

# 15

## Extraction methods in pharmaceutical analysis

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#### Principles of extraction

The analyte is removed from materials in a formulation matrix which would interfere in its analysis using a solvent in which it is highly soluble but the matrix interferents have limited solubility. Further solvent partitioning steps may then be used in order to reduce the interferents.

#### Applications

- Most analyses of pharmaceuticals require an extraction step or extraction steps and optimisation of these processes has an important bearing on the precision and accuracy of the analysis
- Widely used in bioanalytical measurements and for concentrating trace amounts of analyte.

#### Strengths

- A simple and cheap method of removing interferents.

#### Limitations

- Limited selectivity, limited choice of partitioning solvents, large volumes of solvent required. (See SPE keypoints.)

## Introduction

Complex extraction and derivatisation methods are most often applied to bioanalytical procedures and to the concentration of trace impurities in pharmaceuticals rather than to straightforward quality control of active ingredients in pharmaceuticals. Quality control of the active ingredient in a formulation generally utilises a simple extraction procedure and if there is a problem of interference from excipients following extraction, chromatography is able to resolve the active ingredient from the interferants and permit quantitation. However, there are circumstances where low dosage formulations and advanced drug delivery formulations may require more detailed sample handling.

## Commonly used excipients in formulations

The principal reason for conducting extraction prior to analysis is in order to remove materials which might interfere in the analysis. This is a greater requirement if chromatographic separation is not carried out during the analysis. Some non-chromatographic techniques such as NIRA aim to avoid all sample preparation through using advanced computing techniques to screen out interference. Even when chromatographic separation is used, extraction of some type has to be carried out prior to analysis in order to remove insoluble tablet matrix materials or oily excipients in creams and ointments. When low levels of drugs are being monitored in biological fluids, extraction procedures may have to be quite detailed in order to remove interference by endogenous compounds. The major types of interferants in formulations are briefly considered in this chapter.

## Tablets and capsules

Tablets and capsules usually consist largely of a filler except for high dose formulations, such as paracetamol tablets and tablets of other non-steroidal anti-inflammatory drugs, where the active ingredient may compose a large part of the formulation. The most commonly used filler in tablets is lactose and other popular fillers include other sugars or sugar polymers such as cellulose, starch and mannitol. These substances are polar and will dissolve or swell best in water, thus extraction procedures where the drug is water soluble are best carried out in aqueous media so that the drug is efficiently recovered from the sample matrix. The fillers themselves do not absorb UV light so they are not likely to interfere directly in HPLC procedures where for instance in commonly used reversed-phase chromatography procedures they will elute at the void volume with little perturbation of the chromatographic baseline. Similarly, they produce little interference in direct analyses by UV spectrophotometry. If the drug is not completely water soluble, methanol or ethanol may be used for extraction since they will wet the tablet powder quite well and will dissolve many organic molecules.

Lubricants are used in tablet preparation and include magnesium stearate, stearic acid and polyethylene glycol. They only comprise at most 1–2% of the tablet bulk so that their potential to interfere is slight, particularly since their chromophores are weak. The fatty acid lubricants can often be observed if analysis of a tablet extract is carried out by GC-FID. Tablet coatings are often based on modified sugar polymers such as hydroxypropylmethylcellulose. These coatings are used at about 3% of the tablet bulk, are water soluble and do not absorb UV light.

Colourants obviously have the potential to interfere in analysis because they are efficient absorbers of UV/visible radiation. In tablets and capsules, colours tend to be organometallic dyes or metal oxides which are not appreciably soluble in any of the solvents used for extraction and can be filtered off with other insoluble matrix constituents. When capsules are analysed the coloured outer shell is removed before the contents of the capsule is extracted.

## Suspensions and solutions

In suspensions and solutions, the dyes used are water soluble and include natural pigments such as chlorophylls, carotenoids and anthocyanins, and coal tar-based dyes. More effort may be required to remove interference by these materials. Solid phase extraction with ion exchange resins may be useful for removing anionic or cationic dyes, although simple extraction of the drug into organic solvent of moderate polarity may leave such dyes in the aqueous phase. Solutions tend to contain anti-microbial preservatives and anti-oxidants. These are usually either phenols or quaternary amines such as benzalkonium chloride and have strong enough chromophores to interfere in the analysis of a drug. These compounds have to be removed prior to analysis by extraction procedures. Suspensions also contain surfactant materials such as the polyethylene glycol-based detergents but these compounds do not have appreciable UV absorbance and thus have little potential for interference.

## Creams and ointments

Sodium and potassium salts of fatty acids, cationic surfactants and non-ionic surfactants are used in creams and ointments. As discussed above these compounds do not have strong chromophores but, particularly the fatty acids, may interfere in chromatography for instance by contaminating reverse-phase HPLC columns if they are not removed. Contamination of reverse-phase HPLC columns by lipophilic materials can often be observed through a loss of chromatographic peak shape. Creams and ointments contain large amounts of oily triglycerides which have to be removed to avoid interference with the chromatographic process. Extraction of the cream with methanol can partly remove this type of interference, partitioning of the extract between hexane and methanol or methanol water mixtures may also be used, the highly lipophilic material is removed into the hexane layer.

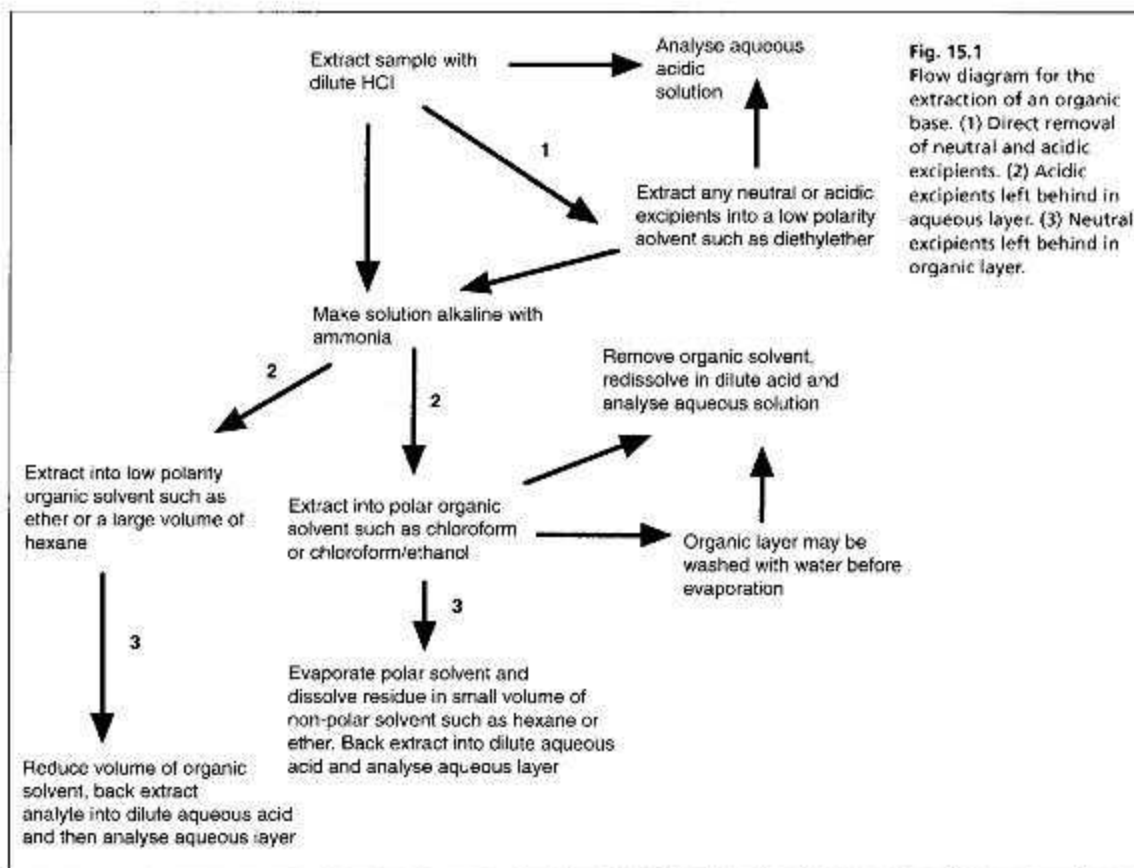
## Solvent extraction methods

Solvent extraction procedures provide simple methods for separating the analyte from excipients in formulations. The analytical method applied to the isolated analyte can be for example either gravimetric, volumetric, spectrophotometric or chromatographic. In most cases in the pharmaceutical industry chromatographic methods are preferred. The extraction method adopted is governed by the need to remove excipients and by the properties of the analyte.

## Extraction of organic bases and acids utilising their ionised and un-ionised forms

Salts of organic bases such as sulphates and hydrochlorides are often highly water soluble and the free bases are usually quite organosoluble, particularly in relatively

polar solvents such as chloroform or mixtures of chloroform and ethanol. Similarly the sodium or potassium salts of organic acids are freely water soluble while the unionised acids are usually quite organosoluble. These properties can be used to advantage in designing an extraction procedure. A flow diagram for the extraction steps which can be used for the separation of an organic base from a formulation is shown in Figure 15.1.



**Fig. 15.1**  
Flow diagram for the extraction of an organic base. (1) Direct removal of neutral and acidic excipients. (2) Acidic excipients left behind in aqueous layer. (3) Neutral excipients left behind in organic layer.

This type of extraction is employed in the BP assay of Cyclizine Lactate Injection: the injection is diluted with dilute  $H_2SO_4$  and then neutral and acidic excipients are extracted with ether. The solution is basified and the cyclizine is extracted into ether leaving the lactate ion, which would not have extracted during the initial ether extraction step, behind in the aqueous layer. For convenience in measurement by UV spectrophotometry and in order to carry out volumetric dilution of the extract, cyclizine is then back extracted into dilute  $H_2SO_4$  and subjected to further dilution.

The same principles apply to the extraction of an organic acid except that in this case high pH values are used to ensure the acid remains in the aqueous layer and low pH values are used to ensure that it is extracted into the organic layer.

### Partitioning between organic solvents

Partitioning between organic solvents is used in the extraction of analytes from oily excipients such as in the extraction of steroid creams prior to HPLC analysis. The

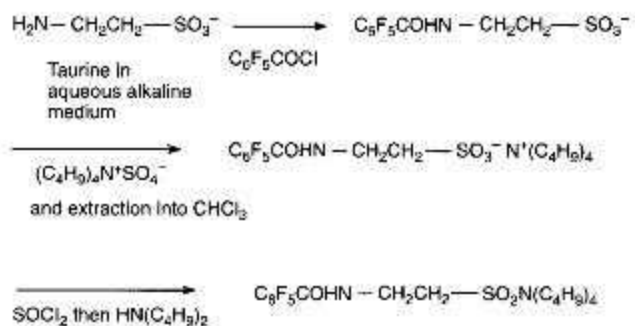
most commonly used systems are methanol/hexane, aqueous ethanol/hexane or acetonitrile/hexane. In the case of analysis of corticosteroids in creams, the cream is usually dispersed by heating in hexane and then extracted with an equal volume of methanol. Methanol and hexane mix only very slightly and the oily excipients remain in the predominantly hexane layer, while the more polar corticosteroid partitions into the methanol layer. Use of an internal standard with a structure closely related to that of the analyte is essential in order to achieve good precision in this type of analysis through compensating for incomplete recovery of the analyte. Examples of pharmacopoeial methods using this type of partitioning include assays of: hydrocortisone acetate cream, fluocinolone cream and beclomethasone cream.

### Ion pair extraction

Ion pair extraction provides a standard method for estimating ionic surfactants either colorometrically or titrimetrically. For example a cationic surfactant such as cetrimide can be estimated by pairing it with a lipophilic anionic dye such as bromocresol purple. The ion pairing creates a coloured lipophilic ion pair, which can be extracted into an organic solvent such as chloroform and a quantitative measurement of the colour extracted can be made spectrophotometrically. This type of assay is described in the BP for Clonidine Injection and Benzhexol Tablets.

Ion pair extraction has also been used to extract polar analytes in bioanalytical procedures. Figure 15.2 exemplifies the determination of the amino acid taurine by gas chromatography-mass spectrometry (GC-MS); this figure also illustrates a useful property of amines (and phenols), which is that they will react more rapidly than water with an acylating reagent in an aqueous environment thus improving their organosolubility. After acylation and ion pair extraction with tetrabutyl ammonium sulphate the taurine is converted to an amide prior to analysis by GC-MS.

**Fig. 15.2**  
Extraction and  
derivatisation of taurine.

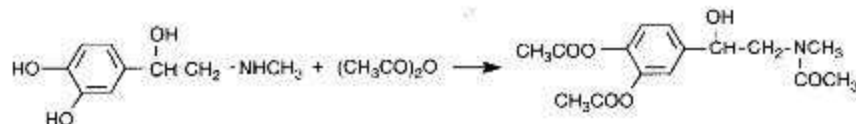


### Derivatisation prior to extraction

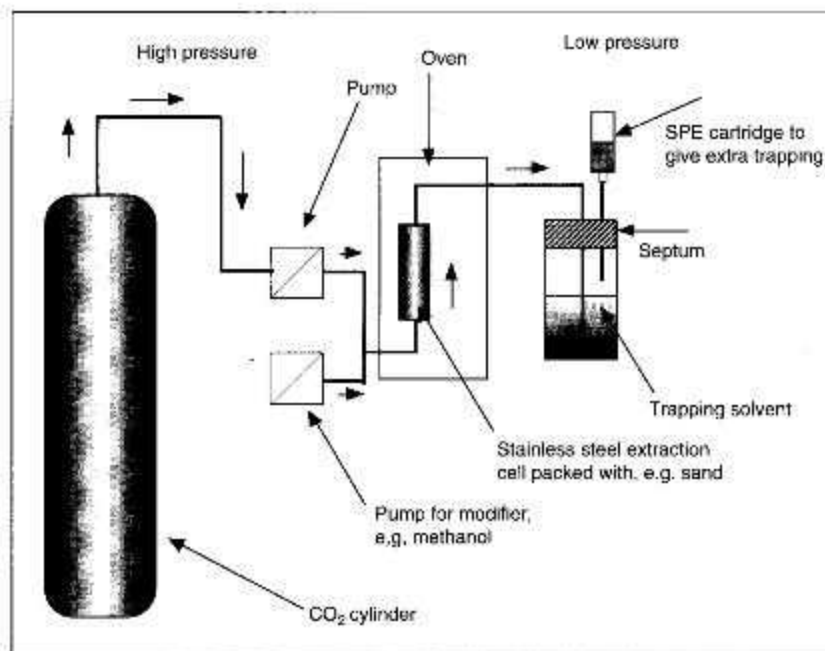
Figure 15.3 shows aqueous phase acylation of adrenaline in an injection with acetic anhydride. The reaction is carried out in the presence of aqueous sodium bicarbonate and is used in a gravimetric determination of (-) adrenaline in Adrenaline Injection BP.

### Supercritical fluid extraction

Figure 15.4 shows a schematic diagram of a supercritical fluid extraction apparatus. The advantages of supercritical fluid extraction (SFE) are as follows:



**Fig. 15.3**  
Aqueous phase  
acetylation of adrenaline  
prior to gravimetric  
analysis.



**Fig. 15.4**  
A supercritical fluid  
extraction apparatus.

- (i) The solvents are used above their critical temperature and pressure but they function almost as effectively as liquid solvents and have the advantage that mass transfer between sample and solvent, i.e. the rate of extraction, is very fast.
- (ii) The solvent strength of supercritical fluids can be increased or decreased by varying the pressure in the extraction vessel thus providing a simple means of producing selective extraction.
- (iii)  $\text{CO}_2$ , which is frequently used as an extraction medium, is a non-toxic, non-flammable solvent which is readily disposed of and its low critical temperature ( $31.1^\circ\text{C}$ ) means that it can be used as an effective solvent for extracting unstable compounds.

The most efficient method of conducting SFE is via the dynamic process illustrated in Figure 15.4. This process enables the addition of a polar modifier such as methanol, which increases the solvent strength of the non-polar  $\text{CO}_2$ . The liquid  $\text{CO}_2$  with about 5% v/v of modifier is passed through a stainless steel cell containing the sample, which may be mixed with inert material so that the sample occupies the whole cell volume. Two recent examples of the utilisation of SFE in the analysis of pharmaceuticals are discussed as follows.

Vitamin A, vitamin E and their acetate and palmitate esters were determined in tablets.<sup>1</sup> The tablet powder was mixed with sand and loaded into the extraction vessel;  $\text{CO}_2$  alone was used for extraction at  $40^\circ\text{C}$  for 15 min. The  $\text{CO}_2$  was vented to



atmosphere after being passed into a vial containing tetrahydrofuran at 0°C to trap the analytes. The process was found to give good recovery and was more selective for the analytes than an established liquid/liquid extraction process. The sample was analysed by HPLC.

An unstable analogue of prostaglandin, PGE<sub>1</sub> formulated in a polybutadiene polymeric matrix, was placed in a SFE cell and extracted with CO<sub>2</sub>/formic acid (95:5) at 75°C.<sup>2</sup> Extraction was continued for 60 min and then the extract was collected in hexane/ethanol (2:1) at 0°C. The advantages of the SFE method were that the solvent effected simultaneous cleavage of the polymer–prostaglandin bond without instability problems and with improved mass transfer enabling good recovery from the polymer matrix.

## Solid phase extraction (SPE)

### KEYPOINTS

#### Principles

The analyte is dead stopped on the SPE medium by loading it onto the cartridge in a solvent of low eluting power. It may then be washed with other solvents of low eluting power and is then finally eluted with a small volume of a strong solvent.

#### Applications

- Particularly useful for selective separation of interferants from analytes, which is not readily achievable by liquid/liquid extraction
- Widely used in bioanalytical measurements and environmental monitoring for concentrating trace amounts of analyte.

#### Advantages in comparison with liquid/liquid extraction

##### *Solid phase extraction*

- The solid phase is immiscible with solvents and thus after loading the sample a range of washing conditions can be used to remove interferants through having a wide choice of washing solvents
- Chemical nature of adsorbant can be varied so that it is selective for a particular functional group in the analyte
- Emulsions are not formed between the two phases
- A sample in a large volume of solution can be trapped on the column (dead stopped) and thus concentrated
- Only small volumes of solvent are required both for washing and elution
- Extraction can be carried out in batches rather than serially
- The expense of the columns can be offset against savings in solvent purchase and disposal.

##### *Liquid/liquid extraction*

- Solvents must be immiscible, hence there is a limited choice of extraction and washing solvents
- Emulsions may form
- Large solvent volumes are required when extraction of large sample volumes is carried out
- The extract may have to be concentrated prior to analysis and then back extracted into an aqueous phase
- Extractions have to be conducted serially.

#### Limitations

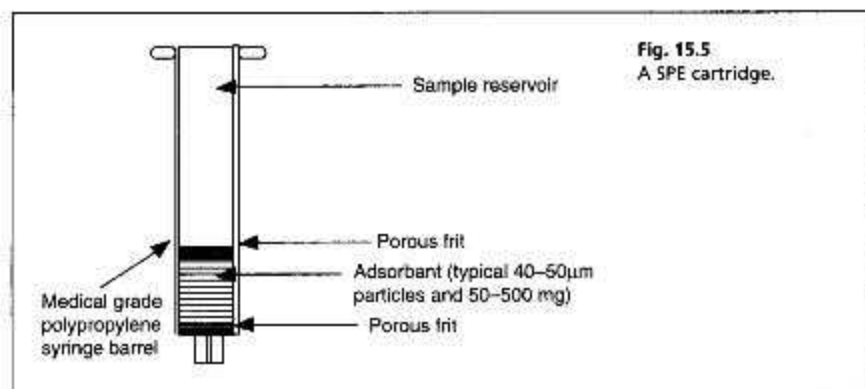
- Although recoveries are generally good, it is probably best to use an internal standard in this type of analysis to compensate for any possibility of irreversible absorption onto the extraction medium
- Silica gel-based SPE columns are unstable to strongly alkaline conditions.

## Introduction

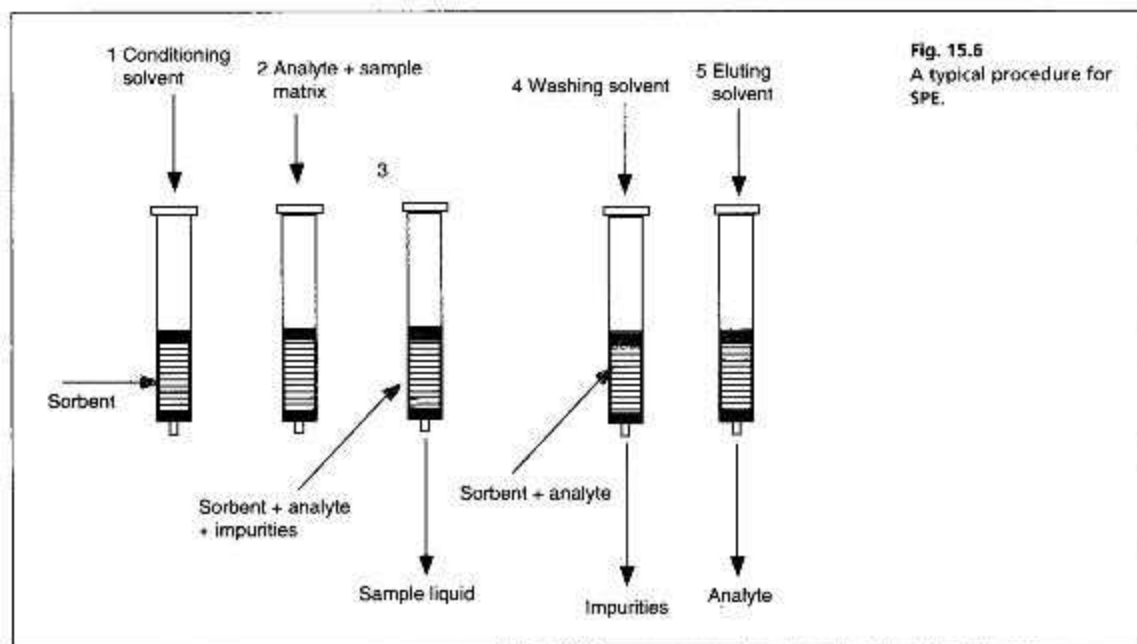
SPE is increasingly being adopted as a useful method of sample preparation where extraction into an organic solvent would have originally been employed. The reasons for this are outlined in the Keypoints box. The technique has been employed more extensively in the 'clean up' of biological samples prior to analysis but there are increasingly useful examples of its application to the analysis of drugs in formulations.

## Methodology

Typically solid phase extraction is based on the type of system shown in Figure 15.5. The volume of sample which can be loaded onto this small column can be increased using a column with a larger sample reservoir. The sample is usually aspirated through the column under vacuum.



The steps involved in a SPE procedure are shown in Figure 15.6:

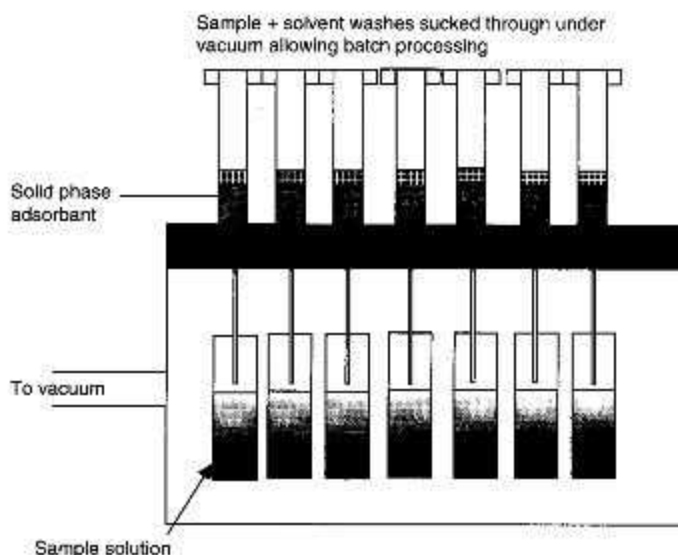




- (i) The column is washed with 5–10 bed volumes of the solvent, which will be used to elute the analyte and if an ion exchange adsorbant is to be used, 5–10 volumes of an appropriate buffer.
- (ii) The analyte is loaded onto the column in an appropriate solvent, which is too weak to elute it from the column.
- (iii) The sample solvent passes through the column leaving the analyte + impurities adsorbed on the stationary phase.
- (iv) The column is washed with solvent, which will elute impurities while leaving the analyte on column. This requires a good understanding of the physico-chemical properties of the analyte and the adsorbant.
- (v) The analyte is eluted with an appropriate solvent, preferably one which will leave further interferants behind on the column.

A vacuum manifold can be used for conducting multiple extractions simultaneously as shown in Figure 15.7.

**Fig. 15.7**  
Vacuum manifold for multiple solid phase extraction.

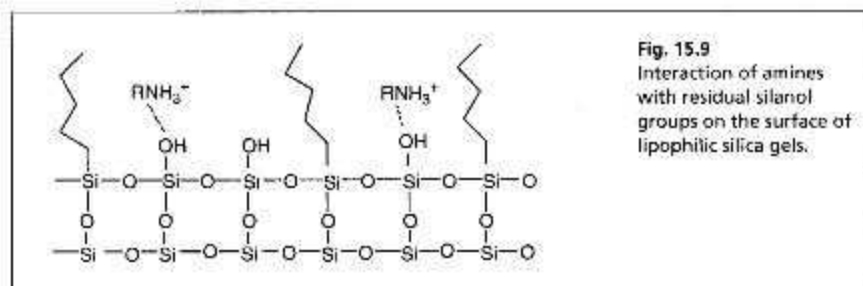
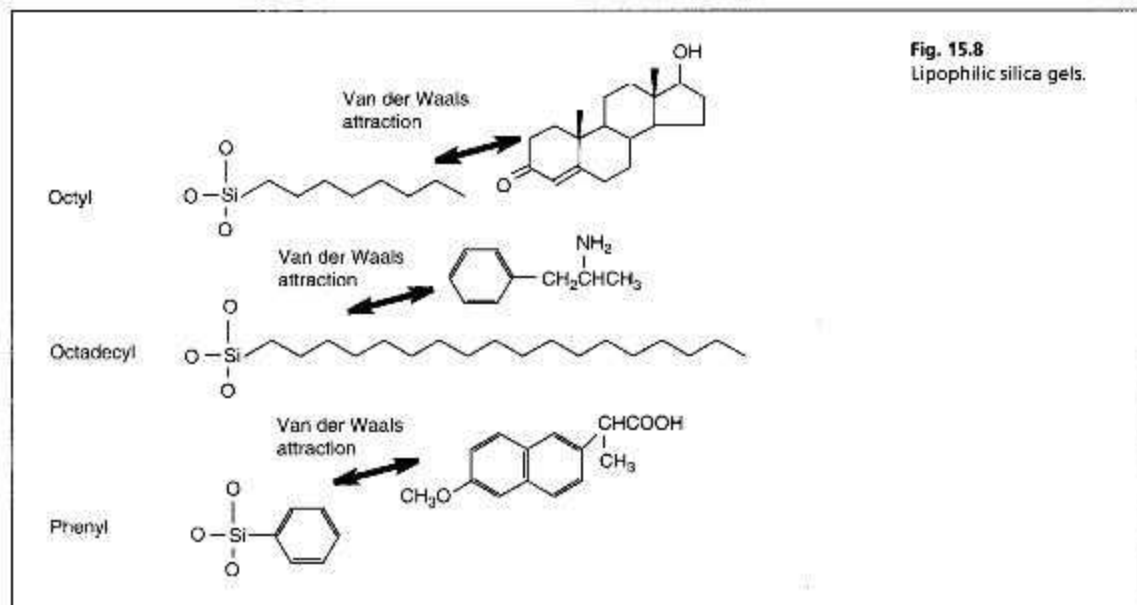


## Types of adsorbants used in SPE

### Lipophilic silica gels

The lipophilic silica gels shown in Figure 15.8 will retain lipophilic compounds, provided they are in an un-ionised state, through van der Waals interactions and in the case of amines, through some degree of polar interaction. These phases are generally not completely endcapped so that there are free polar silanol groups remaining on the surface.

In the case of amines the type of interaction shown in Figure 15.9 may occur. The shorter the alkyl chain length on the silica gel surface the more likely it is that adsorption also plays a part in the extraction. It is possible to buy highly endcapped reverse-phase silica gels where most of the residual silanols have been blocked but it may be better to take advantage of the mixed lipophilic and adsorptive properties of reverse phases which have not been endcapped.



Recently high purity styrene divinylbenzene polymeric gels have become available for use in lipophilic SPE extraction; these types of materials formerly contained monomer materials which could interfere in analyses. These types of gels are much more lipophilic than surface-modified silica gels and also have a higher capacity for sample loading. Their applications are similar to those of the lipophilic silica gels.

### Typical extraction methodologies using lipophilic silica gels

Typically the columns are conditioned by washing with 5–10 bed-volumes of methanol followed by a 5–10 bed-volume of water or a suitable buffer.

**Basic compounds** are adsorbed from aqueous solution by adjusting to alkaline pH with buffer, e.g. a buffer of pH 10 would be suitable for most bases. The column can be washed with further aliquots of alkaline buffer, water or if the compound is highly lipophilic, mixtures of methanol and alkaline buffer can be carried out. The compound is finally eluted with either an acidic buffer or with an organic solvent such as methanol or ethanol. An example of an extraction of an amine using a lipophilic silica gel is shown in Box 15.1.

**Acidic compounds** are extracted from aqueous solution by adjusting to acidic pH. The column can be washed with dilute acid, water and if the compound is highly

**Box 15.1 Typical example of extraction by reverse-phase SPE**

An oral suspension of chlorpromazine has the following composition and must be extracted so that excipients are removed prior to analysis by UV spectrophotometry: Chlorpromazine 0.025% w/v, parahydroxybenzoic acid methyl ester 0.1% w/v, neutral water soluble dye, lipophilic flavouring agent, sodium lactate buffer.

- (i) 5 ml of solution is mixed with 5 ml of 0.5 M ammonia buffer pH 9.5 and the sample is passed through an octadecyl silane (ODS) SPE cartridge. The cartridge is washed with a further 5 ml of ammonia buffer. At this stage the chlorpromazine is in its free base form and has adsorbed onto the lipophilic ODS and the lactate, the dye and the preservative (which are all water soluble, particularly at high pH), have passed through the column.
- (ii) The sample is eluted with 5 ml of 0.5 M phosphate buffer pH 2.0/methanol (95:5) and made up to the volume required for analysis by UV spectrophotometry. These conditions elute the basic chlorpromazine and leave behind any neutral lipophilic compounds, e.g. flavouring on the column.

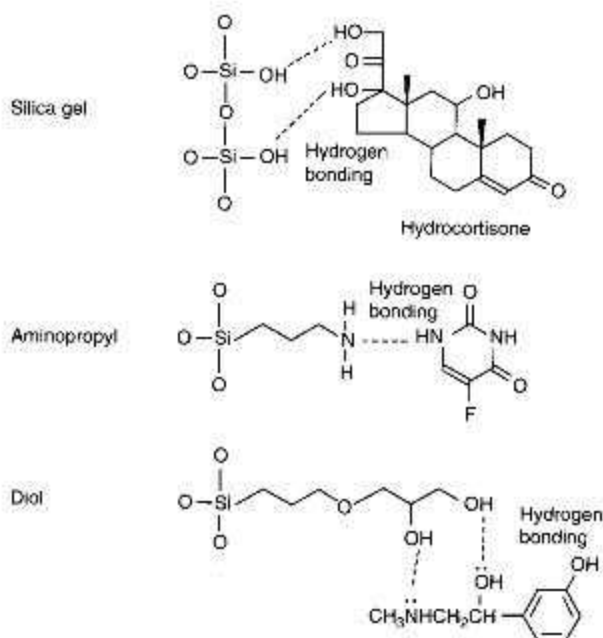
lipophilic, mixtures of methanol and dilute acid. The compound can be eluted with methanol, acetonitrile, tetrahydrofuran (THF) or alkaline buffer.

*Neutral compounds* can be extracted without controlling pH. Washing can be carried out with dilute acid or alkaline buffer (to remove ionisable impurities) and methanol water mixtures. The compounds can be eluted from the column with methanol, ethanol or chloroform.

**Polar surface-modified silica gels**

These silica gels retain analytes through interaction between polar groups; silica gel itself or the surface-modified polar silica gels shown in Figure 15.10 may be used.

**Fig. 15.10**  
Silica gel and polar  
surface-modified silica  
gels.



## Typical methodologies using straight-phase adsorbants

These adsorbants are typically used for polar compounds that are not well retained by reverse-phase adsorbants. The columns are conditioned by washing with 5–10 bed-volumes of the solvent which will be used to elute the analyte. The sample is loaded onto the column in a solvent, which is not sufficiently strong to elute it. Washing of the column is often carried out with a moderate polarity organic solvent, e.g. alcohol-free methylene chloride. Polar compounds are then eluted with methanol or mixtures of methanol and acidic buffer (for basic compounds) or methanol and alkaline buffer (for acidic compounds). Diol columns have been used to good effect in the extraction of polar drugs from pharmaceutical creams.<sup>3,4</sup>

## Anion exchangers based on surface-modified silica gels

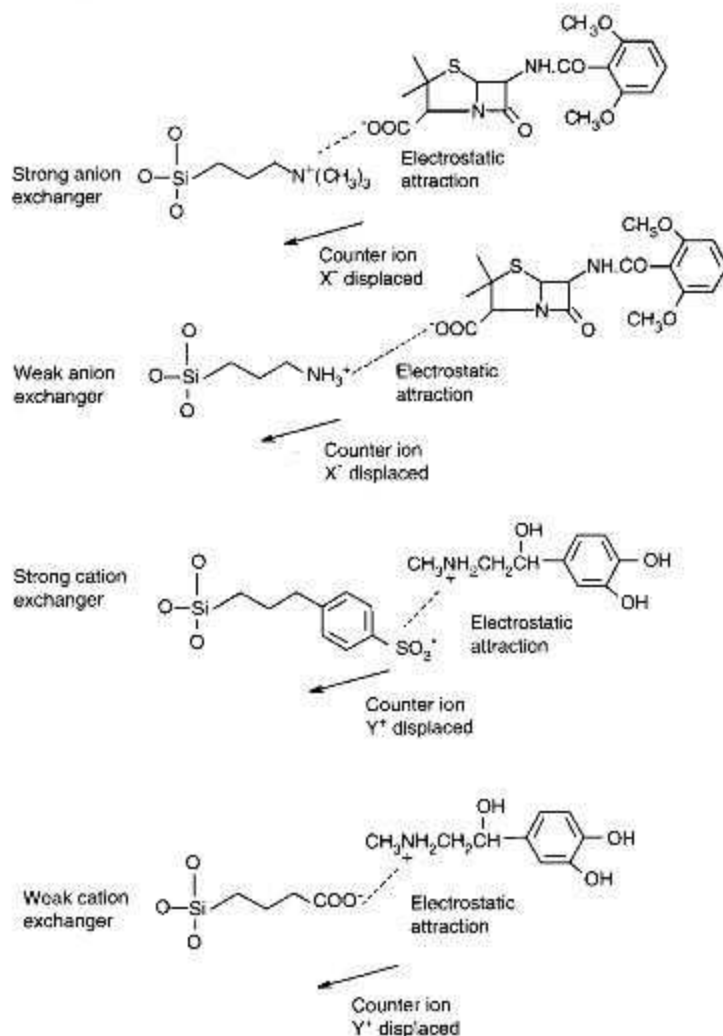
Ion exchangers based on polymeric resins have been in use for many years. Silica gels coated with ion exchanging groups are a relatively recent innovation. They have the advantage that they have less organosorptive properties than the polymeric resins and thus do not require as high an organic component in the eluting solvent to remove organic compounds after adsorption. Typically the anion exchanger is conditioned by washing with 0.01–0.1 M buffer at the pH of the sample solution. The buffer should contain ions which are relatively easy to displace such as OH<sup>-</sup>, C<sub>2</sub>H<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, or F<sup>-</sup>. Ions such as Cl<sup>-</sup>, Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, HSO<sub>4</sub><sup>-</sup> or citrate are not readily displaced. The acidic sample is then applied in a buffer (0.1 M) one or two pH units above its pK<sub>a</sub> value, e.g. for methicillin shown in Figure 15.11, a buffer pH 3.8–4.8 would be used. Methicillin can be extracted with either a strong or a weak cation exchanger since it is a relatively strong acid. The adsorbant can then be washed with further amounts of the buffer, with deionised water or with organic solvent. The sample can then be eluted with a buffer containing a counter ion at a high concentration, e.g. for methicillin 1 M sodium chloride (NaCl) or 1 M sodium citrate. Many organic compounds are likely to have a high affinity for the lipophilic surface of the SPE medium and methanol might be included in the elution buffer. Compounds can also be eluted by ionisation suppression, thus methicillin could be eluted at low pH, e.g. with 1 M hydrochloric acid/methanol, but this would not be advisable in this example because of the instability of penicillins at low pH.

## Cation exchangers based on surface-modified silica gels

The cation exchange column is conditioned by washing with a 0.01–0.1 M buffer at the pH of the sample solution. The buffer should contain K<sup>+</sup>, Na<sup>+</sup> or NH<sub>4</sub><sup>+</sup> ions which are readily displaced from the gel by organic cations; divalent ions such as Ca<sup>2+</sup> or Mg<sup>2+</sup> are difficult to displace from the gel. The sample is then applied in a buffer (0.1 M) one or two pH units below its pK<sub>a</sub> value, e.g. for the extraction of adrenaline (Fig. 15.11) ammonium chloride (NH<sub>4</sub>Cl) buffer pH 8.3 might be used. The adsorbant can then be washed with further amounts of the buffer, with deionised water or with an organic solvent such as methanol. Elution is then carried out with a buffer containing a counter ion at a high concentration, e.g. 1 M ammonium chloride buffer. If the sample has limited aqueous solubility a solvent such as methanol or ethanol can be included in the high ionic strength buffer. An alternative to elution at high ionic strength would be suppression of the ionisation of the amine group by elution with methanolic ammonia; however, in this case the time of exposure of the

readily oxidised catechol group to high pH conditions should be minimised. This type of elution is useful if GC or GC-MS analysis is to be carried out because the analyte is eluted in a salt-free solution.

**Fig. 15.11**  
Silica gel-based ion  
exchange SPE media.



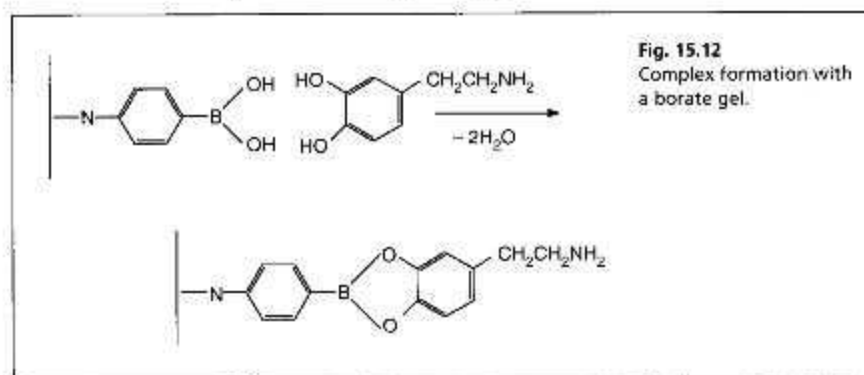
### Factors requiring attention in SPE with silica gels

- Too fast a flow rate does not allow sufficient time for equilibration between the extraction medium and the solvent flowing through it, e.g. the solvent may track through the matrix without contacting the whole surface.
- The capacity of sorbent gels is 1–5% of their mass, e.g. for a 100 mg cartridge 1–5 mg.
- Non-selective gels may have reduced sample capacity for dirty sample matrices, e.g. if a small amount of octadecyl gel is used to extract a sample from a matrix containing large amounts of lipophilic materials, the gel capacity may be exceeded.

- (iv) Attention must be paid to the control of the pH of the sample and washing solutions.
- (v) A careful choice of washing solvents can produce extensive sample 'clean up'.
- (vi) The elution solvent must overcome both the primary interactions of the analyte with the bonded phase and any secondary interactions with silanol groups.
- (vii) The amount of fines (fine particles of silica gel) generated by different manufacturers' cartridges varies. Ideally very little fines should elute from the column with the sample. Fine particles of silica gel can damage HPLC systems and interfere in derivatisation reactions used prior to analysis by GC.

### Borate gels (Fig. 15.12)

These gels are based on immobilised alkyl boronic acids. They have a selective affinity for 1,2- or 1,3-diol groupings such as those found in catechol-containing molecules such as dopamine and in sugars or glycosides.



The analyte is loaded onto the gel in a buffer at *ca* pH 7.0 and the complex formed can then be broken down using a mildly acidic eluent such as 0.1 M acetic acid. This type of extraction has been applied to the determination of dopamine, adrenaline and noradrenaline in plasma and to the determination of the extent of reaction of glucose with serum albumin as a measure of glucose fluctuations with time in diabetics.

### Immunoaffinity gels

These adsorbants are based on immobilised ligands, which have a high affinity for a particular analyte (Fig. 15.13). There are examples where antibodies have been raised to an analyte and then bound to the surface of a SPE matrix. Various types of chemistry permit this type of immobilisation and affinity chromatography is well established in biochemistry. With the proliferation with biotechnological products such as therapeutic peptides, the use of these types of columns for extraction may increase since they can be designed to be highly selective for such compounds.

An example of a highly specific affinity adsorbant of this type is where a monoclonal antibody to a particular compound is immobilised as shown in Figure 15.14. For instance, a gel with a monoclonal antibody to  $\beta$ -interferon attached has been used in industrial scale extraction of the compound from fermentation mixtures.

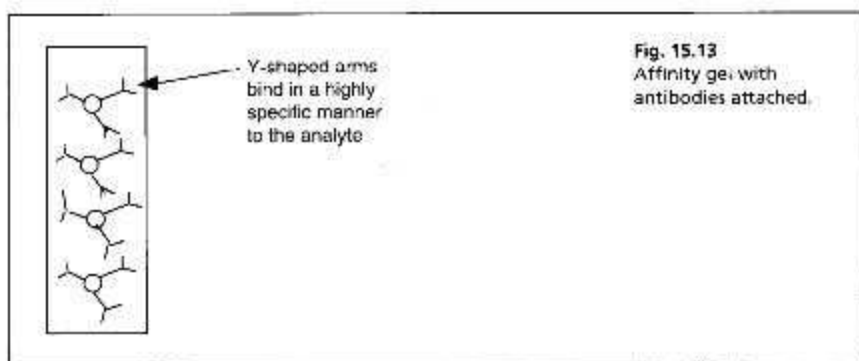
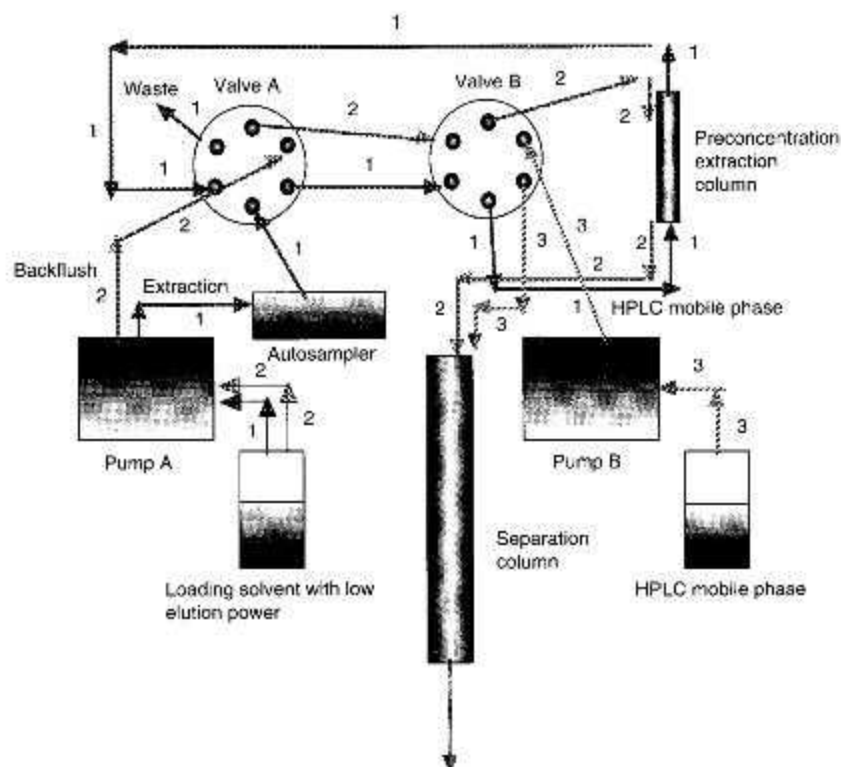


Fig. 15.13  
Affinity gel with antibodies attached.

In most examples in the literature, polyclonal antibodies are used for preparing such columns but the increasing availability of monoclonal antibodies (MAbs) should lead to affinity gels based on MAbs becoming available. Such specificity would be particularly valuable where peptide drugs have to be selectively extracted from biological matrices prior to analysis.

### Adaptation of SPE for automated online extraction prior to HPLC analysis

Fig. 15.14  
An example of an automated SPE system.





Automated extraction can be carried out as illustrated in Figure 15.14. The basics of the system are as follows:

- (i) A solvent of low strength loads the sample so that it is trapped at the head of an extraction column. This column could be non-polar if removal of polar impurities is required before chromatography or polar if removal of lipophilic materials is required.
- (ii) The sample is back flushed with the same solvent which was used in loading it onto the extraction column and is trapped at the head of the chromatography column.
- (iii) The sample is eluted with the HPLC mobile phase, e.g. a method was developed for the analysis of macrolide antibiotics.<sup>5</sup> Extraction was carried out by flushing it onto a cyanopropyl cartridge with phosphate buffer pH 10.5/acetonitrile (90:10). The sample was then backflushed with the same solvent onto an ODS analytical column and then eluted with phosphate buffer pH 7/acetonitrile (46:54).

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#### Further reading

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