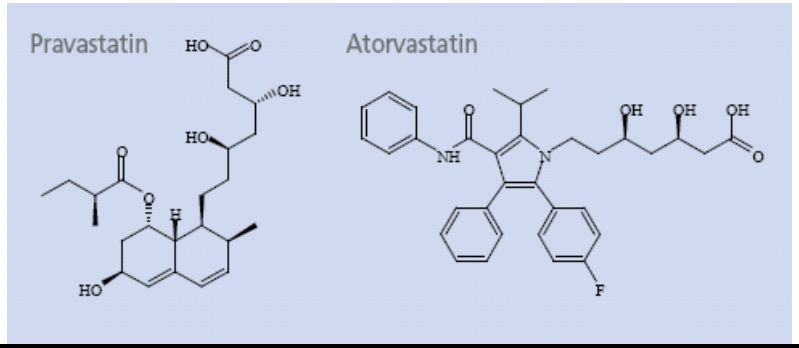
HLB Phase Chemistry:	Hydrophilic modified styrene polymer
SAX Phase Chemistry	Quaternary amine functionalized hydrophilic modified styrene polymer
SCX Phase Chemistry	Sulfonic acid functionalized hydrophilic modified styrene polymer
pH Compatibility:	0-14
Particle Size:	55-60 µm
MS Suitable:	Yes
Surface 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%

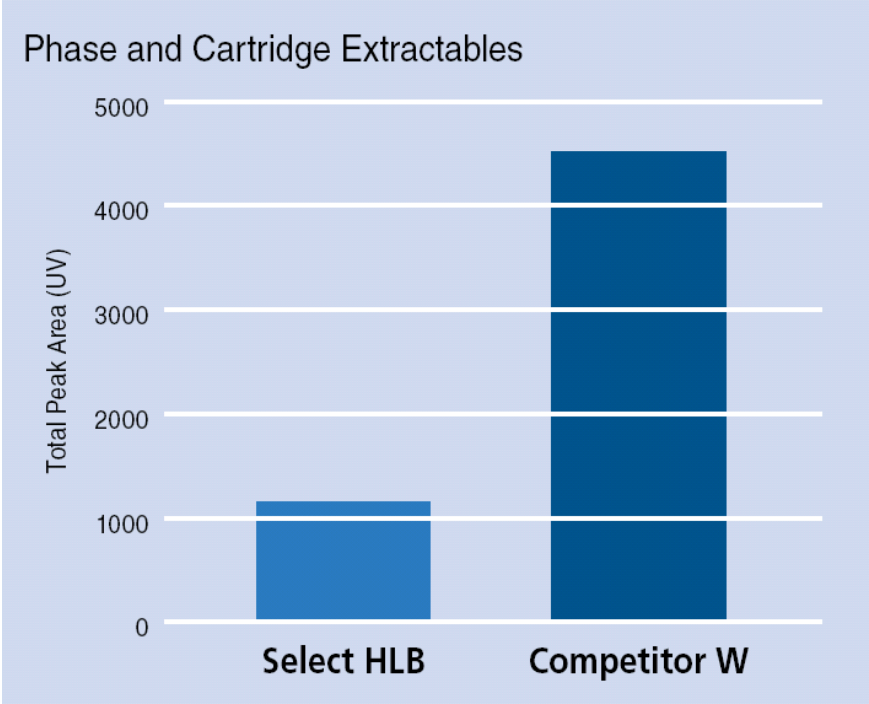
Supel-Select HLB: Minimum Extractables

Assays today require greater sensitivity

SPE phase chemistry and hardware should impart minimum extractables

Each lot is tested for:

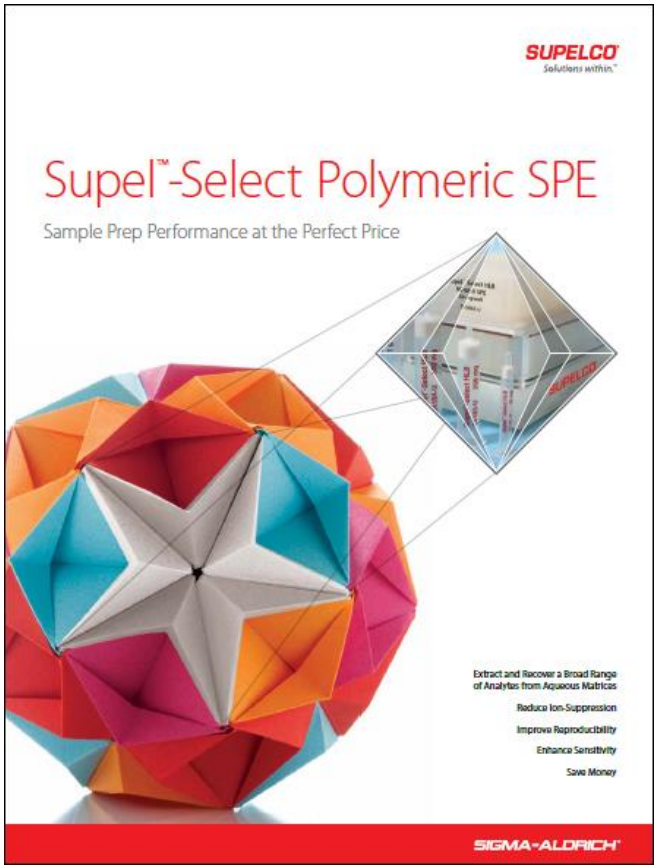
- Recovery
- LC-UV & LC-MS cleanliness
- Particle size
- Density
- Pore size
- Pore volume



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 - removes acidic interferences
- C18 - retains some hydrophobic interferences
- 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

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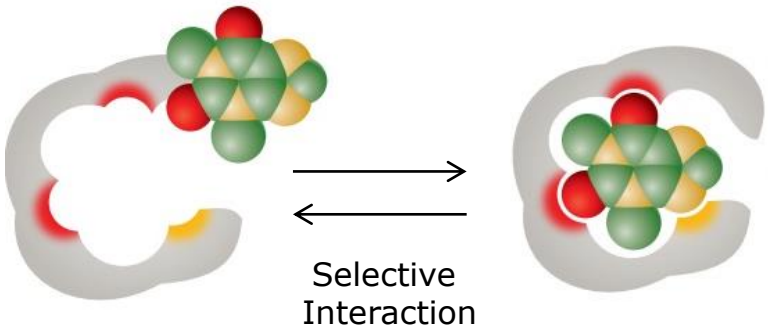
SupelMIP SPE – Molecularly Imprinted Polymer SPE

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

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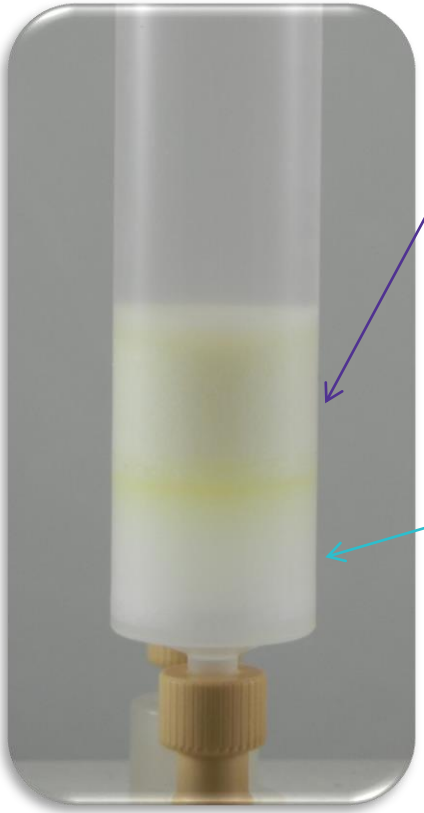
A New Approach: Analysis of Non-Polar POPs in Edible Oils

Supelclean™ EZ-POP NP: Dual-layer SPE Cartridge Containing Florisil® and Z-Sep/C18 Mix



Florisil® layer:
retains background constituents with polar functionality such as fatty acids

Z-Sep/C18 layer:
retains fatty matrix through both Lewis acid/base and hydrophobic interactions

**Note: Yellow Color = Oil Matrix Removed From Sample*

- Easy sample preparation methodology using minimal volume of solvent.
- Final sample extracts compatible with GC or HPLC.

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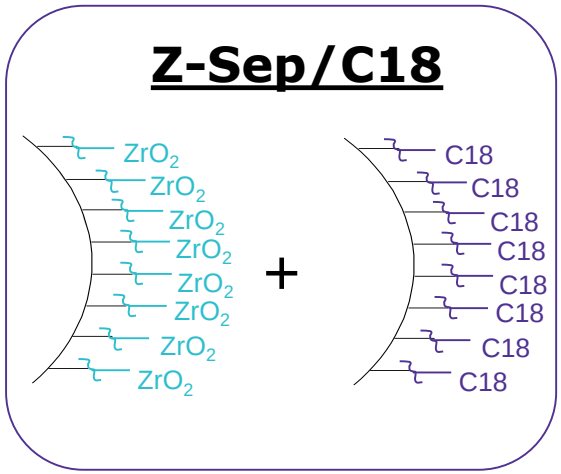
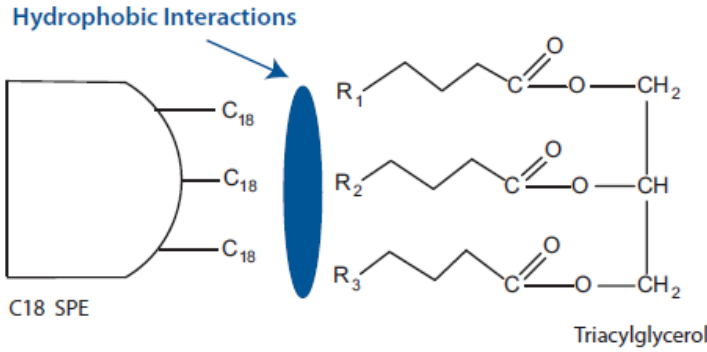
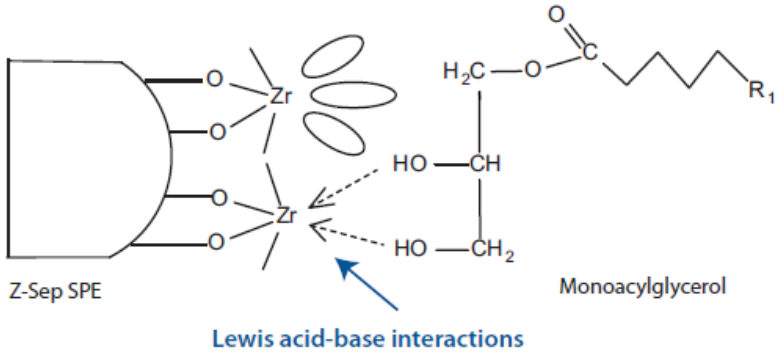
Z-Sep/C18 And Proposed Lipid Retention Mechanism







Supel™ Tox SPE for Mycotoxin Analysis

- Remove interferences associated with **mycotoxin analysis** in food and feed samples.
- **Quick**, **simple**, and **reproducible** sample cleanup solution
- **Compared to** the industry standard **Immunoaffinity Columns** (IAC):
  - Decrease **sample prep time**
  - Increase **reproducibility**
  - More **user friendly**

Complemented by an extensive line of Certified Reference Materials (CRMs)

sigma-aldrich.com/supeltox
sigma-aldrich.com/mycotoxins

- Supel Tox SPE Brochure (PFK)

QUECHERS

for Pesticide Residue Analysis

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QuEChERS Methodology for Pesticide Residue Analysis*….and beyond*

- Stands for: **Qu**ick, **E**asy, **Ch**eap **E**ffective, **R**ugged, **S**afe
- **Loose extraction salts** and **cleanup sorbents** in combination with **shaking** and **centrifugation** for purification
- Pesticide Residue, PAH, PCB, PDBE analysis

Official Methods:

- CEN Standard Method EN 15662
- AOAC Method 2007.01

1	SALTS	Extraction: Phase separation and buffering Cleanup: Water removal
2	SORBENTS	Cleanup: Remove unwanted interferences
3	TUBES	Hold the ingredients and samples during extraction and clean up

SigmaAldrich.com/quechers

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How Does the QuEChERS Method Work?

Weigh 10 g homogenized sample

Add 10 mL Acetonitrile (ACN)

Add Internal Standard

Shake

Add Salt Extraction Tube:
AOAC 2007.01 = Acetate Tube
EN15662:2008 = Citrate Tube

Shake & centrifuge

Transfer ACN layer to cleanup/sorbent tube

Shake & centrifuge

Adjust the pH if necessary

LC or GC Analysis

QuEChERS: What's Inside – Cleanup tube

SORBENTS

In AOAC and EN Official Methods

PSA – "Primary Secondary Amine"
Supelclean™ PSA

- Removes: fatty acids, organic acids, polar pigments, sugars

Carbon ENVI™-Carb

- Removes: chlorophyll, carotenoids

C18 Discovery® DSC-18

- Removes: lipids, non-polar compounds

Unique

Z-Sep Sorbents: Z-Sep, Z-Sep/C18, Z-Sep+

- Removes: lipids, pigments

Role of the sorbent is to trap interferences that co-extract with the analytes.

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QuEChERS Method: the choice of sorbent

Interference	PSA	C18	C18/PSA	ENVI-Carb	ENVI-Carb/ PSA	PSA/C18 /ENVI-Carb
Fats		X	X			X
Pigments	X			X	X	X
Sugars	X		X		X	X
Acids	X		X		X	X

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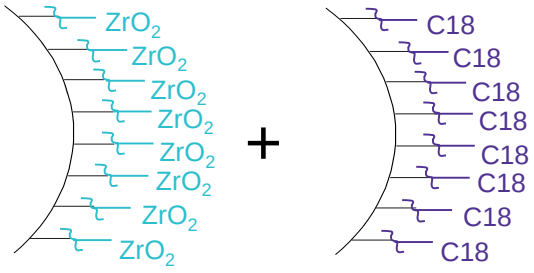
Zirconia-based QuEChERS Sorbents Address Fatty Matrix Interferences

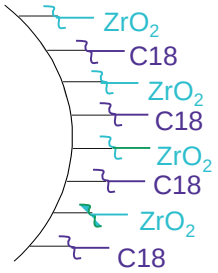
Z-Sep Sorbents for QuEChERS

- Z-Sep/C18: For fatty and/or pigmented matrices containing < 15% fat
- Z-Sep+: For fatty and/or pigmented matrices containing > 15% fat
- Z-Sep: For the analysis of hydrophobic analytes in fatty matrices

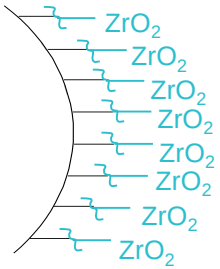
Z-Sep/C18



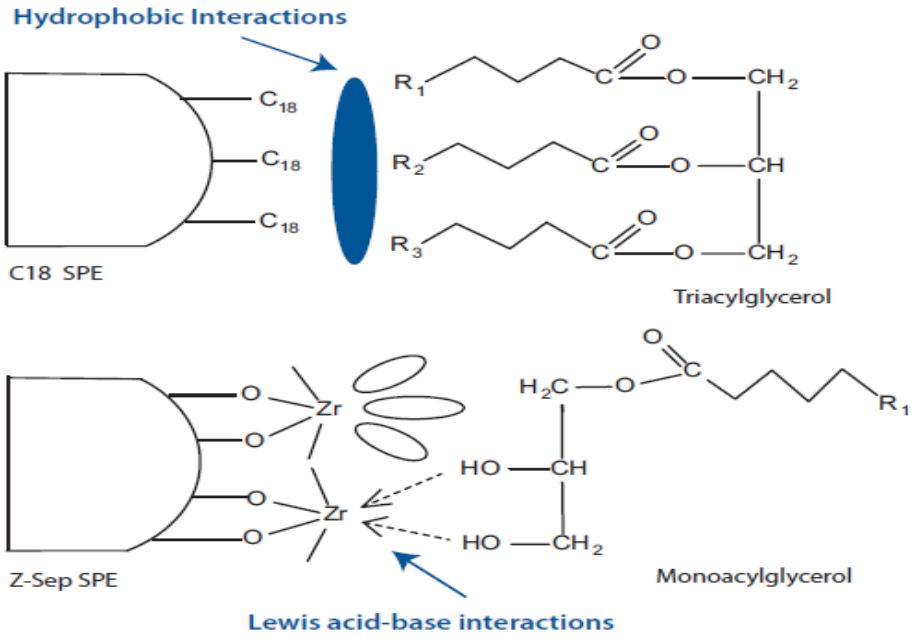
Z-Sep+



Z-Sep



Proposed Retention Mechanism



- Diminishes interferences from fatty matrices
- Removes various colors, specifically orange pigments
- Useful for analysis of pesticide residues
- Useful for analysis of PAH, PCB, PBDE and flame retardant analysis (specifically Z-Sep)

Case Study:

Pesticides in Avocado by QuEChERS & GC/MS

- Pesticide mix included hydrophobic compounds (e.g. organochlorines, hexachlorbenzene) and some other more polar classes all GC/MS amenable.

Extraction and Cleanup Procedures

1. Place 3 g of a homogenized avocado sample into a 50 mL centrifuge tube (Cat. No. 55248-U). Add spike solution if a spiked replicate.
2. Add 25 mL of acetonitrile and shake for one minute.
3. Add the contents of an Acetate Extraction Tube (Cat. No. 55234-U), and shake for one minute.
4. Centrifuge for five minutes.
5. Transfer 3 mL of the supernatant into the appropriate cleanup tube, Z-Sep+ (Cat. No. 55296-U) or PSA/C18 (Cat. No. 55229-U).
6. Shake for one minute, then centrifuge for three minutes.
7. Transfer 1 mL of the supernatant into an autosampler vial for GC/MS analysis.

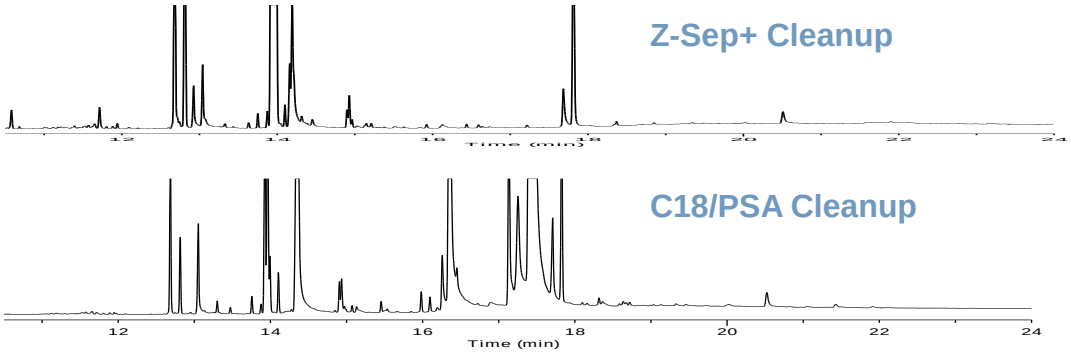
Case Study:

Pesticides in Avocado by QuEChERS & GC/MS

Pigment Removal

Less color remained in the extract cleaned with Z-Sep+ vs. PSA/C18

GC/MS Background

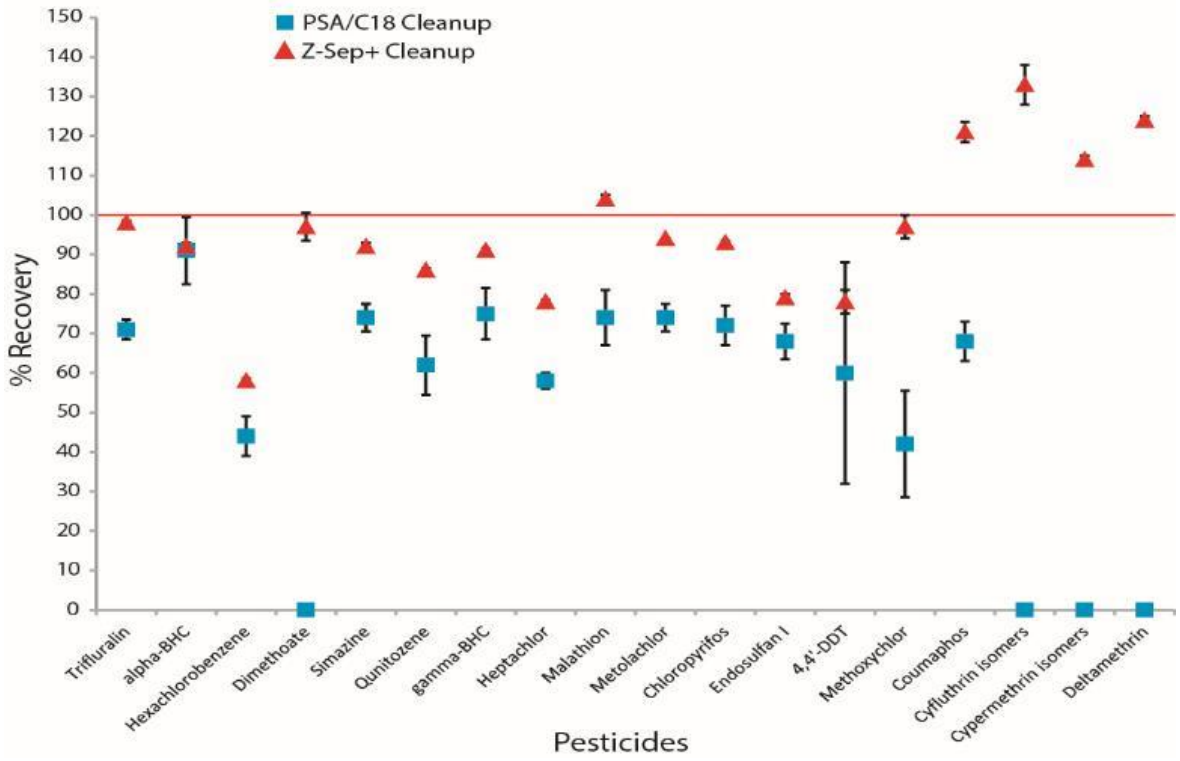


GC-MS analysis of avocado extracts (scan mode) in the same y-scale

Case Study:

Pesticides in Avocado by QuEChERS & GC/MS

Results: Pesticide Recovery (Spiked samples each at 20 ng/g)



- Z-Sep+ showed better recovery overall.
- PSA/C18: matrix interference prevented analysis of cyfluthrin, cypermethrin and deltametrin.
- Z-Sep+ showed better reproducibility than PSA/C18.

SPME

Solid Phase Microextraction

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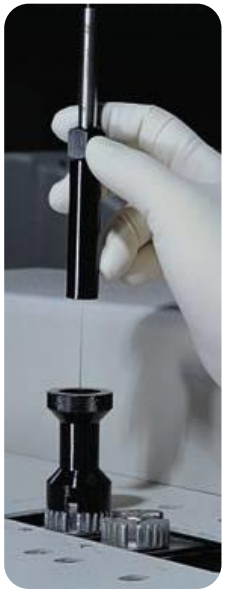
Solid Phase Microextraction (SPME)



- 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

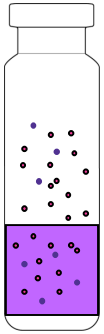
Benefits:

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

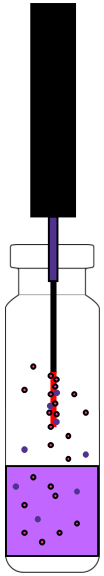
SigmaAldrich.com/spme

How SPME is used

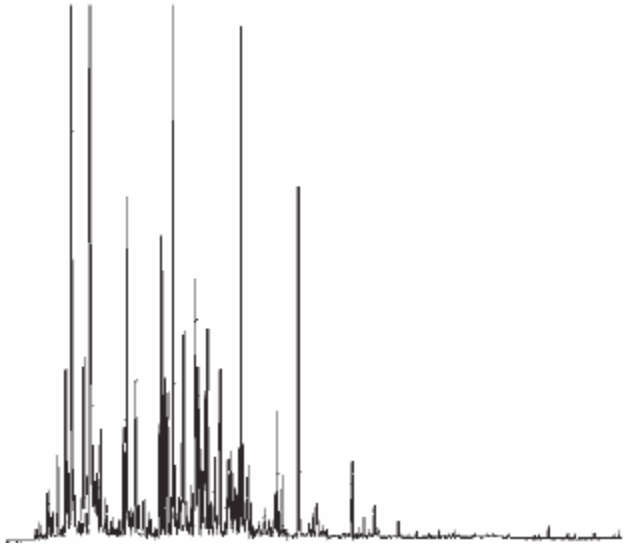
1 *Analyst has a **sample** they want to determine the content of*



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

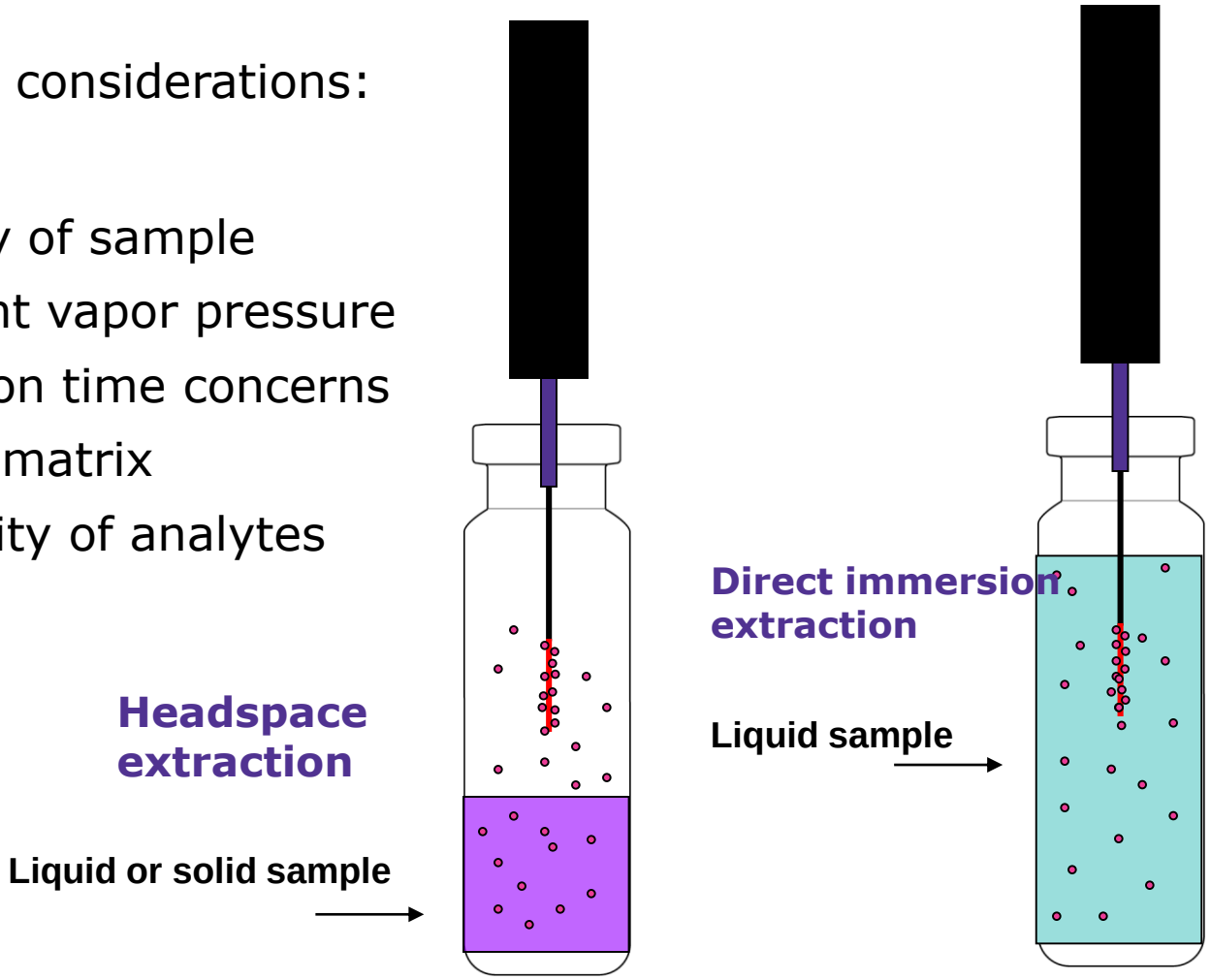
The SPME Concept

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

Sampling Technique: Headspace vs. Direct Immersion

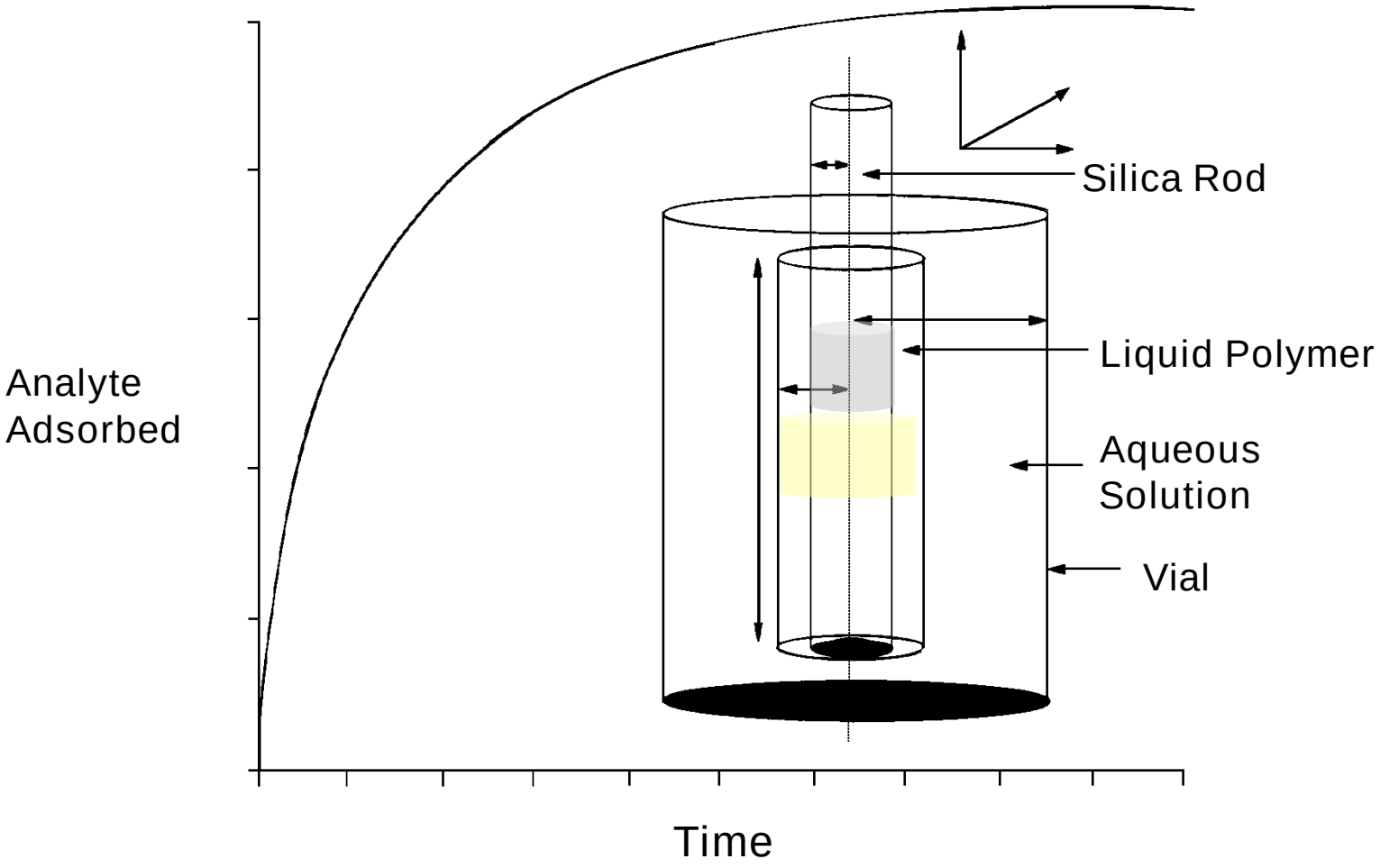
Analytical considerations:

- Volatility of sample
- Sufficient vapor pressure
- Extraction time concerns
- Sample matrix
- Selectivity of analytes



Adsorption Mechanism for SPME

SPME is quantitative!!!

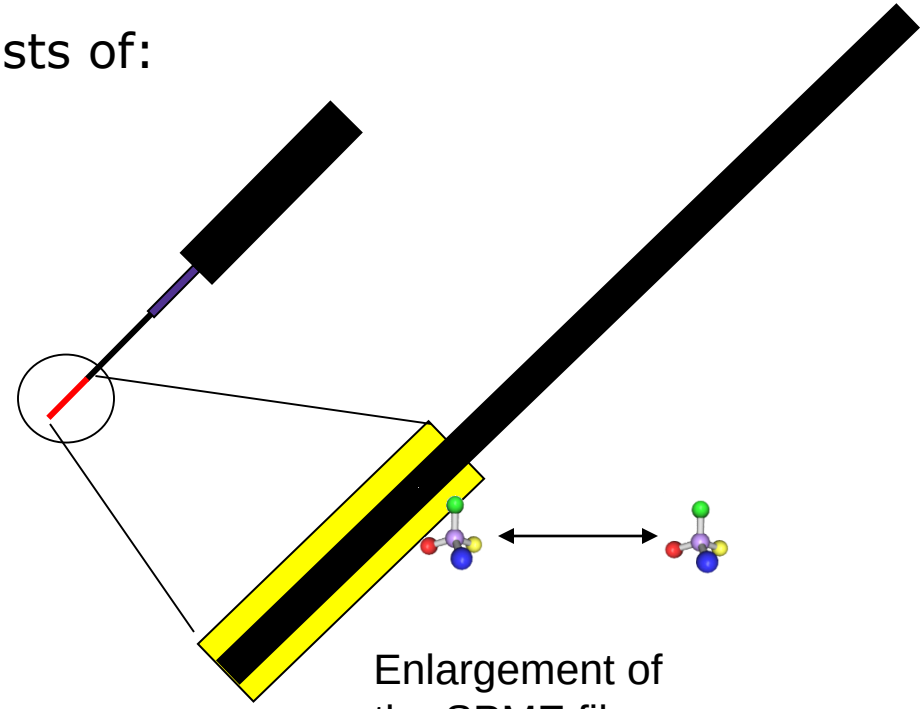


SPME Fiber Coating: The Business End

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

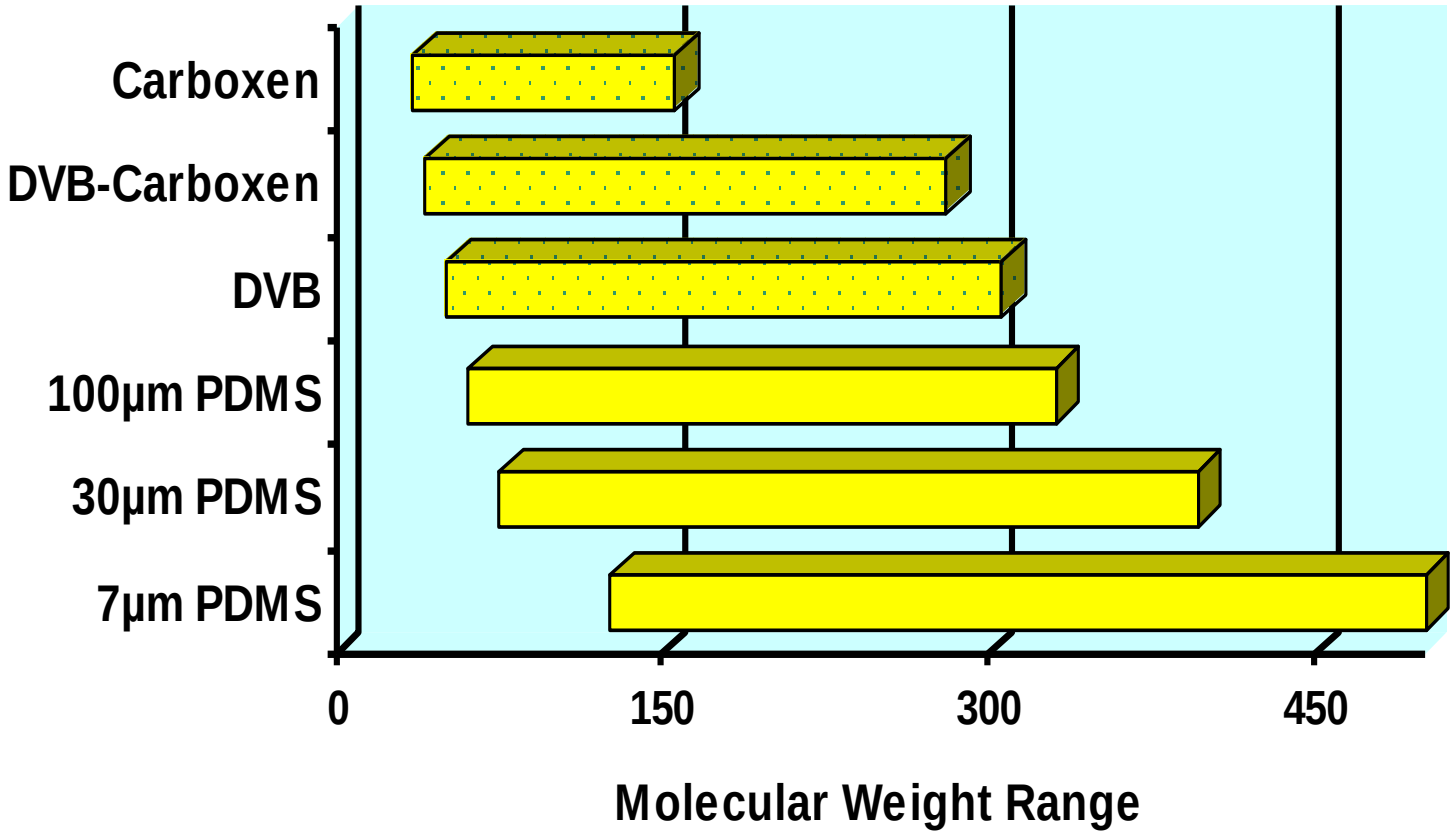
Films – Absorption:

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!