SOLID PHASE EXTRACTION

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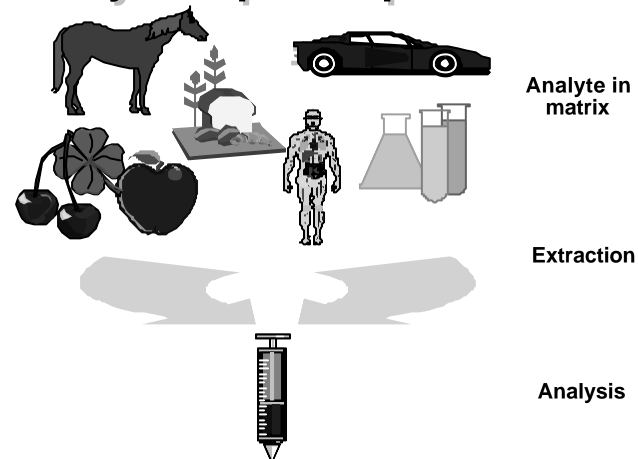
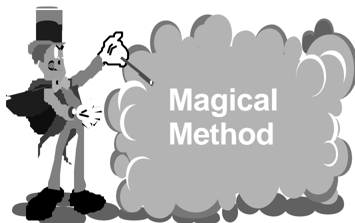
Why Sample Preparation?



Sample Preparation

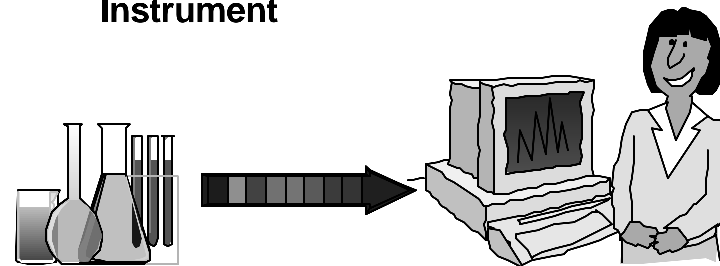
- Typically the most time-consuming step
- Typically the most difficult
- Typically the least amount of effort spent developing a rugged sample preparation method



- **Wouldn't It Be Nice --**

  If We didn't have to Prepare Samples

  Before Injection into the Instrument



Why Perform Sample Preparation?

- **Remove interferences**
  - **e.g. Analysis of drug and metabolite in plasma. Need to remove protein interferences**
- **Concentrate sample**
  - **e.g. Pesticides in drinking water**

- Processing Steps needed to get Sample Ready Before Injecting into the Instrument

^ HPLC ^ GC
^ LC/MS ^ GC/MS
^ AA ^ Others

Sample Prep Techniques

Method	Basis for Selectivity
• Precipitation	Solubility
• Liquid-Liquid Extraction	Partitioning in one of two liquid phases
• Solid-Liquid Extraction (SPE)	Adsorption/partitioning onto solid sorbent
• Dialysis / Ultrafiltration	Molecular weight/size
• Electrophoresis	Charge
• Distillation/Evaporation	Boiling point/vapor pressure
• Supercritical Fluid Extraction	Partitioning into supercritical fluid

Solid Phase Extraction (SPE) Technology

- **Sample Must be in Liquid State**
- **Driving Forces**

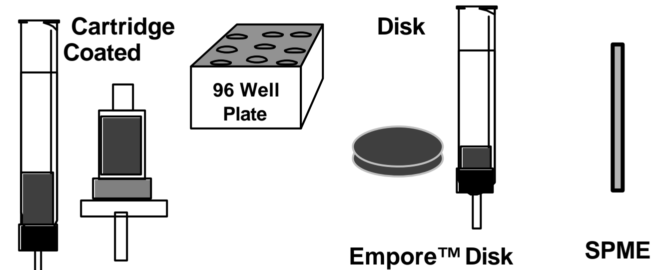
  ^ Gravity

  ^ Pressure

  ^ Vacuum

- **Solid Phase Extraction (SPE)**

- Formats and Configurations



Comparison to liquid-liquid extraction

Faster sample prep - **average time cut by 2/3**

Lower cost - **less solvent and reagent consumption means less hazardous waste**

Greater recoveries - **minimal sample transfer**

Greater accuracy - **no cross contamination**

Less sample handling - **no emulsion problems**

Reduced harm to labile samples - **minimal evaporation**

Improved safety - **due to reduced solvent/sample exposure and glassware**

Easy automation - **simultaneous batch processing of multi-samples**

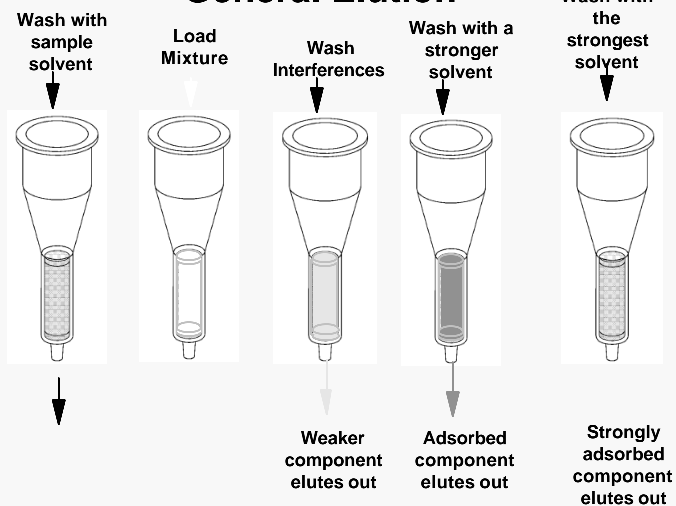
Strategies for Solid Phase Extraction

Active substance can be:

1- Unretained**, while matrix interferences are adsorbed**

2- Retained**, while matrix interferences are washed through**

General Elution



Reversed Phase: General Elution Protocol

1 Solvate the bonded phase with six to ten cartridge hold-up volumes of methanol or acetonitrile. Flush the cartridge with six to ten hold-up volumes of water or buffer. Do not allow the cartridge to dry out.

2 Load the sample dissolved in strongly polar solvent.

3 Elute unwanted components with a strongly polar solvent.

4 Elute weakly held components of interest with a less polar solvent.

5 Elute more tightly bound components with progressively more nonpolar solvents.

6 When you recover all of your components, discard the used cartridge in an appropriate manner.

Normal Phase: General Elution Protocol

1. You may condition the cartridge with six to ten hold-up volumes of nonpolar solvent, usually the sample solvent.
2. Load the sample into the cartridge.
3. Elute unwanted components with a nonpolar solvent.
4. Elute the first component of interest with a polar solvent.
5. Elute remaining components of interest with progressively more polar solvents.
6. When you recover all of your components, discard the used cartridge in an appropriate manner.

* Depending upon your chromatographic conditions, you may also use CN as a packing material for normal phase chromatography.

Ion-Exchange: General Elution Protocol

1. Condition the cartridge with six to ten hold-up volumes of deionized water or weak buffer.
2. Load the sample dissolved in a solution of deionized water or buffer.
3. Elute unwanted weakly bound components with a weak buffer.
4. Elute the first component of interest with a stronger buffer (change the pH or ionic strength).
5. Elute other components of interest with progressively stronger buffers.
6. When you recover all of your components, discard the used cartridge in an appropriate manner.

Chromatographic Modes and Sorbent Types

	NP	RP	IE
Sorbent polarity	High	Low	High
Typical solvent polarity range	Low to medium	High to medium	High
Typical sample loading solvent	Hexane, Toluene, CH2Cl2	H20, Buffers	H20, Buffers
Elution solvents	Ethyl acetate, acetone, CH3CN	H20/CH3OH/CH3CN solutions	Buffers, Salts
Sample elution components order	Least polar components first	Most polar components first	weakly ionized
Solvent change required to elute	Increase solvent polarity	Decrease solvent polarity	Increase ionic strength or pH (AX) up/down

Important Considerations in Protocol Design

Flow Rate

In general, cartridges may be conditioned at flow rates up to 25 mL/min. Sample loading and elution is best done at flow rates below 10 mL/min. Recovery may still be adequate at flow rates up to 20 mL/min (testing should be done to verify this). For ion exchange applications, slower flow rates (1-2 mL/min) are recommended.

Sample Capacity

A typical normal or reversed-phase cartridge may have capacity for up to 100 mg of very strongly retained substances. Note: this quantity includes every substance that may be strongly retained in any given sample, not just the component of interest!

Guide: k'= retention parameter = capacity ratio

The ideal solid phase extraction method:

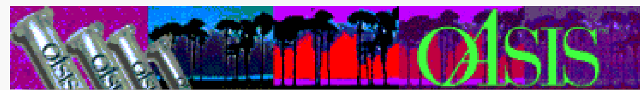
High and reproducible recoveries for acidic, basic, and neutral analytes.

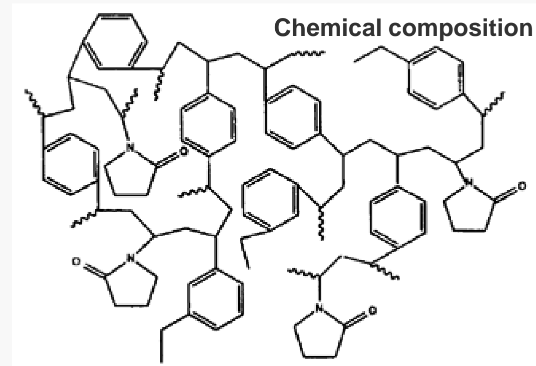
Isolates and allows quantification to pg/mL concentrations of drugs and metabolites from biofluids.

Rugged and easy to automate for large volumes of samples.

Fast and cost efficient.

New Polymeric SPE Cartridges

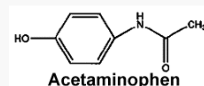




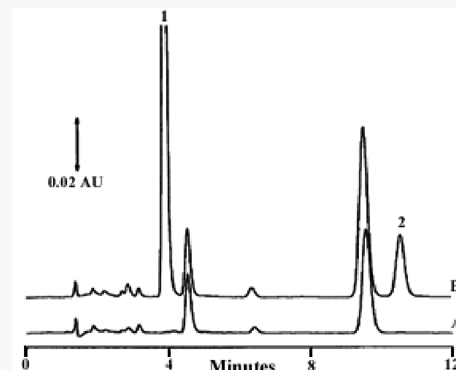
Solid Phase Extraction Procedure OasisTM HLB 1 cc Extraction Cartridge

Condition **1 mL methanol/1 mL water**
Load **1 mL spiked porcine serum**
Wash **1 mL 5% methanol in water**
Elute **1 mL methanol**
Evaporate and Reconstitute **40 °C under nitrogen stream** **200 µL of 50 µg/mL sulfanilamide (I.S.) in water**

Chromatogram of Serum Extracts: A) Blank B) Spiked Sample



HPLC Analysis Conditions:
Column:
SymmetryShieldTM RP8, 3.9 mm x 150 mm with SentryTM Guard column, 3.9 mm x 20 mm
Sample:
50 µL of reconstituted porcine serum extract
Mobile phase:
20 mM potassium phosphate, pH 3.0/methanol 97:3 (v/v)
Flow rate: 1.0 mL/min
Temperature: 30 °C
Detection: 220 nm



General Elution Protocol for Oasis HLV Cartridges-RP mode

Condition:
Apply and draw 1 mL of methanol through the cartridges.
Equilibrate:
Apply and draw 1 mL of water.
Load:
Apply and draw 1 mL of sample.
Wash:
Apply and draw 1 mL of 5% methanol in water. Stop vacuum pump, remove manifold cover and discard waste. Add rack containing collection vials. Replace cover, and turn vacuum on.
Elute:
Apply and draw 1 mL of methanol and collect eluate for analysis.

Differences Between HPLC and SPE

	HPLC	SPE
Particle size	~5 µm	40-80 µm
Packed bed efficiency	high	low
Extra-column volume	low	high
Column length	5-30 cm	~1 cm
Number of plates (N)	~10,000	< 50

Bottom line: HPLC can separate similar compounds. SPE requires a significant selectivity difference between compounds for separation. *Compounds not well resolved by HPLC cannot be separated by SPE with a similar retention mechanism.*

Solid Phase Extraction (SPE) Technology

Comparison of Efficiency - HPLC *vs.* SPE