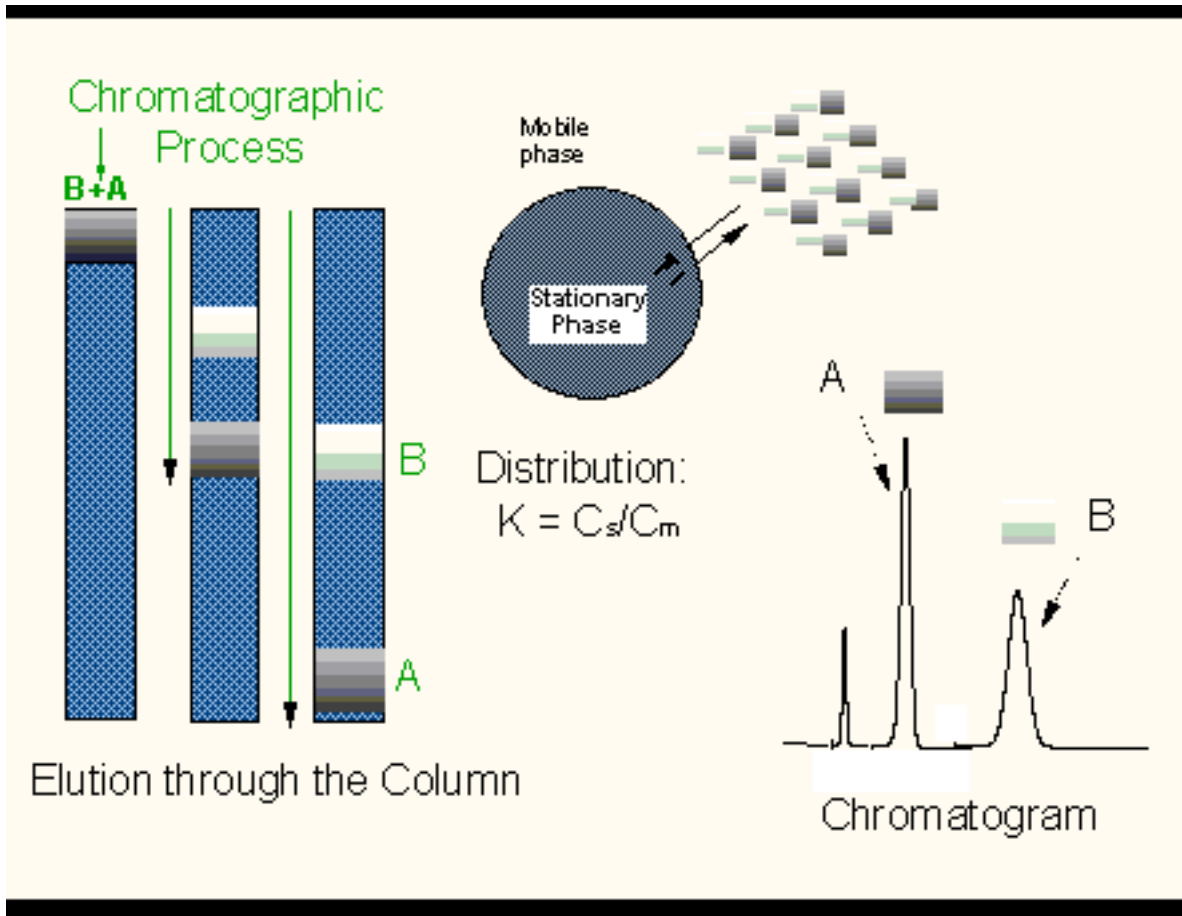
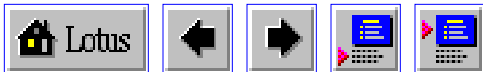




SOLID PHASE EXTRACTION







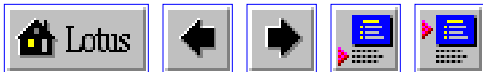
Sample Preparation:

Sample preparation is a key procedure in modern chemical analysis. By some estimates, 60-80% of the work activity and operating cost in an analytical lab is spent preparing samples for introduction into an analytical device.

Solid Phase Extraction (SPE)

Solid Phase Extraction (SPE) is the most widely used sample preparation technique in the analysis of new pharmaceutical compounds and metabolites in blood, serum and urine.

- SPE is necessary to remove interfering matrix constituents such as proteins and quantify to pg/mL levels, particularly for metabolites and bioactive drugs.



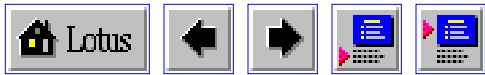
Strategies for Solid Phase Extraction

Active substance can be:

1- Unretained, while matrix interferences are adsorbed

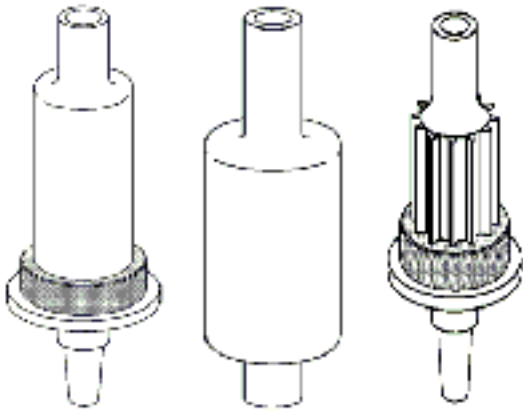
2- Retained, while matrix interferences are washed through

- The first strategy is usually chosen when the desired sample component is present in high concentration. When components of interest are present at low levels, or multiple components of widely differing polarities need to be isolated, the second strategy is generally employed. The second strategy may also be used for trace enrichment of extremely low level compounds and concentration of dilute sample. A complex matrix may be treated by both elution strategies to
- isolate different target analytes.

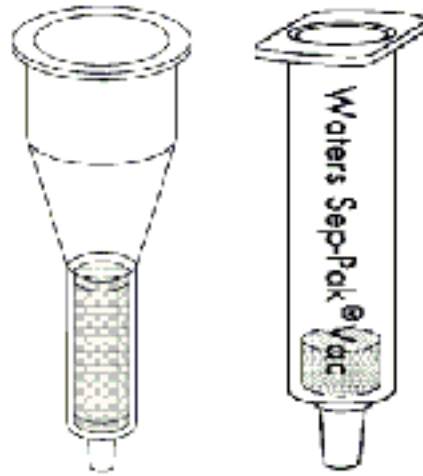


Solid Phase Extraction Cartridges:

Manual



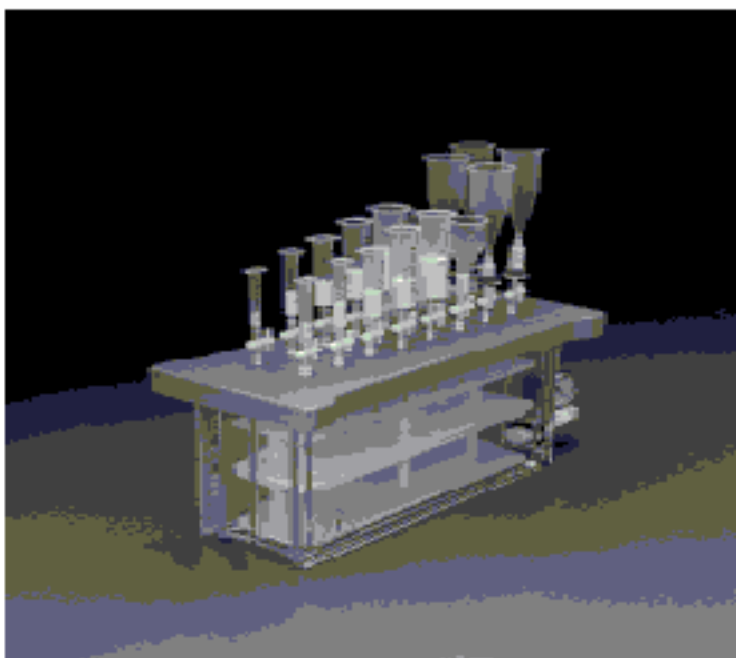
Vacuum



- SPE is typically performed by loading the complex sample onto a preconditioned extraction cartridge containing a chromatographic sorbent.

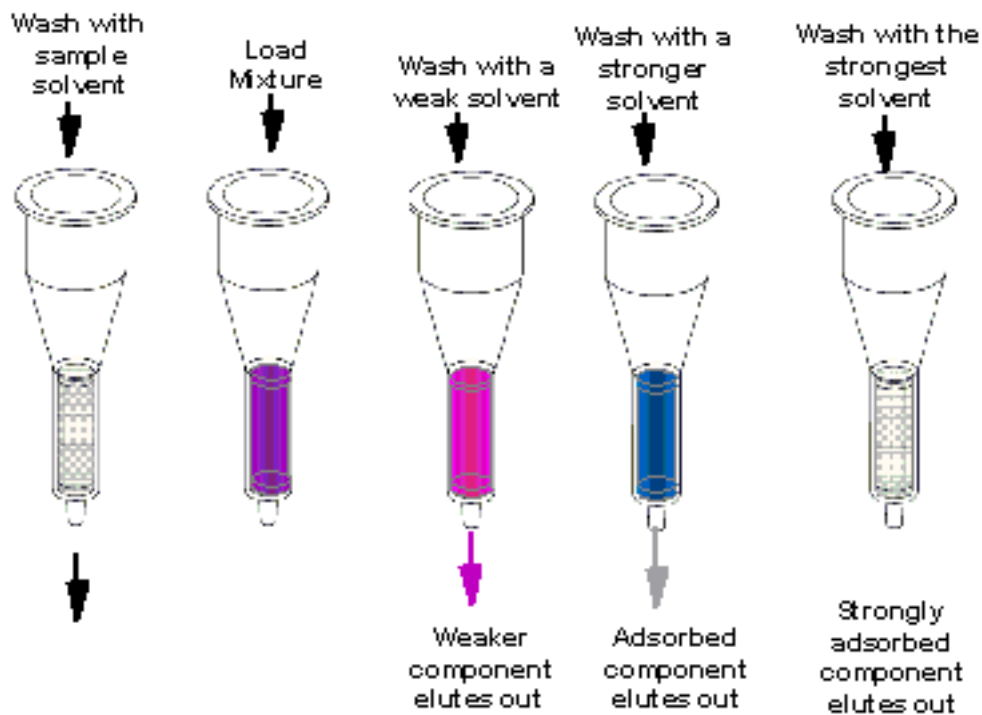


Vacuum Manifold





General Elution





Three major purposes

Sample Concentration: Enrichment

Frequently, the component of interest is present in levels too low for detection. Sample preparation can concentrate the component to adequate levels for measurement.

Contaminants

The presence of interfering matrix elements can mask the analysis of the component of interest. Sample preparation can remove excess contaminants to yield clean, informative chromatograms.

Bring to Solution

For most analyses (HPLC, GC, spectrometry, RIA, etc.), the sample must be properly prepared in solution for subsequent analysis.



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.



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.**
-



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, CH ₂ Cl ₂ | H ₂ O, Buffers | H ₂ O, Buffers |
| Elution solvents | Ethyl acetate, acetone, CH ₃ CN | H ₂ O/CH ₃ OH/CH ₃ CN 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 |



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



Important Considerations in Protocol Design

Flow Rate

Conditioning flowrates: up to 25 mL/min.

Sample loading and elution: flow rates below 10 mL/min.

Ion exchange: 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



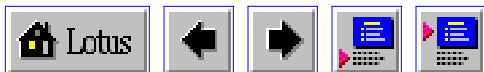
The ideal solid phase extraction method:

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

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

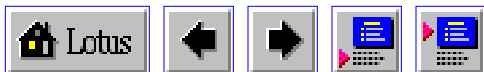
Rugged and easy to automate for large volumes of samples.

Fast and cost efficient.



Stationary Phase Supports

| Stationary phase | Functionality |
|------------------|--|
| C18 | $-\text{Si}(\text{CH}_3)_2\text{C}_{18}\text{H}_{37}$ |
| tc18 | $-\text{SiC}_{18}\text{H}_{37}$ |
| C8 | $-\text{Si}(\text{CH}_3)_2\text{C}_8\text{H}_{17}$ |
| C2 | $-\text{SiC}_2\text{H}_5$ |
| Porapak RDX | -divinylbenzene/vinylpyrrolidone |
| Aminopropyl | $-\text{Si}(\text{CH}_2)_3\text{NH}_2$ |
| Cyanopropyl | $-\text{Si}(\text{CH}_3)_2(\text{CH}_2)_3\text{CN}$ |
| Diol | $-\text{Si}(\text{CH}_2)_3\text{OCH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH}$ |
| Accel Plus CM | $-\text{COO}^- \text{Na}^+$ |
| Accel Plus QMA | $-\text{C}(\text{O})\text{NH}(\text{CH}_2)_3\text{N}(\text{CH}_3)^+ \text{Cl}^-$ |

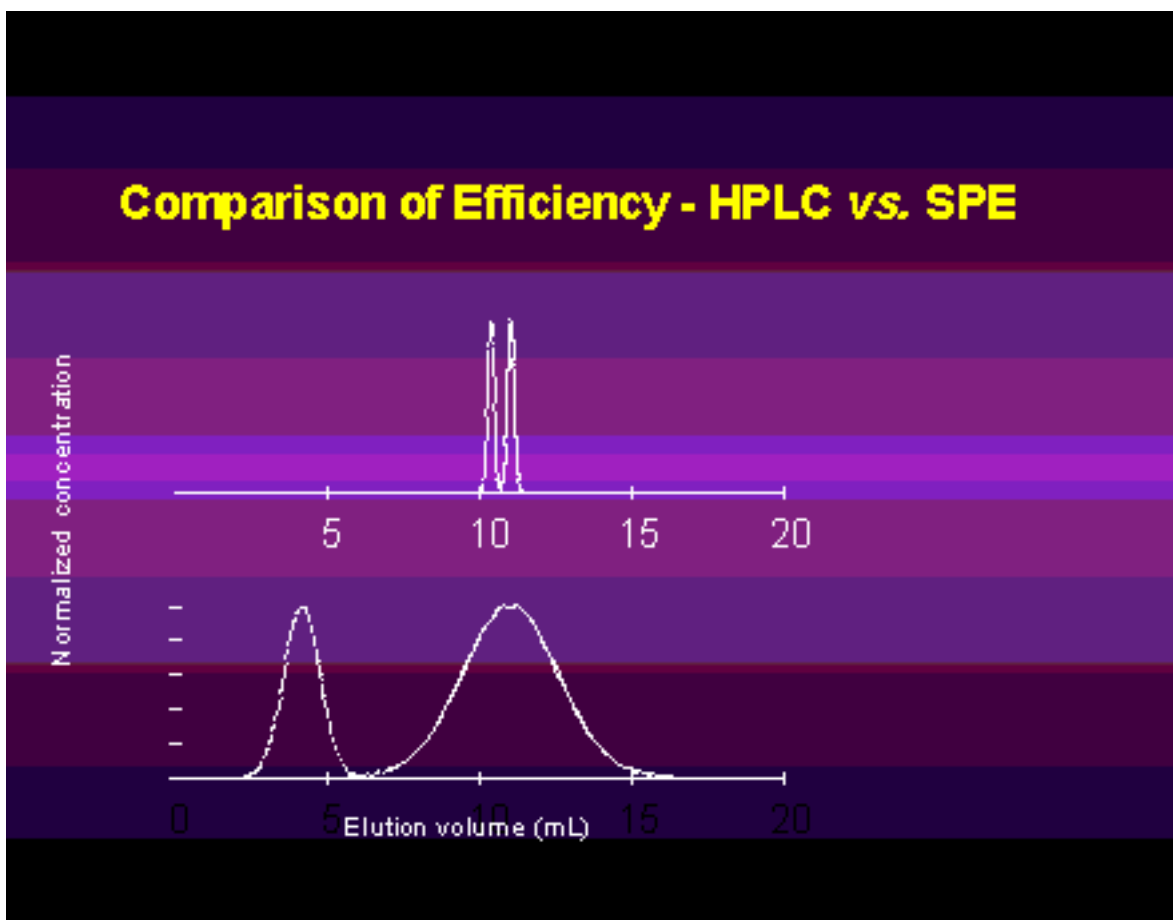
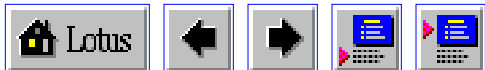


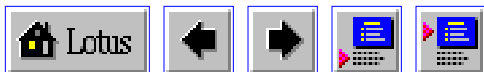
Solid Phase Extraction

- **Same Theoretical basis as HPLC.**
 - ◆ Retention and selectivity unaffected by particle size
 - ◆ Efficiency dependent on:
 - particle size
 - column geometry (extra-column effects)

Typical Number of plates:

- ◆ HPLC ~10,000
- ◆ SPE <50
- ◆
- **Minimum Selectivity (α), for $R_s = 1.2$**
 - ◆ HPLC 1.06
 - ◆ SPE 3.95





How much of surface is used for adsorption of analyte?

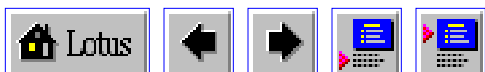
- Calculate maximum surface coverage on C18 Sep-Pak
 - Mass sorbent/cartridge = 100 mg
 - Surface area ~ 300 m²/g ~ 30 m²/cartridge
 - C18 Coverage ~ 3 μmol/m² ~ 100 μmol/device

Assume one C18 ligand can bind one molecule of analyte

- Maximum coverage ~ 100 μmol/cartridge

Assume analyte MW=200

Maximum mass load ~20 mg for full surface coverage

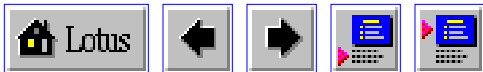


Differences between HPLC and SPE

| | 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) | ~5,000-10,000 | < 50 |

Bottom line: HPLC can separate similar compounds.

SPE requires a significant selectivity difference between compounds for separation.



SPE Strategies

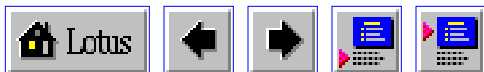
- ◆ **Elute the product of interest, retain interferences**

- want $k' \sim 0$ for analyte
- want k' large for interferences
-

- ◆ **Elute interferences, retain product**

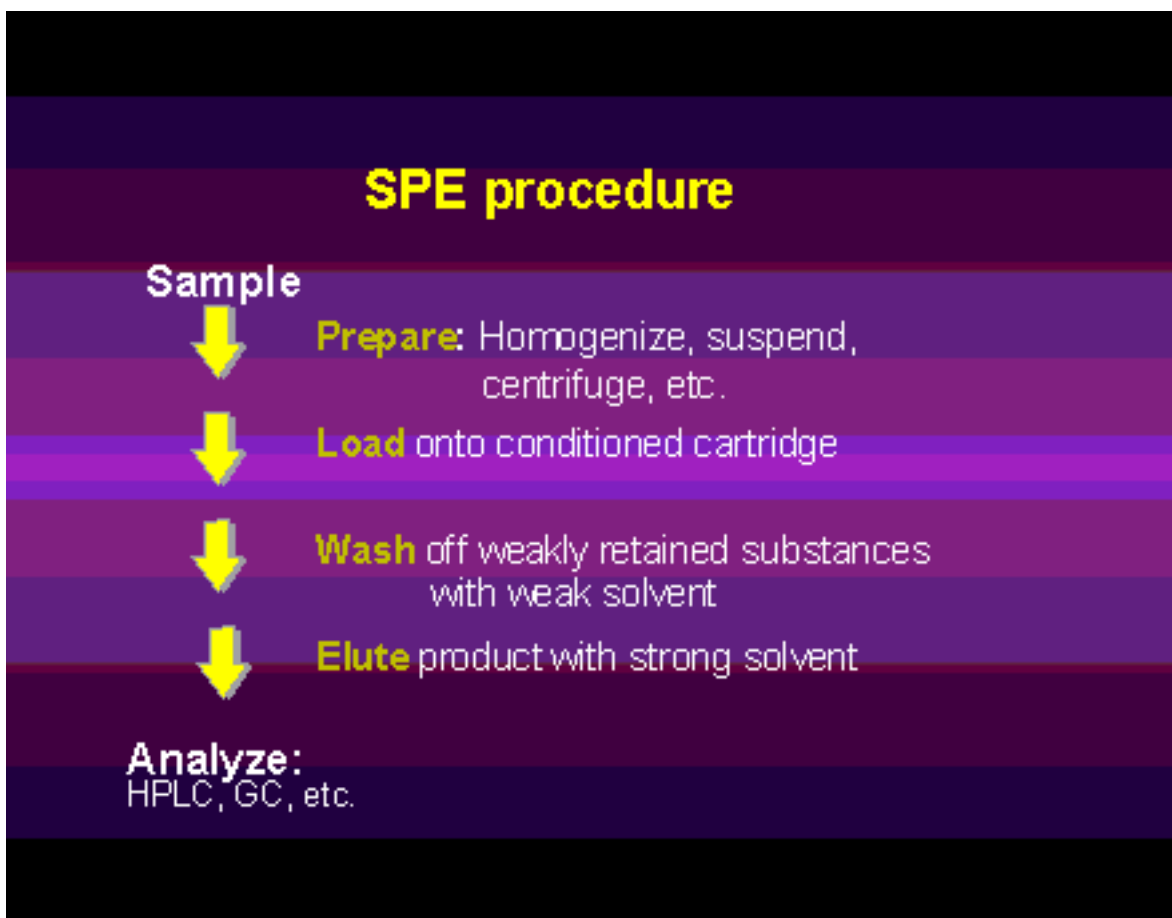
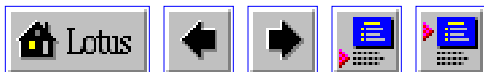
- want $k' \sim 0$ for interferences
- want k' large for analyte

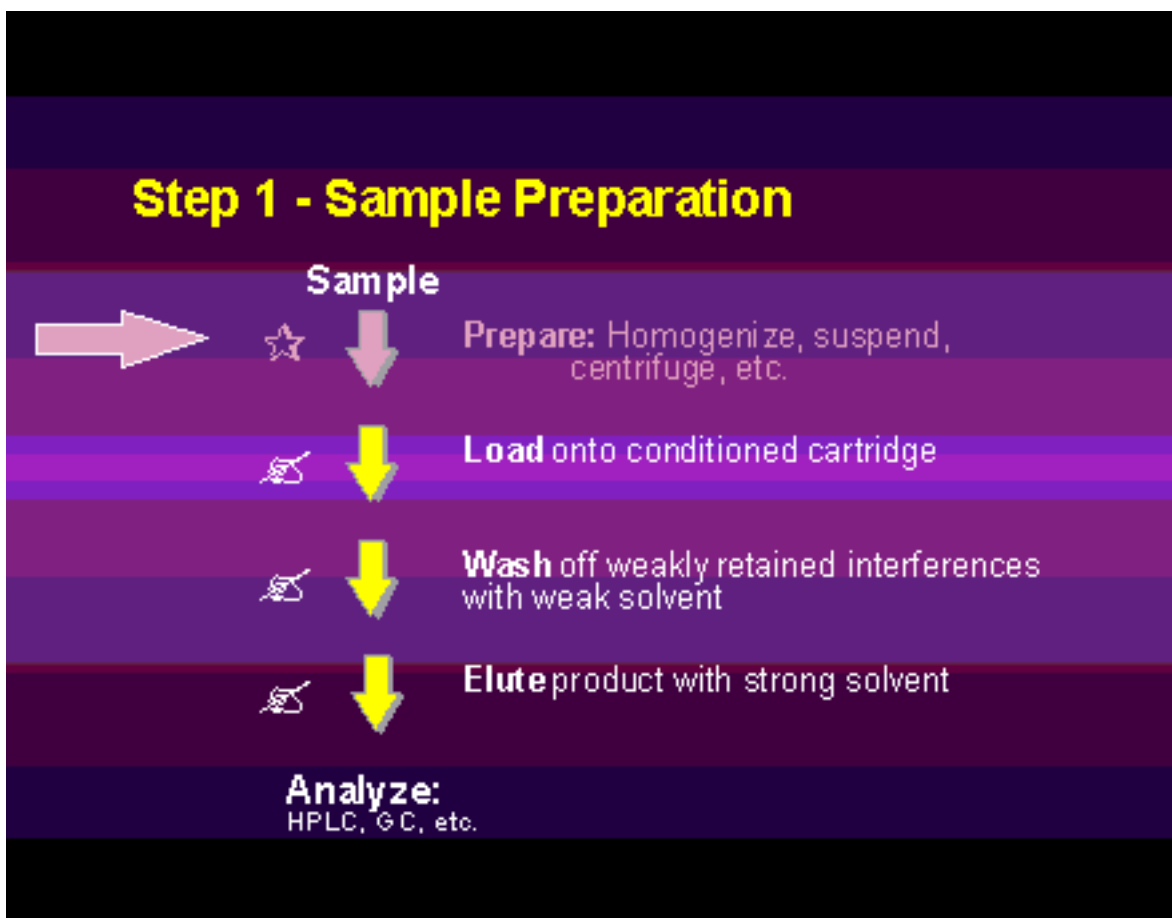
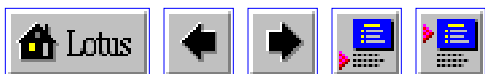
$$k' = \phi \frac{C_s}{C_m}$$



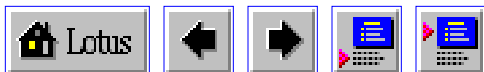
Common Problems in SPE

- ◆ Incomplete removal of interferences
- ◆ Low recovery of analyte(s)
- ◆ High variability





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- tep 1 - Sample Preparation



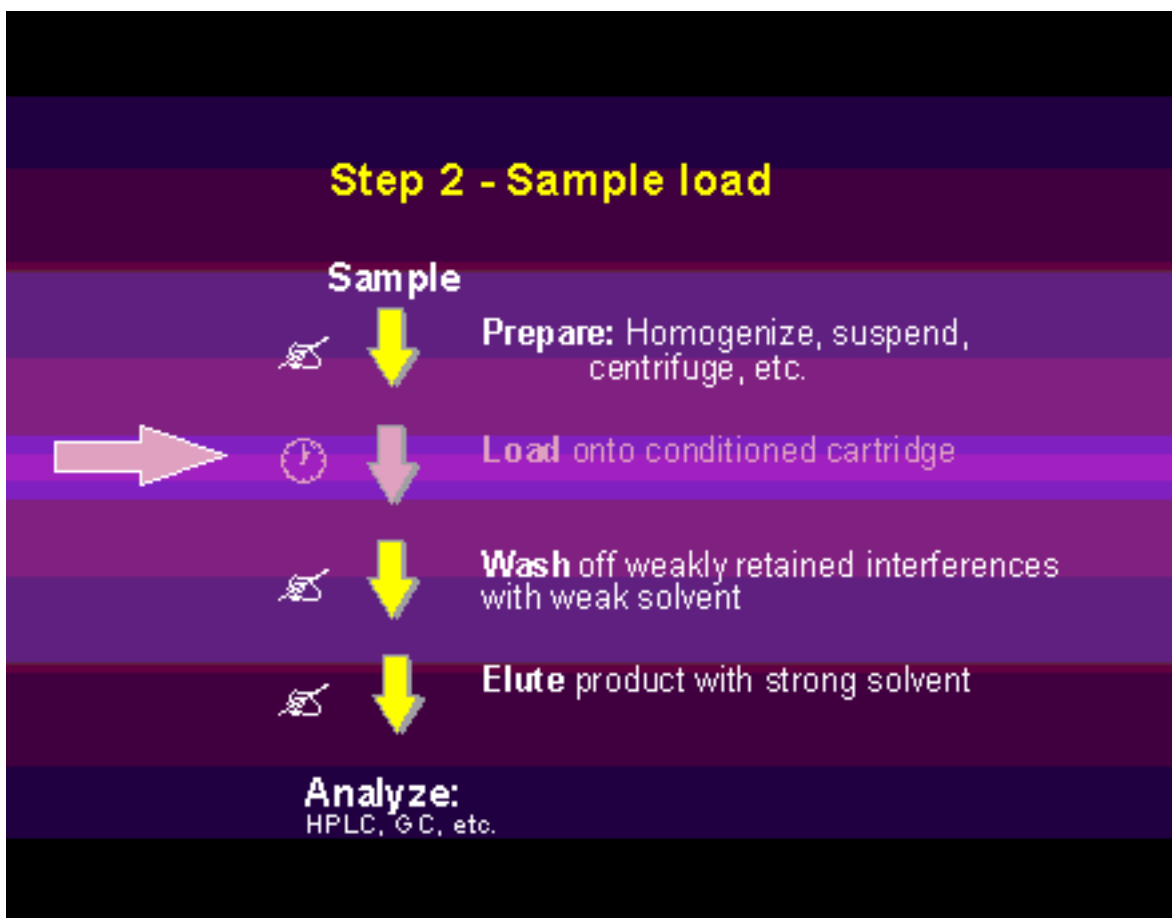
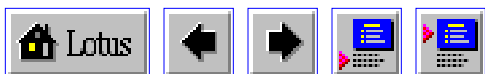
Step 1 - Sample preparation

- **Typical problems**

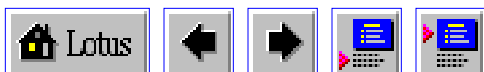
- ◆ Adsorption to test tube walls
- ◆ Adsorption to or inclusion in matrix solids

- **Possible solutions**

- Use silanized or plastic test tubes
- Homogenize more completely
- Add organic solvent to matrix



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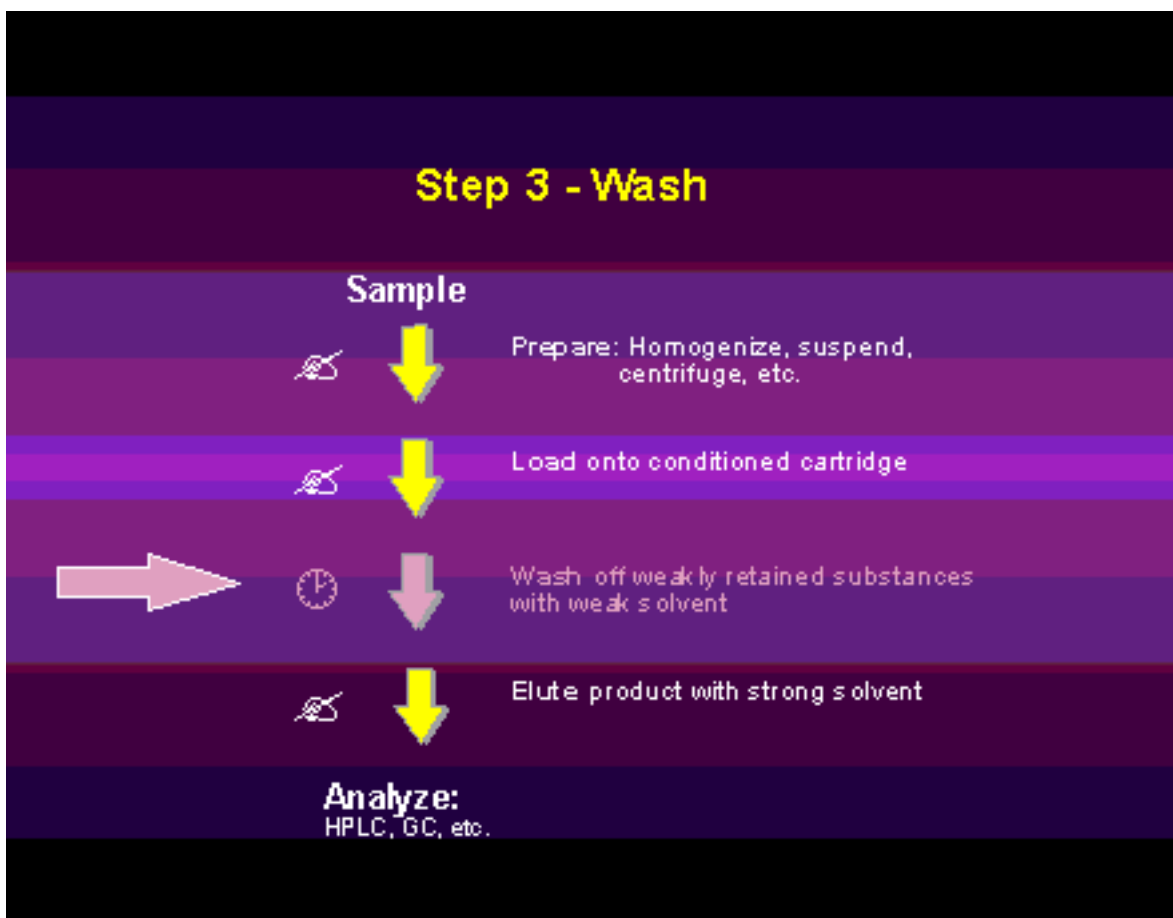
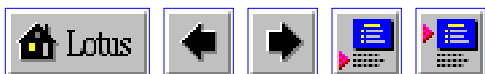
Step 2 - Sample Load

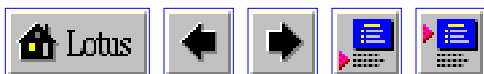
Possible problems

- **Improper conditioning of cartridge**
- **Poor analyte retention**
- **Matrix variability**
- **Volume overload**
- **Mass overload**

Solutions

- Condition cartridge as appropriate. Do not let dry.
- Dilute with weaker solvent, use stronger sorbent, use larger cartridge (Cs/Cm)
- Buffer sample to constant pH, ionic strength
- Decrease load volume, use larger cartridge
- Decrease load volume, use larger cartridge





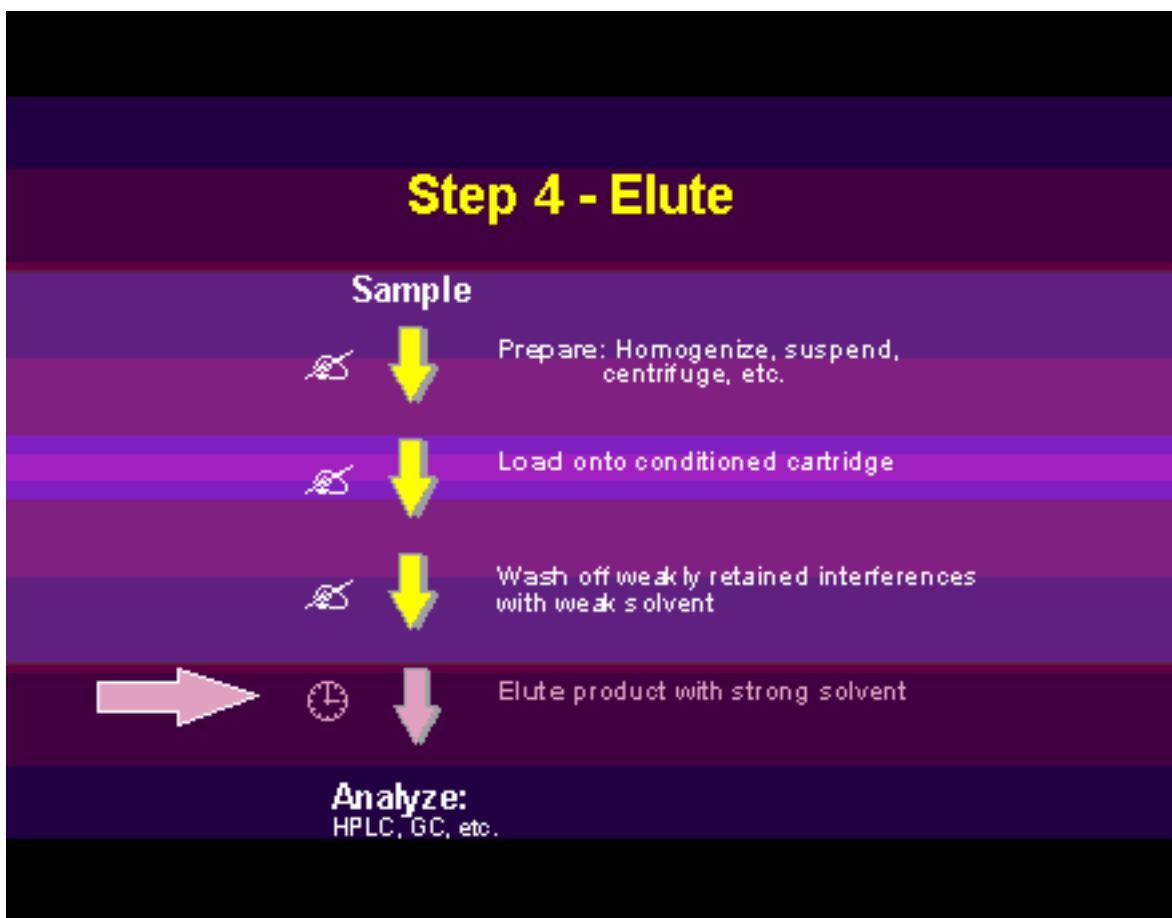
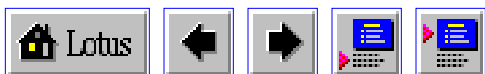
Step 3 - Wash

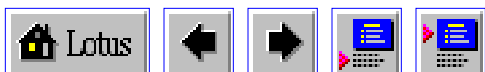
Possible problems

- Poor analyte retention
- Matrix variability
- Volume overload
- Mass overload

Solutions

- Use stronger sorbent, use larger cartridge
- Buffer sample to constant pH, ionic strength
- Decrease load volume, use larger cartridge
- Decrease load volume, use larger cartridge





Step 4 - Elution

Possible problems

- Too strong retention

- Silanophil interactions

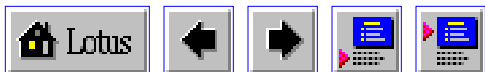
- Interferences coeluting during analysis

Solutions

- Use stronger elution solvent or increase volume of elution solvent

- Change pH of elution solvent, increase H-bonding strength of elution solvent, increase ionic strength, add competing amine

- Change mode of SPE separation



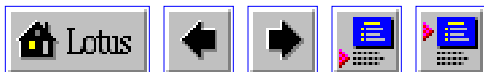
The Silica Surface

Silanophilic Interactions

The diagram illustrates a silica surface network. It features a central chain of silicon atoms (Si) connected by oxygen atoms (O). Each silicon atom is also bonded to oxygen atoms, some of which are part of silanol groups (Si-OH) and others that are bonded to R groups. The structure is shown in a perspective view, with some atoms and bonds extending outwards. The background of the slide is a gradient of purple and blue.

Characteristics of typical silica gels for SPE

- Surface area: $300 \text{ m}^2/\text{g}$
- Pore size: 125 Å
- Pore volume: 1 cc/g
- Degree of hydroxylation: 6-8 $\mu\text{mol}/\text{m}^2$
- Metal contaminants: Fe, Al, Na, Ca at 5-500 ppm



Mixed Mode Effects

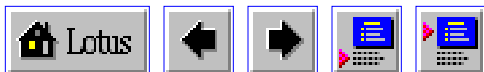
- **Silica surface prior to bonding**

- 6-8 μmol silanols per square meter

- **Bonding chemistry**

- 2-4 μmol bonded phase per square meter

- **Thus chromatographic surfaces range from 33% to 75% silanols (25% to 67% alkyl silanes)**

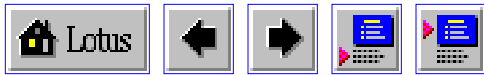


Incomplete Removal of Interferences

Generally due to similar retention behavior of interferences and analytes

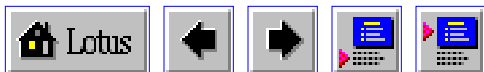
Examine method:

- ◆ Does pH/ionic strength affect selectivity?
- ◆ Adjust wash protocol
 - ◆ Elute more weakly retained interferences, but not analyte
- ◆ Adjust elution protocol
 - ◆ Elute analyte, but not more strongly retained interferences
- ◆ Use different SPE sorbent with different "chromatographic handle"



Low Recovery

- Poor conditioning procedure
- Poor adsorption of analyte (volume overload)
- Mass overload
- Incomplete elution
- Adsorption of analyte to matrix



High Variability / Poor Reproducibility

- Lot-to-Lot variability in sorbent chemistry
- Variability in matrix
 - pH
 - Ionic strength
 - Specific / Non-specific adsorption
- Method / Procedure not robust
- Operator variability



Conclusions

- ◆ SPE is fundamentally liquid chromatography
 - ◆ Typically less than 50 plates
 - ◆ Alpha greater than 4 required
 - ◆ Silanol/Mixed mode interactions
- ◆ Maximize analyte retention for adsorption
- ◆ Minimize analyte retention for elution

Conclusions

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