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ANALYTE ISOLATION BY SOLID PHASE EXTRACTION (SPE) ON SILICA-BONDED PHASES

Classification and Recommended Practices

(Technical Report)

Prepared for publication by

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Analyte isolation by solid phase extraction (SPE) on silica-bonded phases: Classification and recommended practices (Technical Report)

Synopsis

This article reviews the technique of solid phase extraction (SPE) using silica bonded phases. In the first section, a classification is given of the different analyte isolation techniques and SPE is defined. An overview is given of the different kinds of sorbents available, especially focussing on the the chemically bonded silica phases. Further sections deal with SPE in practice: a description of a typical extraction column, the outlines of off-line SPE, the importance of flow rate, the capacity of the sorbent and what the important factors are for method development. Attention is paid to the different binding mechanisms in SPE as selectivity is a function of the interaction among the matrix, the analyte and the sorbent. Selectivity can be further enhanced using a combination of more than one solid phase, ion-pair SPE or solvent changeover. Furthermore the possibilities of trace enrichment and the fractionation of complex mixtures are considered. Two critical points in SPE, namely the between-batch and between-manufacturer variation and the possible elution of interfering compounds from the cartridge housing are discussed. In the section on automation, a description is given of the available equipment for off-line and on-line SPE. Finally, the applications of SPE are outlined.

OBJECTIVES AND CLASSIFICATION OF PRETREATMENT METHODS

Quantitative or qualitative analysis of an analyte usually requires an analyte isolation. The aim of the isolation step is to make the test portion suitable for the subsequent analysis and can include the isolation of the analyte from matrix components, concentration of the analyte, class fractionation or a combination of these objectives. The extent of isolation, purification and concentration of the analyte is determined by:

- the matrix itself (complexity, composition)
- the concentration of the analyte in the matrix
- the selectivity and sensitivity required in the subsequent analysis
- the analytical objectives (e.g., screening, quantitative or qualitative analysis)

An isolation scheme can consist of many different techniques, each of which can be classified according to their function in one of four major groups. The first group comprises those techniques whose aim is to release the analyte from the biological matrix and includes hydrolysis using either acids, bases or enzymes. A second comprises all techniques for the removal of endogenous compounds, such as deproteinisation, dialysis, liquid-liquid extraction or solid-phase extraction. The third group comprises procedures for liquid handling and include dilution, evaporation, dissolution, filtration etc.. Finally a group of techniques for enhancing the selectivity and sensitivity of the analysis can be distinguished, with, e.g., pre- and post-column derivatization [1].

The technique of solid phase extraction (SPE), an operation primarily enabling the removal of endogenous material, is surveyed herein.

DEFINITION OF SOLID PHASE EXTRACTION

A solid phase extraction (SPE) consists of bringing a liquid or gaseous test portion/solution in contact with a solid phase, or (ad)sorbent, whereby the analyte is selectively adsorbed on the surface of the solid phase. The solid phase is then separated from the solution and other solvents (liquids or gases) are added. The first such solvent is usually a wash to remove possible adsorbed matrix components; eventually an eluting solvent is brought into contact with the sorbent to selectively desorb the analyte.

In this article, attention will be focussed on SPE with the use of liquid test portions/solutions and solvents in the wash and elution steps.

Solid phase extraction is the term mostly encountered in the literature for this isolation method, but many synonyms exist, e.g., liquid-solid extraction (emphasizing the use of liquids and solvents instead of gases), column extraction, digital chromatography, bonded phase extraction (emphasizing the use of silica-bonded phases) or selective adsorption techniques [2-4].

SYNTHESIS AND TYPES OF SILICA BONDED PHASES

The classical adsorbents have been used for many years, e.g., carbon, magnesium silicate (Florisil), alumina or celite, macroreticular polymers and ion-exchanging resins. However a major advance in SPE was the introduction some 15 years ago of silica-bonded phases. Only the latter types of sorbents will be discussed herein. This does not mean that other possibilities do not exist. One can, for example, expect that polymeric phases will become more important and in inorganic analysis one uses columns loaded with complex formers, such as 8-hydroxyquinoline loaded on silica or bis(2-ethylhexyl)hydrogen phosphate loaded on plastic polymers.

The silica-bonded phases used in solid phase extraction are similar to those used in liquid chromatography (LC), except that the mean particle sizes of the silicas are different: for SPE irregularly particles with a particle diameter ranging between 30 and 60 μm is used [5], whereas the silica for HPLC usually has a particle diameter of 3 to 10 μm and can be irregular or spherical in shape.

The silica-bonded phases are synthesized by the reaction of silica surface silanol groups with chloroalkyl- or alkoxyalkylsilanes (Fig. 1) [8,9]. A reaction with monofunctional silanes can only result in a monolayer, while one with di- or trifunctional silanes can result in mono- or polymer layers (monomeric-bonded and polymeric-bonded phases), depending on the reaction conditions. In the synthesis not all surface silanol groups are bonded with an alkyl chain (through Si-O-Si-C bonds); as unreacted silanol groups are polar, acidic sites remain on the surface, which makes the interaction with analytes heterogeneous and more complex. Therefore, some sorbents are 'end-capped', i.e., a further

a) Reaction with mono-, di- or trifunctional silanes resulting in monolayers.

b) Reaction with di- and trifunctional silanes resulting in polymer layers.

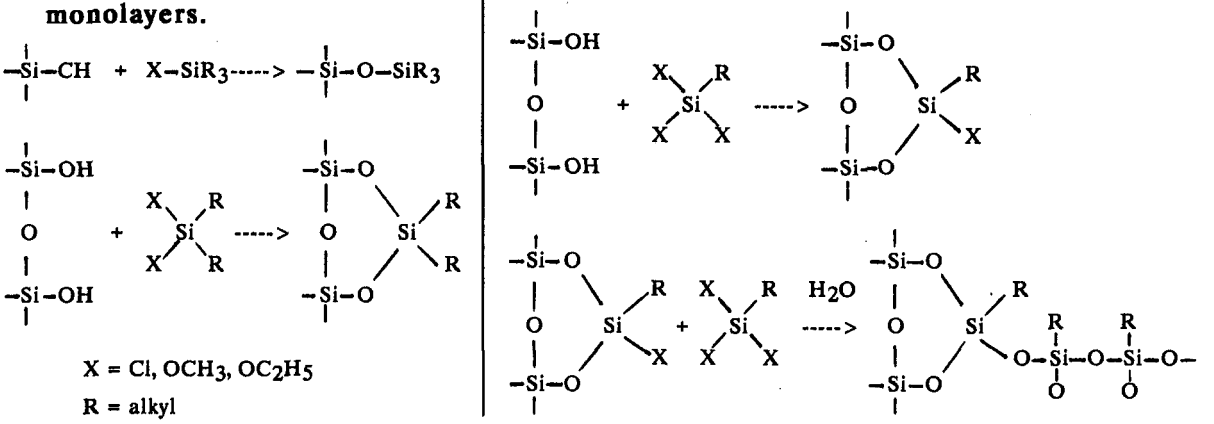


Figure 1 : Reaction of silica surface silanols with mono-, di- or trialkylchlorosilanes (8)

reaction is carried out on the residual silanols with trimethylchlorosilanes (Fig.2) [9,10]. These smaller chlorosilanes can still react with the surface silanol groups even when steric factors prohibit the larger long-chain chloroalkylsilanes. However, even after endcapping about 30 % of the silanol groups remain and can possibly interfere with an extraction of particular analytes [1].

Depending on the nature of the organochlorosilane reagent, bonded phases with a large range of functionalities can be prepared. They are commonly divided into three groups, depending on the main interaction mechanism :

1) apolar sorbents; 2) polar sorbents; 3) ion-exchange sorbents.

By analogy with HPLC, it is usual to distinguish two modes of SPE operation, namely the reversed phase mode when the sorbent is apolar and the solvents polar and the normal phase mode using polar sorbents with apolar solvents.

Table 1 gives a summary of the main commercially available silica-bonded sorbents . To this table one could add some mixed function phases, which possess both apolar and ion-exchange moieties and are employed in screening methods [11-13]. With such a wide range of sorbents, highly

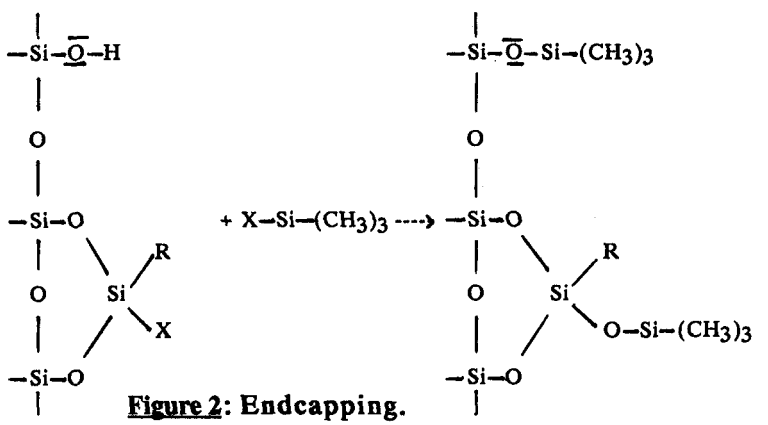
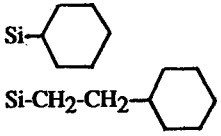
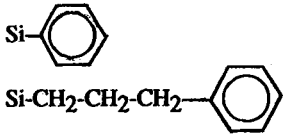
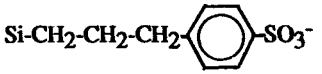
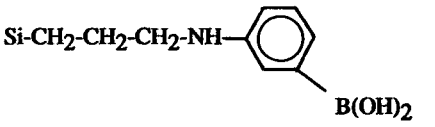
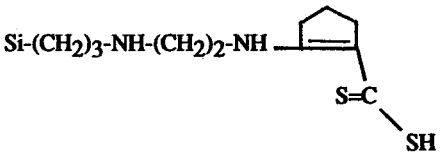


Figure 2: Endcapping.

Table 1: Alkyl-bonded functionalities of commercially available silica bonded phases [6,10,22]

Phase		Bonded moiety
APOLAR PHASES		
C_1	methyl	Si-CH_3
C_2	ethyl	Si-CH_2-CH_3
C_3	propyl	Si-$(CH_2)_2$-CH_3
C_4	butyl	Si-$(CH_2)_3$-CH_3
C_6	hexyl	Si-$(CH_2)_5$-CH_3
C_8	octyl	Si-$(CH_2)_7$-CH_3
C_{18}	octadecyl	Si-$(CH_2)_{17}$-CH_3
CH	cyclohexyl	
PH	phenyl	
POLAR PHASES		
Si	silica	Si-OH
CN	cyanopropyl	Si-CH_2-CH_2-CH_2-CN
2OH	diol	Si-CH_2-CH_2-CH_2-O-CH_2-CHOH-CH_2OH

Phase		Bonded moiety
ION EXCHANGE PHASES		
CBA	carboxylic acid	Si-CH_2-COO^- Si-CH_2-CH_2-COO^-
SCX	benzenesulphonic acid	
PRS	propanesulphonic acid	Si-CH_2-CH_2-CH_2-SO_3^-
NH_2	aminopropyl primary amine	Si-CH_2-CH_2-CH_2-NH_2
PSA	*N*-propylethylenediamine primary/secondary amine	Si-CH_2-CH_2-CH_2-NH-CH_2-CH_2-NH_2
DEA	diethylammoniopropyl tertiary amine	Si-CH_2-CH_2-CH_2-NH^+-$(CH_2$-$CH_3)_2$
SAX	trimethylammoniopropyl quaternary amine	Si-CH_2-CH_2-CH_2-N^+-$(CH_3)_3$
COVALENT BINDING		
PBA	phenylboronic acid	
METAL-LOADED PHASES		
ACDA	2-amino-1-cyclopentene-1-dithiocarboxylic acid modified silica	

selective extractions should be possible. The selectivity is further increased when making use of bonded phase silica capable of selective binding with the analyte, such as a phenylboronic acid bonded phase for the extraction of molecules with cis or coplanar adjacent hydroxyls (e.g., catecholamines, carbohydrates, nucleotides or ecdysteroids) [2,3,10,14]. High selectivity is also obtained with metal-loaded SPE phases : an ion-exchanging or ligand-exchanging moiety is bound to the silica and a metal ion (e.g., Hg(II), Ag(I), Pt(IV)) is immobilized on it. The interaction consists of a complex formation between the metal ion and the analyte [6,8].

USE OF A SPE COLUMN

The original way of performing a solid phase extraction was batchwise or static, i.e., by mixing the sorbent and the test solution or solvents in a tube and separating both phases by centrifugation or filtration. The batchwise extraction of catecholamines on alumina is a typical example [15-18]. In the alternative, dynamic way, the test solution or solvents are passed through the sorbent which is packed in a column. The latter is now the preferred method.

The sorbent can be packed manually, either dry or in a slurry, into a syringe barrel or a Pasteur pipette. However, several manufacturers produce disposable SPE columns containing different amounts of sorbent, packed between two frits (made of polyethylene, stainless steel or PTFE) (Fig. 3). Different sizes are available: the amount of sorbent packed ranges from 50 mg to 10 g and the volume of the corresponding reservoir varies from 1 mL to 60 mL. Larger volumes can be passed through a SPE cartridge either by using an adaptor to connect a larger volume or refilling the cartridge reservoir. The shape of the cartridge may differ depending on the manufacturer, on how the liquids are pushed through the sorbent and on the volume of the reservoir. The cartridge shown in Fig. 3A is the most common design. The solvents can be drawn through the cartridge by use of a vacuum device, offering the possibility to process between 8 and 30 test portions simultaneously. The flow of the solvent through the sorbent may also be obtained by centrifugation. The cartridge shown in Fig. 3B is a typical design for the preparation of single test portions using a syringe although such cartridges can be used with an

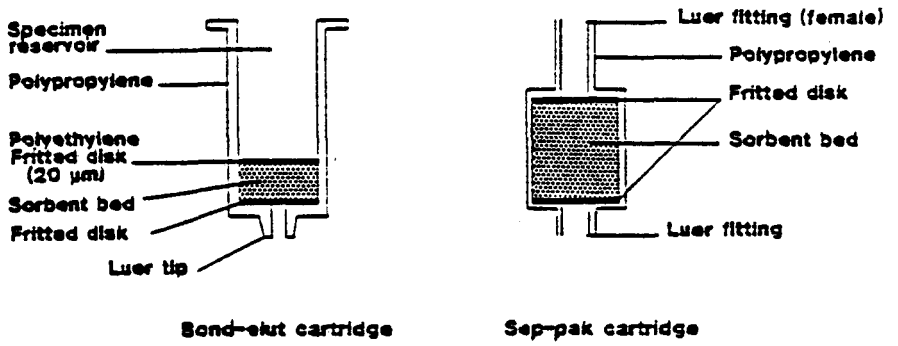


Figure 3: Design of disposable SPE columns(2).

appropriate vacuum manifold. Other cartridges have a shape compatible with the use of automated instruments or they are specially designed for use with robotics.

The most recent way to present the sorbent is as a disk, similar to a membrane filter, whereby the silica-bonded phase is impregnated in a PTFE matrix [19,20].

SPE IN PRACTICE [refs. 1–3, 5, 6, 10, 21, 22]

A solid phase extraction using silica bonded sorbents consists of five steps:

1) *Wetting the sorbent.*

First, a solvent capable of wetting the alkyl chains is brought in contact with the sorbent. In the dry state the bonded alkyl chains are twisted and collapsed on the surface; on contact with a suitable solvent, they are solvated so that they spread open to form a bristle. This ensures a good contact between the analyte and the bonded phase in the adsorption step. It is important that the sorbent remains wet in the following two steps. Failure to perform this stage will result in poor recoveries of the analyte.

Apolar and ion-exchange sorbents can be prewetted with five to ten volumes of a water miscible solvent like methanol. In the normal phase mode, i.e., with polar sorbents, five to ten void volumes of a nonpolar solvent (e.g. the solvent in which the analyte is dissolved) can be used.

2) *Conditioning of the sorbent.*

Five to ten void volumes of a solvent or buffer similar to the test solution that is extracted is pulled through the SPE column. In reversed phase mode extraction, the solvent used for wetting can be replaced by water or buffer. For ion-exchange sorbents a buffer must be used with a pH at which the analyte and the functional ionic (fixed) site have an opposite charge. Failure to carry out this stage can result in the first portion of the test solution conditioning the phase which could result in inefficient recovery.

3) *Adsorption step.*

The test solution is passed through the sorbent bed at a controlled flow rate. To ensure a complete retention of the analyte(s), it may be necessary to change the test solution characteristics (by changing pH, polarity or viscosity).

4) *Wash step.*

By passing a suitable solvent through the SPE column possible interfering matrix components can be eluted, while the analyte of interest remains adsorbed.

5) *Elution.*

In this last step the analyte is desorbed from the solid phase with an appropriate solvent.

METHOD DEVELOPMENT AND INTERACTION MECHANISMS [refs. 1, 2, 10, 11, 22]

The selectivity of a SPE procedure is determined by the sorbent and the solvents used in the adsorption, wash and elution steps. The development of a SPE method requires the selection of the correct sorbent as well as the optimum adsorption, wash and elution conditions. To achieve this it is necessary to consider a number of analyte and matrix characteristics, as well as the possible interaction mechanisms among the analyte, the matrix and the sorbent :

a) *analyte characteristics* : pK_a values if any, solubility, molecular mass, polarity, molecular structure and the presence of special acid or basic functions.

b) *matrix characteristics* : polarity, presence of co-extractable compounds.

c) If the analyte is subsequently chromatographed, *data about the HPLC* system to be used (which stationary phase, mobile phase) and retention data.

d) *interaction mechanisms* : Van der Waals binding forces are non-specific attractive forces between any two atoms when they are less than 0.3 - 0.4 nm apart. They can only become significant when numerous analyte atoms can simultaneously come close to numerous sorbent atoms, meaning that they must be sterically complementary [23,24]. Hydrophobic interactions are van der Waals binding forces between apolar groups (apolar analyte and apolar sorbent) that are enhanced in an aqueous medium. Owing to the high affinity among the water molecules (hydrogen bonding), apolar molecules are excluded from the water and tend to associate by means of van der Waals forces. These apolar binding forces are weak and non-specific : they are typically found between the carbon chain bonded on the silica surface and the carbon skeleton of an analyte. Breaking these forces can easily be performed by replacing the water molecules, ordered around the analyte retained on the carbon chain of the solid phase, with less polar molecules.

Polar interactions include hydrogen bonding, dipole-dipole interactions and induced dipole-dipole interactions. A hydrogen bond is an especially strong dipole-dipole interaction, whereby a hydrogen atom forms a bridge between two strongly electronegative atoms (e.g., O, N, F). These polar interactions occur on the silica sorbent and sorbents with a cyano, diol or amino functional group and are enhanced in an apolar medium. Their binding energy is somewhat higher than that for apolar interactions. Water molecules compete with the analyte for the polar binding sites on the sorbent.

If an analyte can be ionised, an electrostatic interaction on an ion-exchanging sorbent can result in a strong and selective retention. Disrupting electrostatic binding needs a pH change to suppress the ionic character of the analyte or the ion-exchange sorbent or the use of a competitive ion.

The strongest possible interactions are those by covalent bonding. In SPE these covalent bonds are rare. An example is the binding of catecholamines on the phenylboronic acid bonded phase [2,3,14].

Table 2: Interaction forces and the corresponding binding energies.

Interaction mechanisms		Binding energy in KJ/mol (3,5)
apolar	dispersion forces or Van der Waals forces	4-20
polar	hydrogen bonds	20-40
	dipole-dipole interaction	10-40
	induced dipole-dipole	8-25
anion- or cation-exchange		200-1050
covalent bonds		410-3360

Also the interaction on a metal-loaded phase is a coordination bond: a complex is formed between an analyte and a metal ion, immobilized on a silica bonded ion-exchanging or ligand-exchanging phase. The interaction can be disrupted by a pH change or the introduction of a competing ligand or a solution of a metal salt [6].

The choice of a proper sorbent and adsorption, wash and elution conditions must be made after consideration of the four items (a to d).

In the adsorption step the analyte-sorbent interaction must be enhanced, while the matrix-analyte and matrix-sorbent interactions must be reduced. An example of a matrix-analyte interaction is the protein binding of a drug. A matrix-sorbent interaction is, for example, the binding of compounds present in urine or plasma on an ion-exchange phase, thus blocking the ion-exchange sites of the sorbent and hindering the adsorption of the analyte. Pretreatment of the test portion, such as dilution, deproteinisation, precipitation of salts or filtering, can reduce these analyte-matrix and matrix-sorbent interaction. On the other hand, the analyte-sorbent interaction may be increased by altering the pH or polarity of a test solution. Changing the pH influences the dissociation of a molecule and its polarity. The apolar binding of a compound on an apolar, reversed phase sorbent can be improved by increasing the polarity of the adsorption medium (e.g., the addition of water or a salt) and the polar binding on a polar sorbent can be improved by decreasing the polarity of the adsorption medium (e.g., removing excess of water, adding low polarity organic solvents).

In the choice of an appropriate wash and eluting solvent, the pH, ionic strength and polarity also play a key role in determining the retention or elution of an analyte.

In the wash step, the conditions must be chosen in such a way that the analyte is retained, while other, possibly interfering matrix compounds elute. This requires again a good prior knowledge of what interfering compounds might be present in the matrix and the analyte properties. For a C_{18}-cartridge , which retains compounds by means of van der Waals forces, the choice of washing solvents is restricted

to polar solvents because a decrease in solvent polarity causes co-elution of the analyte. In reversed phase extraction water containing the maximum amount of organic solvent at which the analyte is still adsorbed is often used. In contrast, polar cartridges can be washed with an appropriate apolar, organic solvent. An ion-exchange sorbent, retaining an ionic analyte through strong electrostatic forces, can be washed with polar as well as apolar solvents to remove both polar and apolar interferences, as long as the pH and ionic strength remain adjusted for the analyte's retention.

The eluting solvent must desorb the analyte in as small a volume as possible to avoid the need for a concentration step later in the procedure. The conditions are opposite to those in the adsorption step: if for instance a high polarity is required to adsorb an analyte, a low polarity will facilitate its elution, or vice versa. The dispersion forces which retain analytes on an apolar solid phase can be broken with organic, apolar solvents (methanol, acetonitrile, ethyl acetate, hexane, methylene dichloride...); for polar interactions solvents as water, methanol, 2-propanol, acetic acid, amines or high ionic strength buffers are mostly used. The elution from an ion-exchanging phase normally requires a pH modification so that the analyte or the bonded phase functionalities become uncharged. Additionally, the ionic strength can be increased or counterions added with a high affinity for the ion-exchanging phase, so that the analyte will be displaced from the sorbent.

Both the eluotropic strength and the compatibility of the solvent with the subsequent analytical method used to quantify or identify the extracted analytes must be considered. If the extract is analysed by HPLC, the solvent strength of the eluent must be smaller or equal to the strength of the mobile phase to avoid band broadening. Otherwise additional stages may be required, e.g. dilution with the mobile phase.

The development of an SPE procedure is not always easy. Most sorbents are capable of binding compounds through more than one interaction mechanism. An apolar C_8 - sorbent would be expected to bind molecules by the primary interaction of apolar forces, but secondary polar interactions can occur at the residual silanol sites. A sorbent such as the aminopropyl-bonded phase can have three types of interactions occurring simultaneously: anion exchange at the aminopropyl function, apolar binding at the carbon chain and polar interactions at the residual silanol sites. The extent of secondary interactions at the residual silanol groups is affected by steric hindrance : the residual silanol groups are less accessible on, e.g., a C_{18}-sorbent than on a cyano-bonded phase. Secondary interactions become important in an extraction depending on the physico-chemical properties of an analyte and on the adsorption and wash conditions. Silanol activity can, for example, be reduced by masking these acid sites with positively charged bases, which are brought in contact with the sorbent in the conditioning step, or by working at a pH where the silanol sites or the analyte are uncharged [1].

The evaluation of an analyte isolation scheme during method development involves the control of each step for possible losses and the determination of the overall elution recovery of the analyte. This can be done by collecting the solvents in each SPE step and analysing them using the analytical technique to be applied for measurement, e.g., HPLC. Practical problems can however arise during the evaluation: for instance, the liquid collected after the adsorption step may still contain a large number of matrix compounds, resulting in many interfering peaks on a chromatogram and making it difficult to measure the analyte. Such problems can be avoided if one can make use of a radiolabelled form of the analyte and determine the radioactivity by liquid scintillation counting on each collected SPE step [1,2,25].

SELECTIVITY OF SPE

The selectivity of a sorbent (or wash and eluting solvent) is determined by the difference in affinity for the analyte compared to the matrix compounds.

The octadecyl sorbent is generally considered to be the least selective, as almost all molecules (analytes and interfering matrix compounds) can to some extent bind through van der Waals forces. Selectivity is enhanced when switching to more polar sorbents with shorter alkyl chains. For a lipophilic analyte, having no potential interaction with selective phases (such as ion-exchange or covalent binding), Doyle [26] advises first to investigate a C_2 solid phase instead of a C_{18}. The C_2 phase is often apolar enough to retain the analyte, whereas endogenous interfering compounds will be less well retained. A second advantage is that elution will be easier from a C_2 phase, provided no secondary interaction at the residual silanol groups occurs. The possibility of using a sorbent of intermediate polarity was also demonstrated in the work of Musch and Massart [27] when a SPE method was developed for the isolation of basic drugs from an aqueous medium or plasma using a cyano-bonded phase which resulted in clean plasma extracts. Acceptable recoveries for 24 of 30 drugs were obtained when eluting with methanol containing 0.1 % propylamine or with a phosphate buffer at pH 3, I = 0.05/methanol (1:1 volume ratio). If an analyte is ionisable, the use of ion-exchange phases can be considered to achieve a more selective isolation of the analyte. It is however too simple to conclude that the selectivity of a sorbent increases with the binding strength of the potential interaction between the analyte and the solid phase, as the selectivity also depends on the matrix. Whether the SPE of an ionic analyte on an ion-exchanging phase is selective or not will depend on the ionic strength of the matrix and the affinity of other ions present for the ion-exchanging sorbent.

The highest selectivity is obtained via covalent binding with the sorbent; the binding energy of a covalent interaction ensures that the matrix has less impact on the selectivity. The use of silica-bonded, metal-loaded phases can result in selective SPE methods, provided that the test solution contains low

concentrations of both competing ligands or metal ions and inorganic anions capable of forming a complex with or precipitating the immobilized metal ion. An example is the clean-up of river water matrices, whereby the anilines, which interfere in the analysis of phenylurea herbicides, are selectively removed on a Pt^{IV}-silica bonded phase. The Pt forms a complex with the primary amines (anilines), whereas the substituted ureas are unretained [6].

If insufficient selectivity is obtained with one sorbent, a sequential use of two different sorbents is possible, which is referred to as dual cartridge mode or chromatographic mode sequencing. Each sorbent should have a different primary interaction mechanism, such as in the combination of an apolar phase with an ion-exchanging phase. Süss [28] needed to switch to a dual cartridge method using a C_{18} sorbent and a strong cation-exchanging sorbent in series for the extraction of benperidol from plasma because the plasma extracts using a C_{18} cartridge alone contained substantial amounts of impurities. Bradbury et al.[29] described the extraction of 25-hydroxyvitamin D and its metabolite from plasma using first an apolar C_{18} sorbent, followed by a polar silica cartridge.

For small molecular mass, lightly polar or ionic compounds the retention on an apolar sorbent can be increased by ion pair formation. The principle of ion-pair liquid chromatography [9] can simply be transferred to the SPE cartridge. A counter-ion of opposite charge to the analyte is adsorbed on the sorbent in the conditioning step and is added to the test solution, possibly also to the wash solvent. The solvents must be buffered at a pH where both analyte and counter-ion are charged. The ion pair formed is more apolar than the initial analyte and therefore better retained through van der Waals forces. Desorption can be effected with organic solvents. The counter-ions used for ion-pair SPE are the same as in ion-pair HPLC (e.g., alkylammonium salts for acids and alkanesulphonate for bases). Ion-pair SPE has, for instance, been used to extract preservatives and the artificial sweetener saccharin from foodstuffs [30], the herbicide paraquat in rat brain [31] or vinca alkaloids from serum and urine [32].

Although a C_{18} sorbent should, theoretically, be used in reversed phase mode, and a cyano-bonded phase in normal phase mode, SPE methods have been described using these phases in either mode, which also contributes to the selectivity of the extraction. The solvent of a test solution normally dictates the mode used in the adsorption and wash step (reversed phase for aqueous test solutions, normal phase for apolar solvents). Afterwards, the sorbent is dried completely by aspirating air through it and in the elution step a solvent changeover is made (from polar to apolar or vice versa), switching to the alternative mode. Because of the drying step, even immiscible solvents can be used in the adsorption and elution step. Lehr and Damm [33] made use of such a solvent changeover in the extraction of a broad-spectrum antimycotic drug, ciclopirox, from plasma: the methyl derivative of the drug, dissolved in hexane, was adsorbed on a CN cartridge, washed with toluene, the sorbent was dried by aspiration and

the analytes eluted with a mixture of water and acetonitrile. Another example is the extraction of fluconazole from rodent diet [34] whereby the food was first extracted with methylene chloride which was filtered and then aspirated through a conditioned CN cartridge. After drying the sorbent, fluconazole was eluted with a methanol-water mixture. For the extraction of corn and soybean herbicides from well water, Schuette et al. [35] adsorbed the herbicides on a C_{18} sorbent and eluted with methylene dichloride. The methylene dichloride was passed through an anhydrous sodium sulfate bed to eliminate water residues.

In some cases, SPE may be combined with other pretreatment techniques as deproteinisation, salting-out or liquid-liquid extraction for additional selectivity.

FLOW RATE DURING SPE

The importance of the flow rate and whether the flow rate needs to be strictly controlled during SPE is not always made clear in the literature. For the extraction of pesticides from water matrices on 100 mg C_{18} sorbent, developed by Junk and Richard [36], flow rates as high as 250 mL/min could be employed in the adsorption step, without any effect on the recoveries : they stated that the small particle size and the high surface area of the C_{18} phase ensured a good contact between the analytes and the octadecyl functional group, even at high flow rates. On the contrary, other authors claim that the flow rate can have an influence on the recoveries, because both the adsorption and elution steps involve an equilibrium process between the solid phase and the analyte [1,5,11]. At higher flow rates, non-equilibrium conditions may exist, which ultimately result in lower recoveries, lower breakthrough volumes and larger elution volumes. Recommendations have been made to keep the flow rate beneath 2 mL/min [11] or to keep the vacuum pressure of the manifold lower than 30 kPa [1].

CAPACITY OF THE DISPOSABLE SPE COLUMNS

The capacity of a sorbent can be defined as the maximum amount of material (analytes and interferences) retained by a given mass of sorbent from a specific test solution [10,22]. The capacity depends on the type of solid phase used and the bed volume and is related with the analyte's retention, which in turn is dependent on the solvents or matrix the analyte is dissolved in [5,6]. Estimates of the column capacity of silica bonded phases lie in the mg/g range.[10,22]. As the concentration of analytes extracted from pharmaceutical, biomedical, environmental or food material usually lie in the ng/mL or µg/mL range, the capacities of the available sorbent quantities should be sufficient for most applications, even with all matrix interferences that can, together with the analyte, be adsorbed [5,6,10,37]. Choosing a larger sorbent mass (1 g instead of 100 mg for instance) can be of interest if large volumes need to be extracted (e.g., measurement of organic compounds in waste water) or to compensate for a weak affinity

of a solid phase for the analyte [4]. If a large volume of a test solution is aspirated through the cartridge, the loading of the analyte occurs according to the principle of frontal chromatography: at the start, the analyte is retained on the sorbent in a small band. Continuing the loading, all adsorption sites become gradually occupied by the analyte, until no sites are left and the analyte can no longer be retained and it 'breaks through'. This breakthrough occurs more rapidly for weakly retained analytes. By selecting a larger sorbent mass, the number of sites is increased and consequently the 'breakthrough volume', or the amount of analyte loaded on the solid phase, is increased. It should, however, be emphasized that the breakthrough volume is primarily controled by factors that must be optimised during the method development (appropriate wetting and conditioning of the solid phase, the solvent and pH of the test solution and the selection of the sorbent for extraction), and changing the mass sorbent is the last possibility to be attempted to increase the breakthrough volume. The breakthrough volume of the cartridge can be determined experimentally by means of a frontal analysis chromatogram or breakthrough curve. The breakthrough volume is thereby determined as the first deviation from the baseline. Equations permitting the estimation of the breakthrough volume have been developed [5,6].

TRACE ENRICHMENT WITH SPE

The sensitivity of an analytical method is primarily determined by the physicochemical properties of the analyte and the detection system used, but can be improved if the analyte isolation includes, besides a selective clean-up and isolation, a concentration step. SPE offers the possibility of concentrating the analyte by performing the elution with a volume smaller than the volume loaded on the cartridge. In practice, this concentration step, or trace enrichment, is especially useful for the analysis of organic compounds, herbicides and pesticides in water (drinking water, lake or river water) [6,35,36]. In these applications volumes up to 1 liter of water are pulled through a cartridge, while the volume for the elution is less than 5 mL. Thus concentration factors of up to 1000 are attained.

In biomedical applications, the volumes of the test portions are mostly limited to a few milliliters, so that the maximum achievable concentration factor is much lower.

Another way to concentrate the analyte is to elute with an organic, relatively volatile (low boiling point) solvent, which is evaporated to dryness. The residue can then be dissolved in a smaller volume of an appropriate solvent [27]. However, due to the additional evaporation step, the advantage of speed with SPE is lost. If the subsequent analysis consists of HPLC, then it is possible to elute in a larger volume of a solvent that is weaker than the mobile phase and concentrating the extracted compounds at the head of the column. The disadvantage of both working concentration methods is that all co-eluted interfering compounds are also concentrated.

THE FRACTIONATION OF TEST PORTIONS USING SPE

Besides the isolation and purification of the analyte of interest, SPE on disposable silica bonded phases can be used to fractionate a complex mixture into compound classes. A fractionation can be achieved by using solid phases with varying selectivities for the different compound classes and/or by using eluting solvents with varying selectivities. An example is the fractionation of combustion-generated analytes into four compound classes on a cyanopropyl cartridge, using eluents with increasing polarity. The most apolar hexane fraction contained the aliphatic hydrocarbons, alkyl-substituted aromatics and 2- and 3-ring aromatic hydrocarbons; in the subsequent benzene fraction the 3-6 ring PAH were eluted; the methylene dichloride fraction contained the 7-10 ring PAH and, finally, the very polar compounds were eluted with methanol. Each fraction could be separated further by HPLC [38]. Ecdysteroids, extracted from insects, ticks, cestodes and nematodes, can be fractionated into classes containing the free ecdysteroids, the conjugated derivatives and the acidic metabolites, using a combination of a silica (NP) and a C_{18}-phase (RP) and eluents of varying polarities [39]. For the identification of color additives in candy, gelatin dessert and drinks, a method is available whereby a mixture of dyes is fractionated on a C_{18}-cartridge using 2-propanol and water in different ratios as eluting solvents [40]. Commercially available 'mixed function' or 'mixed mode' sorbents have both hydrophobic and ion-exchanging moieties bonded on the silica particles, so that fractionation of a test portion becomes possible on a single SPE column, by adjusting the polarity and pH of the solvents. Such mixed function phases have been applied for the toxicological screening of acidic, neutral and basic drugs in plasma or urine [13].

REPRODUCIBILITY OF SPE

Differences in recovery have been observed when transferring a SPE method developed on sorbents of one manufacturer to sorbents of another manufacturer [41-44]. Even for batches of sorbents of the same manufacturer varying recoveries have been observed [42,45,46]. This between-manufacturer and between-batch variation for SPE sorbents is probably comparable with the variations observed for HPLC columns [47-49] as the synthesis of the two types of bonded silica is similar and both use the distribution of the analyte between solid and liquid phases. A difference in the percentage coverage with functional groups and the percentage of residual silanols, or the degree of endcapping, are most often ascribed to be the cause of these between-batch and between-manufacturer variations [1,41,44]. The support porosity and the monomeric or polymeric structure of the chemically bonded phase [50] are two other sorbent characteristics reported to indirectly influence the recovery with SPE. For silica with wider pores, a higher coverage density was obtained, resulting in changes in analyte recoveries. The differences observed in analyte recovery on a monomeric and a polymeric-bonded phase were explained by the difference in the number of unblocked silanols. The presence of 'highly energetic' silanol groups,

responsible for irreversible binding of basic compounds, was also put forward. Further characteristics expected to be important in connection with the reproducibility of silica bonded phase are: the mean particle size and pore size and their distributions [1], the surface area, the original silica particles, the methods for synthesis of the bonded phases and the mass of sorbent packed per cartridge.

Some manufacturers add a quality assurance report to each box of SPE columns, mentioning items such as the particle size distribution and the mean particle size, the specific surface area, the percentage surface coverage, the turbidity and the colour of a methanol extract.

CONTAMINATION FROM THE CARTRIDGE

One of the problems experienced with SPE is the co-elution of interfering compounds from the solid phase packing and the cartridge. Musch [27] reported an impurity co-eluting from the CN-cartridge when acetonitrile was used in the eluent and Kikawa et al. [51] mentioned the appearance of artefact peaks in the chromatographic analysis after SPE using silica cartridges. Pre-washing the cartridges could prevent the occurence of the interfering peaks. Junk et al. [52] investigated the possibly interfering compounds extracted from the polypropylene housing, the polyethylene frits and the C_{18}-bonded silica phase. The main compounds identified in extracts of the cartridges were remnants of the polymerisation process (alkanes and alkenes), plasticizers and antioxidants. The degree of interference depended on the solvents used in the SPE and varied between cartridges from different batches and different suppliers. Manufacturers have responded to the problem of plasticizer contamination by the introduction of inert glass cartridges with PTFE frits [12] or cartridges with stainless steel frits.

AUTOMATION

Automation of analyte isolation makes it possible to analyse routinely large numbers of test portions, as the analyses can continue overnight and a test portion can be extracted while the analyte of a previous portion is being quantitated or identified.

In addition to the higher throughput, an automated analysis is, compared to manual methods, more consistent in the sense that fewer outliers occur [53]. Higher throughput and higher consistency in the results are related to the limited number of manipulations in an automated method [1,54]. A classical SPE method, performed off-line and manually, requires at least the five steps as described above, and often additional manipulations such as dilution or evaporation are necessary. Compared with liquid-liquid extraction, the time of the extraction and the number of manipulations are already significantly decreased. The number of manual steps can be further decreased with the automation of the SPE procedure. An on-line solid phase extraction combined with HPLC only requires the loading of the test aliquots, possibly previously centrifuged, in the autosampler [7,55].

If an SPE method is performed manually, one can handle one test solution at the time using a syringe, or up to 30 test solutions can be processed simultaneously using a vacuum manifold. Two types of vacuum devices are available: with the simplest system, the waste solvents are collected in the vacuum device and tubes or vials for collection of the eluent are placed in the manifold prior to the elution step. In a second system the cartridges are placed on a disk that can be rotated to a waste or a collect (elution) position. In the waste position the solvents passing the sorbent are collected in the vacuum device, in the collect position the eluent is directed into tubes.

Manual SPE is off-line, which means that the extraction and the subsequent analysis are completely separate and the extract must be transferred to the chromatograph or other analytical cquipment. The alternative is to work on-line, whereby the eluent of the SPE column is automatically directed into the chromatograph for the separation and quantitation of the analytes of interest.

For automated SPE, two categories of equipment can be distinguished : instrumentation mimicking the off-line SPE procedure and the on-line SPE procedure with column switching.

The first category of equipment mimicks the different manual manipulations of the SPE by means of a robotic arm : test solutions can be mixed, different liquids can be pushed through a cartridge under positive pressure, condition, adsorption and wash solvents are directed to the waste, and the eluents are collected in vials. The volumes and the order in which the different solvents must be dispensed are programmed. Sometimes it is possible to filter the test solution or evaporate solvents, or to inject the final extract automatically for the chromatographic analysis [56]. In the latter case, the automated off-line SPE is integrated with the subsequent analysis and turned into an on-line procedure. With this equipment it is possible to automate any manually developed SPE method.

In on-line SPE with column switching a SPE column is placed in front of the HPLC column and the apparatus is extended with an additional pump, a solvent selection valve and a high pressure switching valve. A simple scheme for column switching is shown in Fig. 4 [7]. The solvent to condition the sorbent is first pumped through the SPE column and directed to waste, then the test solution is loaded on the cartridge and rinsed with an appropriate solvent. In the adsorption and rinsing step the percentage of organic solvent must be kept low to ensure the analyte's retention. For the elution, the valve is switched so that the mobile phase passes through the SPE column and flushes the analytes on the analytical column. While the analytes are separated on the chromatographic column, the high pressure valve is switched back and the cartridge is reconditioned to start the extraction of the next test solution.

The SPE column used in an on-line SPE system with column switching is commonly refered to as the 'pre-column' [6,7]. This term can be confused with the precolumn used in HPLC based on liquid-liquid partitioning: this is a guard column placed between the HPLC pump and the injector and

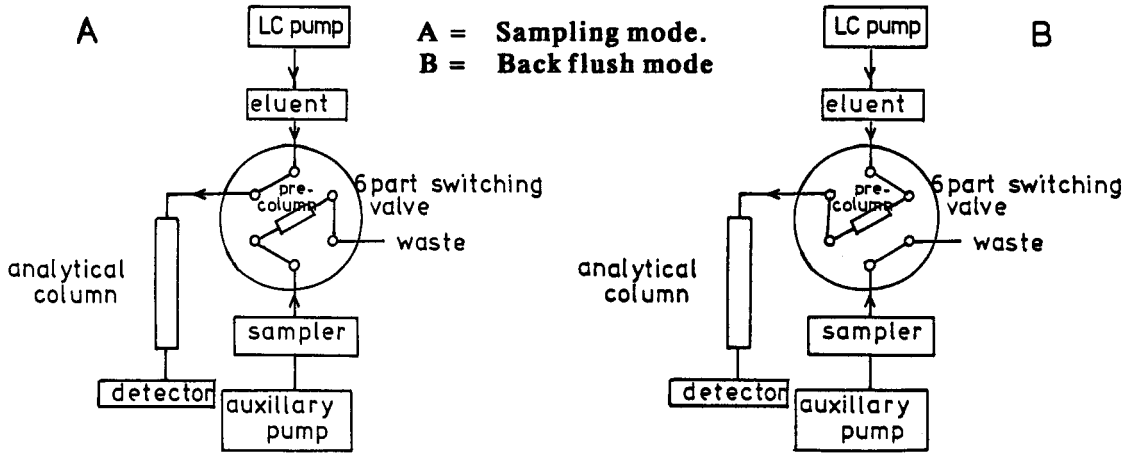


Figure 4 : Column switching scheme for atuomatic test solution processing with a SPE column. Adapted from reference 7.

presaturates the mobile phase with the stationary phase [9]. Therefore the more explicit term 'SPE column' is preferred to the commonly used term 'pre-column'.

On-line SPE has some advantages over the off-line procedures: the number of manipulations decreases, which favours the precision of the analysis, lowers the risk of contamination and reduces human input errors and labour costs. If the on-line SPE is performed by means of a column switching procedure, a closed system is formed from the injection port up to the chromatography, offering additional advantages. First, the closed system also prevents contamination. Secondly, the total amount of analyte concentrated on the SPE column reaches the analytical column. Off-line SPE or an on-line robotic SPE system, on the contrary, can involve a loss of sensitivity because only a part of the extract is injected on the analytical column. Some methods require evaporation of the eluent, which can be a source of losses due to thermal decomposition [6] or adsorption to the vessel. On the other hand, a SPE method involving column switching is more complex to develop as many factors need to be optimised and controlled. Special attention must be paid to the breakthrough volume in the adsorption and wash step and to peak compression on the top of the analytical column. As the choice of the eluent is restricted to the mobile phase used in the subsequent HPLC analysis, peak broadening must be avoided by means of a proper choice of the type and length of the SPE column, the type of column switching, i.e. forward or back flushing, whether the SPE column is eluted with an isocratic mobile phase or by means of a gradient elution or a stepwise gradient and the duration of the flushing. Sometimes it is necessary to pretreat the test portion prior to injection in the on-line SPE - HPLC system, e.g., to disrupt protein binding or to remove suspended particles [6,7].

As a SPE column is used many times in column switching, its lifetime depends on several factors such as the nature of the matrix, the injection volume, the wash solvents used and the duration of the

rinsing [7]. Reusing the cartridges several times has some drawbacks: interfering matrix compounds can elute slowly from the sorbent or its surface may change in function of the different injections, giving rise to an altered adsorption-elution [56]. Memory effects are possible, which means that a small amount of the analyte, not desorbed in a first extraction, will elute during a second extraction. Some types of phases, such as the ion-exchanging or metal loaded phases, are difficult to regenerate [37]. In general the reuse of SPE columns is not recommended, unless it has been proven that washing and regenerating steps can eliminate memory effects and that the overall performance of the SPE column has not changed. On-line SPE with column switching on disposable SPE columns, which are replaced after each SPE, can be performed by means of an automated cartridge exchange module [37,56].

APPLICATIONS OF SPE

SPE has become a widely used isolation technique with applications in different fields such as the quality control of pharmaceutical products, applications in the biomedical area (such as therapeutic drug monitoring, pharmacokinetic and pharmacological studies, measurement of endogneous compounds for diagnostic purposes, screening for forensic analysis and non-medical use of drugs, veterinary residue analysis), and in environmental, food and drinking water analysis. Selective SPE procedures have been described for a wide range of analytes : drugs belonging to almost all pharmacological classes; endogenous compounds such as peptides, catecholamines, steroids or lipids; food additives and pesticides. Most SPE methods are developed for a single compound, possibly with an internal standard (often a structure analogue), but also the simultaneous extraction of apolar and polar compounds using SPE is described: e.g., the simultaneous extraction of relatively hydrophilic and relatively lipophilic beta-blocking agents [57] ; a method for the screening of urine for beta-blocker abuse [58] or the simultaneous extraction of an apolar drug and its more polar metabolites [59].

Depending on the complexity of the matrix, the concentration of the analyte in the test portion and the analytical method to determine the analytes after extraction, SPE can be used as the single analyte isolation step or in combination with other techniques. The isolation of *p*-hydroxybenzoic acids from cosmetics consists of an extraction in ether followed by a clean-up on a C_{18} silica bonded phase to remove co-extracted lipids [60]. To isolate sulfonamides from swine tissue, Haagsma [61] first ground the test material, extracted the analytes in an organic solvent, filtered the solution and finally extracted selectively the amphoteric compounds on an aromatic sulphonic acid cation exchange phase, prior to HPLC analysis. On the other hand, for the monitoring of plasma concentrations of caffeine, theophylline and theobromine a simple SPE on a C_{18}- cartridge yields a sufficiently clean extract for HPLC analysis [62]; a simple SPE on C_{18}, with basic acetonitrile as wash solvent and acid acetonitrile as eluting solvent, has been described for the extraction of aflatoxin M_1 from milk [63].

Examination of the literature reveals that for the majority of the applications a non-selective apolar sorbent such as the octadecyl-bonded silica is chosen. Such simple SPE methods can be found for all kind of drugs (benzodiazepines [64,65], antibiotics [66], non-steroidal anti-inflammatory drugs [67,68], acetazolamide [69], cimetidine [70,71], xanthine derivatives [62] beta-blocking agents [57,72], ascorbic acid [73], benzimidazoles [74,75]), for food colors [25,76] and organic pollutants [6,26]. For some applications higher selectivity is required, which can be obtained with a more selective sorbent, with the dual cartridge methods, ion-pair SPE or a solvent changeover, as described in the section on the selectivity of SPE.

Besides selective SPE methods set up for the specific isolation of a compound or group of compounds, Musch and Massart [28] developed a general strategy for the SPE of basic drugs. SPE on a cyano-bonded phase was evaluated for a set of more than 30 basic drugs, using 0.1 % propylamine in methanol or a mixture of methanol and phosphate buffer pH 3 as eluents. For about 80 % of the drugs a satisfactory recovery could be obtained.

CONCLUSION

Solid phase extraction can be classified as a technique for isolating the analyte from the matrix or for the removal of endogenous compounds. Further differentiation can be made according to the following criteria:

1. the type of sorbent :
   - classical sorbents such as alumina, magnesium silicate, carbon, resins, etc.
   - polymeric stationary phases
   - bonded silica phases
2. the method of performing SPE : static or dynamic
3. in case of dynamic SPE : off-line or on-line

This classification is schematically presented in Figure 5.

This review has only dealt with the SPE on bonded silica phases, since these phases are commercially available in prepacked cartridges and SPE has been widely used for a variety of applications. The technique has the advantage of being easy to perform, relatively rapid (even when performed off-line and manually) and easy to automate. With the large choice of bonded functionalities and with applications as dual cartridge SPE , ion-pair SPE or solvent changeover, selective extraction and clean-up procedures or fractionations can be developed. Innovations in SPE introduced in the last few years are the introduction of phases designed for specific applications (e.g., for monitoring cannabis misuse), cartridges made of glass and PTFE or stainless steel frits to prevent plasticizer contamination [12] and recently the SPE disks [19]. Because of all these advantages and the ongoing developments it is concluded that SPE will continue to be a frequently used analyte isolation technique.

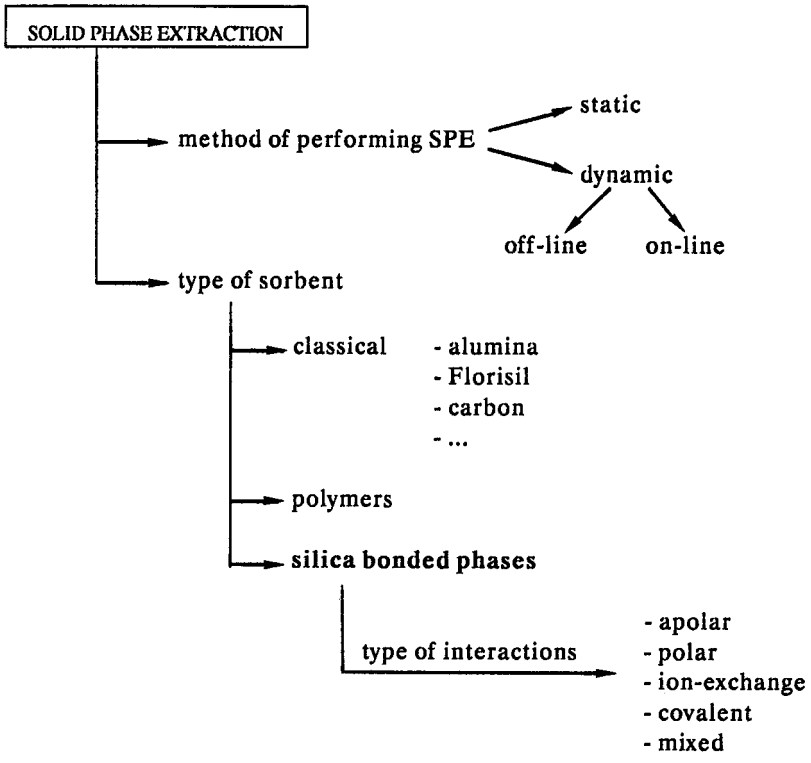


Figure 5 : Classification of solid phase extraction procedures.

PROPOSED DEFINITIONS, TERMINOLOGY AND RECOMMENDED PRACTICES

Definitions and terminology

Solid phase extraction (SPE) consists of bringing a test portion/solution (liquid or gaseous) in contact with a solid phase, or (ad)sorbent, whereby an analyte is selectively adsorbed onto the surface of the solid phase. The solid phase is usually packed in a small column. The liquid or gaseous phase is then separated from the solid phase and other solvents (liquids or gases) are added. The first such solvent is usually a wash solvent that removes possible adsorbed matrix components. Eventually an eluting solvent is brought into contact with the sorbent to desorb the analyte selectively.

The following terms should be used to describe the five steps that characterize a SPE procedure:

1) Wetting (the sorbent): A solvent capable of solvating the solid phase is brought in contact with the sorbent. This is nearly always the case for chemically bonded silica phases. Wetting is necessary to maximize the interaction between the sorbent and the solvents or analytes in the subsequent steps.

2) Conditioning (of the sorbent): A solvent similar to the test solution that will be extracted is passed through the sorbent, in order to prepare the solid phase for the subsequent adsorption step.

3) Adsorption (step): The test solution is brought in contact with the sorbent, allowing the adsorption of the analyte.

4) Wash (steps): Possible interfering matrix components can be washed off the sorbent with (a) suitable solvent(s), while the analyte of interest remains adsorbed. This step is optional.

5) Desorption (step): The analyte is desorbed from the solid phase and eluted with an appropriate solvent.

Silica bonded phase for SPE: a solid phase consisting of apolar, polar, ion-exchanging or special moieties covalently bound on silica particles with an average diameter of usually 30 - 60 μm.

Off-line SPE: the extraction and the subsequent analysis are separate processes and the extract must be transferred manually to the analytical instrument.

On-line SPE: the eluent from the SPE is automatically directed into the analytical instrument. SPE and the subsequent analysis are integrated together. If SPE is combined with a chromatographic analysis, a column switching valve can be used to direct the eluent from the SPE column to the analytical column.

SPE cartridge or column: these terms refer to a column (which may be disposable) containing a solid phase and used for on-line or off-line SPE.

Pre-column: a commonly used term for the SPE column in an on-line SPE system with column switching. It may be confused with a precolumn used in HPLC based on liquid-liquid partitioning and which is placed between the pump and the sample inlet to presaturate the mobile phase with the stationary phase. Therefore the more explicit term SPE column is preferred.

Recommendations concerning publication

A publication should specify the following items concerning the SPE procedure :

- the type of sorbent(s) used, the amount, the supplier and the batch number
- the pretreatment of the test portion prior to loading on the sorbent
- the exact description of the SPE method :
    * all solvents and their volumes used in the different steps
    * the way the test solution or solvents are brought into contact with the solid phase: static or dynamic
    * in case of a dynamic SPE : the vacuum pressure and/or the flow rate (if applicable, the different vacuum pressures and/or flow rates in the different steps)
    * on-line or off-line
    * the equipment used to perform the SPE
- the manipulation of the extract prior to analysis
- in case of a column switching method whereby the SPE column is used several times, the estimated life- time of a SPE column, namely an approximation of the total volume of the test portion that can be extracted without such difficulties as memory effects, blocking of the SPE column, interferences of slowly eluting compounds, etc.

In the summary, the abstract or the list of keywords of a publication, the SPE should be described by specifying :

- the type of sorbent(s)
- off-line or on-line
- in case of a dynamic SPE, this should not be specified because it is the usual method; static SPE should be specially mentioned e.g.,

    on-line SPE on a C_{18} silica bonded phase

    off-line SPE on a C_{18} and NH_4^+ silica bonded phase

Recommendations for manufacturers

It is recommended that each batch of silica-bonded SPE columns contain a quality assurance report with as many as possible of the items listed below. These items are considered to be relevant when developing a SPE method and when using different batches of SPE columns. For these items methods of

determination are described, but an appropriate procedure should be selected and internationally recognized.

- average mass of the solid phase
- mean particle size and the particle size distribution
- mean pore size and the pore size distribution
- the specific surface area
- the % carbon loading
- end-capped or non end-capped phase
- monomeric or polymeric-bonded phase
- the material from which the frits and cartridge housing are manufactured
- whether these products are manufactured using the general ISO 9000 guidelines for quality assurance

The following items would be useful to add in a quality assurance report, but an appropriate method of determination should be developed.

- the percentage of residual silanol functions
- a test to evaluate possible batch-to-batch variation of the SPE columns and the extent of the different types of interactions. This test could be performed by means of selected test solutes, each of them interacting with the silica-bonded phase through a specific, known interaction force. e.g.,
- a test solute known to interact only with the residual silanol groups under specific SPE conditions
- a test solute interacting only through van der Waals forces under specific SPE conditions

Differences in the extent of different types of interactions could be discerned between batches of solid phases by evaluating the retention or elution profiles of these selected test compounds under well defined SPE conditions.

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