

SPE Column Preparation

SPE is a form of digital (step-wise) chromatography designed to extract, partition, and / or adsorb one or more components from a liquid phase (sample) onto a stationary phase (adsorbent or resin). An adsorbed substance can be removed from the adsorbent by step-wise increase of elution strength of the eluent (step gradient technique). SPE extends a chromatographic system's lifetime, improves qualitative and quantitative analysis, and the demand placed on an analytical instrument is considerably lessened.

In general, SPE is used for three important purposes in state-of-the-art analyses:

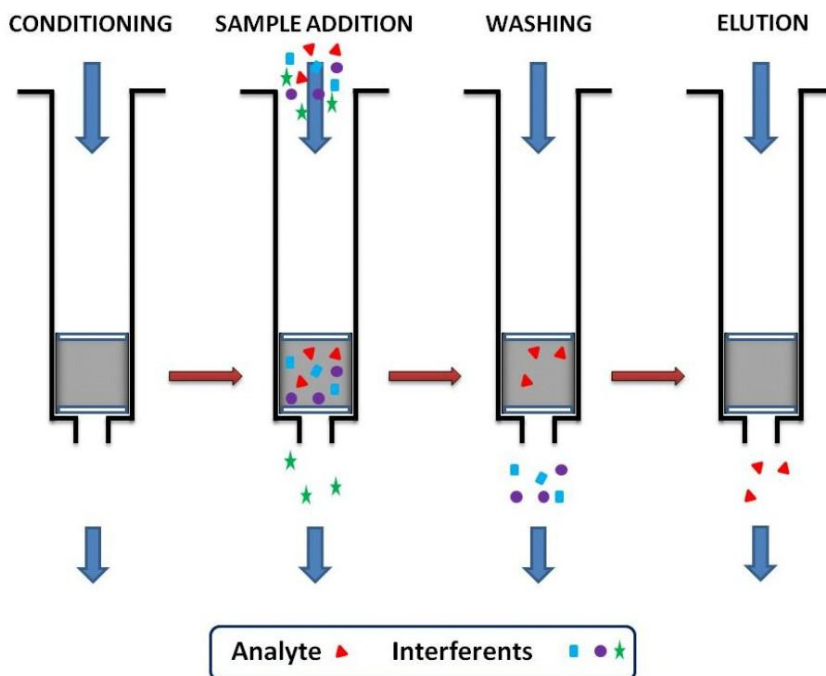
- Concentration of the analyte (up to factor 10.000 - increase of chromatographic sensibility / improved limits of detection)
- Removal of interfering compounds (protection of subsequent analyses like HPLC, GC, TLC, UV or IR spectroscopy)
- Changing an analyte's environment to a simpler matrix more suitable for subsequent analyses.

Advantages of SPE compared to classical liquid-liquid extraction:

- Lower consumption of solvents
- Faster - enormous time savings
- Lower costs per sample
- Potential for automation
- High consistency in individual sample handling
- More specific selectivity because of the broad range of adsorbents and different retention mechanisms
- Optimisation of extraction by variation or adjusting of the solid phase and chromatographic conditions

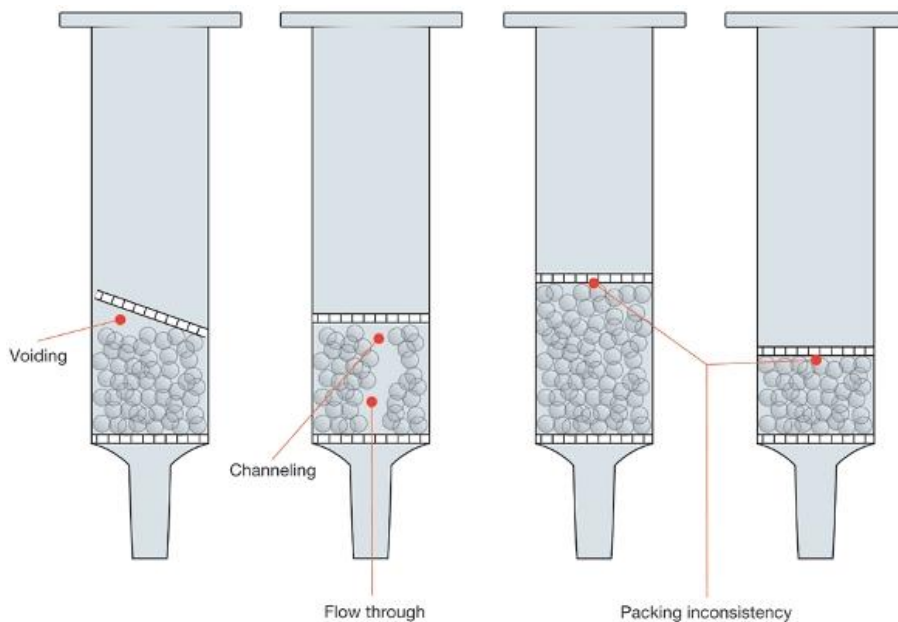
General Steps of SPE Preparation Process:

- **Sample Pre-treatment:** Dependent on compound of interest, sample matrix, and nature of retention chemistry; involves pH adjustment, centrifugation, filtration, dilution, buffer addition, etc.
- **Conditioning:** Solvent is passed through the SPE material to wet the bonded functional groups => ensures consistent interaction.
- **Equilibration:** Sorbent/ phase is treated with a solution that is similar (in polarity, pH, etc.) to the sample matrix => maximizes retention.
- **Sample Load:** Introduction of the sample = analytes of interest are bound/ extracted onto the phase/sorbent.
- **Washing:** Selectively remove unwanted interferences co-extracted with the analyte without prematurely eluting analytes of interest.
- **Elution:** Removing analytes of interest with a solvent that overcomes the primary and secondary retention interactions between sorbent and analytes of interest.
- **Evaporation of eluent/ reconstitution with mobile phase (optional).**



Important Questions

Conventional SPE cartridges and plates are packed with a loose powder (typically polymeric material or silica) positioned between two frits. During the production process, it is possible to over- or underfill the holder with the required amount of material; once packed, these beds are prone to settling and voiding during production and transport.



How to select solid phase extraction columns?

SPE columns may be classified, using the practice in HPLC, as reverse phase type, normal phase type, ion exchange type, and mixed mode type. If the sample is aqueous (such as water, urine, plasma, juice), C18 columns (reverse phase type) are most commonly used. If the sample is in organic solvents, a normal phase column (Florisil, Silica gel, Alumina etc.) should be used.

How to select solvents for activation, equilibration and collection in solid phase extraction?

Activation solvent should be able to wet the SPE column sorbent well and compatible with the solvent for equilibration. For columns of reverse phase type, methanol, iso propanol or other polar solvents can be used. For columns of normal phase type, hexane, toluene, or other low polarity solvents can be used. Solvents for equilibration should be compatible with the activation solvent, wet the sorbent well, and of low elution power. If the elution power is high, targeted compound will not be trapped well on the column. For reverse phase column, water or buffer solution is often used for equilibration. For normal phase column, a low polarity solvent is often used for equilibration. Solvents for collection should be compatible with previously used elution solvent, can remove the targeted compounds with small volume and easy to evaporate.

Packing SPE Step by Step

1. Loading Bottom Frit

➡ Push the bottom frit to the bottom of the cartridge using a push rod.



2. Filling Sorbent

➔ Keep the cartridge upright, place stemmed funnel onto the cartridge, add required amount of sorbent to the cartridge via the funnel. Remove the funnel after all sorbent slips into the cartridge.



3. Loading Top Frit

➔ Push the top frit horizontally to the top of the cartridge using a push rod.



4. Final Control

➔ Check the all parts in your column.

