Table 7 Common HPLC separation problems

Observation	*Problem source*	*Solution*
Poor peak shape	Wrong silica type	Use type B silica
	Blocked frit or column void	Replace frit, backflush column
	Silanol interactions	Use amine additives, change pH, use end-capped stationary phase
Excessive peak width	Bad column	Replace column
	Column overload	Reduce injection volume or mass
	High molecular weight	Normal
	Unresolved peaks	Improve separation
Inadequate retention	Mobile phase too strong	Use lower %*B*
	Column too weak	Switch to C_{18}
	Samples ionized	Change pH
	Samples too polar	Change to normal phase
	Gradient starting too strong	Start at lower %*B*
Excessive retention	Mobile phase too weak	Use higher %*B*
	Column too retentive	Switch to C_8, C_4 or CN
	Samples too hydrophobic	Change to normal phase
	Gradient stops too soon	Stop at higher %*B*
Excessive retention range	Acids and bases or bases and neutrals in sample	Use ion pairing
	Too broad of polarity for isocratic method	Use gradient elution
Inadequate resolution	Retention too short	Increase k
	Poor selectivity	Change α
	Plate number too low	Use longer column or smaller particle size

Troubleshooting Common Problems

Table 7 highlights some of the commonest causes of chromatographic problems likely to be encountered in the HPLC method development.

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ESSENTIAL GUIDES TO METHOD DEVELOPMENT IN SOLID-PHASE EXTRACTION

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Solid-phase extraction (SPE) is a sample preparation technique combining nonlinear modes of chromatography for the separation, purification, concentration and/or solvent exchange of analytes of interest. SPE is the removal of chemical constituents from a flowing liquid sample via retention on a solid sorbent, and the subsequent recovery of selected constituents by elution from the sorbent. SPE was developed as an heterogeneous (two-phase) alternative to homogeneous (one-phase) liquid–liquid extraction (LLE) for the isolation of solutes from solution.

Background

The modern era of SPE began in October 1977 when prepackaged, disposable cartridges/columns containing bonded silica sorbents were introduced by Waters Associates. This technique was featured on the cover of *Laboratory Equipment* in May 1978 and the first peer-reviewed method was published in the *Journal of Chromatography* that same year. The term solid-phase extraction wasn't actually popularized until the early 1980s.

Unlike the meagre resources available to early SPE researchers, there are currently thousands of publications for analytes of pharmaceutical and environmental interest that may be consulted for examples of developed SPE methods. Many manufacturers publish bibliographies of methods developed using their products that are available in print or via the Internet. However, it is sometimes difficult to recognize the process used to arrive at published protocol, and, it is still common that an SPE method for the solute–matrix combination required has not been previously developed. Even if an appropriate method exists, it is advisable to understand thoroughly the principles of SPE method development in order to evaluate properly published methods.

Elsewhere in this volume, there are articles dealing with specific SPE topics (**Table 1**) that should be consulted for detailed descriptions. This contribution addresses method development issues in SPE.

Principles

SPE method development requires exploitation of analyte properties, selection of the appropriate sorbent and recognition of limitations imposed on the analysis by the sample matrix. The distribution of the analyte between the sample matrix and the sorbent in SPE is determined by physical and chemical properties of the analyte and the sorbent. The sample matrix can be manipulated to influence the distribution. In SPE method development, identification of the characteristic properties of the analytes of interest is a necessary first step before the sorbent can be selected or the sample matrix can be modified to effect the recovery.

SPE consists of a basic four-step approach (**Figure 1**):

1. Sorbent preparation or pre-wash: stationary phase conditioning;
2. Retention: analyte adsorption;
3. Sorbent post-wash: removing undesirable contaminants;
4. Elution: analyte desorption.

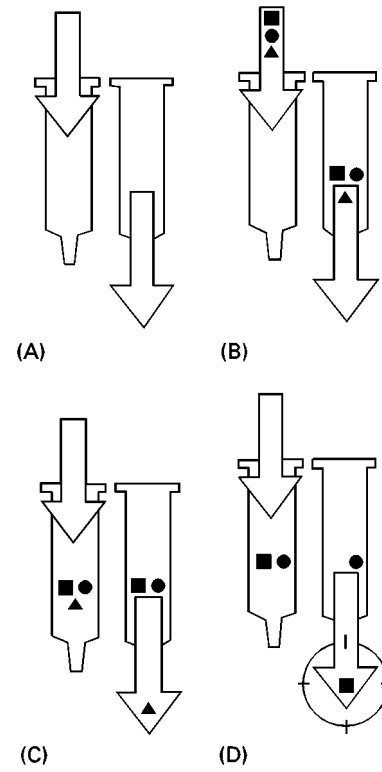

Figure 1 Solid-phase extraction consists of four basic steps: (A) conditioning, (B) retention, (C) rinsing and (D) elution. (A) Conditioning the sorbent prior to sample application ensures reproducible retention of the compound of interest (the isolate). (B) Squares, adsorbed isolate; circles, undesired matrix constituents; triangles, other undesired matrix components. (C) Triangles, rinse the columns to remove undesired matrix components. (D) Circles, undesired components remain; squares, purified and concentrated isolate is ready for analysis. (Reproduced with permission from http://www.varianinc.com/spp/shared/4step.jpg at http://www.varianinc.com/spp/solphase.html Copyright 1999 Varian, Inc.)

Table 1 Articles on solid-phase extraction appearing in the *Encyclopedia of Separation Science*

Article
Extraction
Solid-phase extraction (SPE)
Automation of SPE
Bioanalytical applications of SPE (excluding drugs of abuse)
Classical SPE
Covalent SPE using immobilized boronic acids
Disk approach to SPE
Drugs of abuse
Herbicides
Insecticides
Medical applications (treatment of blood): SPE
Mixed-mode SPE
Molecular imprints for SPE
Polycyclic aromatic hydrocarbons
Phenols
Restricted-access media (SPE)
Sorbent selection for SPE

The four-step process can be as simple as this or may become more involved as one or more of these stages includes additional phases, such as selective adsorption or selective desorption. SPE method development can be tedious because the retention and recovery processes are interdependent. Retention and elution are confounded during method development because the overall analyte recovery is dependent upon both the retention efficiency and the elution efficiency. If the analyte is only recovered in part by an SPE technique, it will be initially unclear whether the problem lies with the retention process or with the elution process.

Because of this quandary, SPE method development can be approached in two ways. An iterative approach to protocol development emulating the classical analytical approach to change one variable at a time can be used. Retention parameters are held constant at selected values while optimizing the elution process (**Table 2**). Once elution is optimized, then the most favourable conditions for retention are determined. The procedure is repeated until the desired results are obtained.

Alternatively, a factorial experimental design approach to determine extraction efficiency is an efficient method development technique. Parameters important to SPE, such as sample pH, elution solvent strength, ionic strength of the sample, addition of organic modifier to the sample, elution by gravity or vacuum, sorbent retentivity, selection of sorbent mass, sample volume, elution volume and sample concentration, representing effects on both retention and elution, may be selected as factors that influence analyte recovery. The factors are usually tested at two or three levels. As a screening procedure, the factorial design can quickly pinpoint significant effects important to SPE. The objective of the factorial design approach is to obtain as much information as possible from few analyses.

Table 2 Factors affecting SPE retention and elution

Retention
Analyte character
Sorbent type
Matrix additives
Sample volume
Sorbent mass
Elution
Eluting solvent identity
Eluting solvent volume
Elution rate

Table 3 SPE sorbent–analyte interaction mechanisms

Primary mechanism	*Sorbents*
Van der Waals	Octadecyl, octyl, ethyl, phenyl, cyclohexyl, styrene-divinylbenzene
Polar–dipole/dipole	Cyano, silica, alumina, Florisil
Hydrogen-bonding	Amino, diol
Electrostatic	Cation exchange, anion exchange

Fundamentals

Stationary-phase Conditioning

Each different SPE sorbent requires conditioning or pretreatment in order to activate or prepare the sorbent to retain the analyte. Conditioning of hydrophobic stationary phases requires a two-step process of treatment with organic solvent followed by an aqueous wash. The conditioning solvent is prepared to mimic the chemistry of the sample matrix, that is, matching the pH and/or the ionic strength of the matrix. The volume of each conditioning solvent passed through the sorbent is usually about five times the dead volume of the sorbent. In addition to activating the sorbent, the conditioning solvent(s) also removes undesirable contamination potentially remaining in the sorbent during manufacture.

Retention

Originally, hydrophobic bonded-silica sorbents were the first materials introduced specifically for SPE, but currently, a suite of sorbents in varying formats are available with nonpolar, intermediate nonpolar/polar, polar, strong and weak anion and cation exchange, and steric exclusion (restricted access) properties. SPE sorbents are designed to retain analytes by a primary mechanism (**Table 3**) but often exhibit a secondary mechanism as well. For example, bonded-phase ion exchange sorbents primarily exhibit anionic or cationic exchange mechanisms but the analyte also experiences nonpolar interactions with the bonded ligand. Also, nonpolar bonded silicas exhibit a secondary polar interaction due to the silica backbone and unreacted surface silanol groups. Knowledge of the dual retention mechanisms encountered in SPE can work to the analyst's advantage. Mixed-mode sorbents (different ligands on the same sorbent) capitalize on dual retention mechanisms by design.

Understanding the mechanism(s) of retention and the selection of an appropriate sorbent depends on a thorough understanding of the character of the analyte. The solute properties of principal importance to retention by SPE are hydrophobicity, polarity and ionogenicity.

Unionized chemicals Many organic compounds are not ionized in water. An unionized organic compound is formed entirely of covalent bonds. SPE sorbent selection and recovery of unionized compounds is known generally to depend on the hydrophobicity of the analyte. The most widely measured parameter used to represent solute hydrophobicity is the octanol/water partition coefficient (K_{ow}), which is the ratio of the analyte concentration in octanol (o) relative to its concentration in an aqueous (w) phase. The logarithm of K_{ow}, also referred to as log P, ranges from -3 to 7 for organic chemicals. Analytes with log K_{ow} values less than 1 are hydrophilic; analytes with log K_{ow} values greater than 4 are highly hydrophobic; analytes with log K_{ow}s between 1 and 4 are intermediate in hydrophobicity/hydrophilicity.

Aliphatic hydrocarbons and mono- and polycyclic aromatic hydrocarbons (PAHs) are nonpolar compounds that tend to increase in hydrophobicity (log K_{ow}) with increasing molecular weight; that is, they tend to be more distributed in the organic, octanol phase, rather than in the aqueous phase. As oxygen- and/or nitrogen-containing functional groups are added to these compounds, they may (but not always) become more polar relative to the parent compound and would distribute more equally between the octanol and water phases, or even prefer to exist in the aqueous phase, thereby decreasing the value of log K_{ow}.

Sorbents for extraction of unionized compounds fall into two general categories: nonpolar extraction sorbents and polar extraction sorbents, depending on the polarity of the functional groups present. Commercially available reversed-phase bonded-silica sorbents for SPE are produced in ranging polarities. The identity of the hydrocarbon covalently bonded to the silica gel backbone may be varied. Common nonpolar ligands bonded on the silica gel surface include aliphatic hydrocarbons of one, two, eight, or 18 methylene, cyclohexyl or phenyl groups. The greater the number of methylene groups in the aliphatic chain, the greater the hydrophobicity of the sorbent generated, i.e. $C_{18} > C_8 > C_2$. For nonionized compounds, the hydrophobicity of the analyte and the hydrophobicity of the sorbent selected for SPE are inversely related; that is, less hydrophobic sorbents are used for highly hydrophobic analytes; conversely, the most hydrophobic sorbents should be used for more polar analytes.

Bonded-phase silica sorbents are known to exhibit mixed-mode retention mechanisms due to silanophilic (silanol) sites that remain on the sorbent after the initial hydrocarbon is bonded to the surface. The presence of silanol groups reduces the hydrophobic character of the surface. Silanol groups on the surface of bonded silicas interact with electron-rich hydroxyl, carbonyl, nitrile and nitro functional groups in analytes. Some of the silanol groups on the surface may be masked by a subsequent reaction with a short chain hydrocarbon in a manufacturing process termed end-capping. Consequently, reversed-phase sorbents that are end-capped are more hydrophobic in character than those that are not end-capped. When a less retentive, less hydrophobic sorbent is desired, a nonend-capped product should be tested. Other polar sorbents are produced by adding oxygen- or nitrogen-containing functional groups such as cyano, hydroxyl or amino to the hydrocarbon bonded phase, or functionalized polymeric phases are produced to enhance polarity. Unmodified silica, alumina and Florisil sorbents are polar extraction sorbents.

Matrix additives influence retention in SPE. For many nonionized chemical compounds, increasing the ionic strength of the sample matrix by the addition of sodium chloride decreases analyte solubility in the sample matrix and increases adsorption on to the nonpolar sorbent via a 'salting-out' effect. Increased salt content in the sample matrix may also produce silanol masking. Silanol groups remaining on the surface can also be deactivated through ion pairing by the addition of masking reagents such as tetrabutylammonium hydrogen sulfate to the sample matrix.

Adding water-miscible organic solvents such as methanol or acetonitrile to the sample matrix reduces the surface tension of the sample matrix, thereby decreasing the retentiveness of highly hydrophobic compounds. The addition of salt to increase the ionic strength of the sample matrix and the concomitant addition of methanol to decrease surface tension can be useful in developing methods for samples having multiple compounds that vary widely in their hydrophobic character.

Ionized chemicals Organic chemical compounds that undergo ionization are comprised of derivatives of acidic organic alcohols, carboxylic acids or basic amine functional groups. Substances that ionize dissociate into their conjugate acid or base in aqueous solution. Ionizable compounds, including organic acids such as acetic acid or benzoic acid, and organic bases such as ethylamine or aniline, are weak electrolytes and are incompletely dissociated in water. The acid dissociation constant, K_a, expresses the ratio of ionized to unionized analyte; therefore, the greater the value of K_a (or the logarithm of K_a, the pK_a) the more ionized material is present. Just as in LLE, the dependence of SPE

recovery on sample pH is a function of the pK_a of the analyte.

The relative per cent of unionized analyte to that in the ionized form exhibits a pH-dependent dissociation that can be exploited by SPE methodology. The relative concentrations of dissociated and nondissociated forms of ionizable analytes in aqueous solution are equal when the solution pH is equal to the pK_a. Therefore, in aqueous solution an organic acid is 99% unionized when the pH of the sample is two log units below the pK_a; and it is 99% ionized when the pH of the sample is two log units above the pK_a. Two log units is a good rule of thumb for considering retention of ionizable compounds. However, if the sorbent (whether nonpolar, polar or ion exchange) exhibits primary or secondary, nonpolar van der Waals-type interactions, the overall hydrophobicity and size of the ionized form of the analyte can also have an effect upon recovery and influence the two log units generalization.

In SPE method development for ionogenic compounds, the decision to be made is whether to retain the analyte as the dissociated or the undissociated form. If a compound is ionizable, the extraction may be performed using ion exchange of the dissociated form. Alternatively, if the analyte can be converted to an undissociated form by ion suppression or ion pairing, then SPE can be conducted on nonpolar sorbents, as described in the earlier section on unionized chemicals.

Organic acids lose protons in aqueous solution, ionizing to form anions, and are retained on cation exchange sorbents. Organic bases gain protons in aqueous solution, ionizing to form cations, and are retained on anion exchange sorbents. Strong and weak ion exchange sorbents are available for SPE. Ion exchange sorbents developed for SPE retain analytes not only by ionic (electrostatic) attraction, but also through secondary van der Waals (nonpolar) interactions between the analyte and the atoms comprising the bridge that links the charged functional group to the silica gel backbone.

Ion suppression refers to adjusting the pH of the sample matrix to influence the chemistry of the analyte of interest. If the ionizability of the analyte is suppressed by controlling the pH of the sample matrix relative to the pK_a of the analyte, then the analyte can be retained in the unionized form and nonpolar or polar extraction sorbents are used instead of ion exchange sorbents.

Ion pairing involves using a reagent added to the mobile phase to accomplish two objectives: to neutralize the analyte charge by combining with an oppositely charged counterionic solute; and to use a hydrophobic, bulky group on the counterion to form an ion pair that is hydrophobic enough to be retained on nonpolar extraction sorbents. The ion-pairing strategy applies to ionized organics as well as metal cations.

Ionizable and nonionizable compounds may coexist in the same sample to be analysed. Selective adsorption of either type of chemical in the presence of the other can be accomplished by controlling the sorbent through a mixed-mode approach or by chromatographic mode sequencing (the use of differing SPE sorbents in tandem), such as using both ion exchange and nonpolar mechanisms to extract the analytes. The use of selective adsorption can be applied to compounds differing in hydrophobicity, charge and structure.

In tandem mode, ionizable and nonionizable compounds may be fractionated by adsorbing nonionizable analytes on nonpolar extraction sorbents and ionized analytes on ion exchange sorbents. Once retained on different sorbents in tandem, they can be physically separated and eluted individually, thereby separating the compounds. Such fractionation often improves the ease of subsequent chromatographic analyses.

Selective adsorption of nonionized compounds in the presence of ionogenic compounds can also be accomplished with pH control of the sample matrix. By selecting a sample pH at which ionogenic compounds exist in the ionized form, it may be possible selectively to retain either the ionized or the nonionized components depending on the type of sorbent selected.

Values for hydrophobic parameters ($\log P$) and acid dissociation constants (pK_a) can often be obtained from the analyte's manufacturer. Alternatively, they can be measured in the laboratory or predicted by numerical estimation methods.

Sample volume and sorbent mass SPE retention is dependent on the relationship between sorbent mass and sample size. The strength of the interaction (whether nonpolar/polar or ion exchange) between the analyte and the sorbent, as influenced by the sample matrix solvent strength, determines the amount of the sample that may be passed through the sorbent before analyte breakthrough occurs. As the strength of the interaction increases and as the sorbent amount increases, the breakthrough volume increases. Breakthrough can be controlled and monitored by attaching a second check cartridge in tandem with the primary extraction cartridge, and eluting them separately.

To establish the dependence of retention on sample loading volume, variable volume samples (each of which comprises a constant molar amount loaded)

are passed through a constant sorbent mass and per cent recovery is plotted as a function of sample loading volume. Repeating this procedure for different sorbent masses will establish the dependence of retention on sorbent mass. Factorial design experiments can also be used to screen for the sorbent mass required relative to sample size. Optimizing the amount of sorbent necessary for the analysis will control analytical costs.

Adsorbed Contaminant Removal

During the retention process, undesirable contaminants in the sample matrix may become associated with the sorbent, or may remain behind in the interstitial spaces between sorbent particles. When the post-wash solvent is identical to the conditioning solvent and to the sample matrix, adsorbed contaminants are not likely to be removed. However, matrix components remaining in the interstitial spaces between sorbent particles will be flushed from the sorbent. The volume of the post-wash solvent should be at least equal to or preferably twice the void volume of the sorbent to ensure that the pore space is entirely replaced with the desired solvent.

If a blank, i.e. uncontaminated, sample matrix is available, it can be used to screen for potential column post-wash solvents. To remove undesirable contaminants in the sample matrix that became associated with the sorbent during the retention process, solvents of greater eluotropic or eluting solvent strength than the conditioning solvent must be used. Even a small amount of the elution solvent can be a post-wash solvent if the effects on the retained analytes of interest are monitored.

Elution

Elution solvent strength and volume The ability of a solvent to overcome the interaction between the analyte and a chromatographic sorbent, thereby causing elution to occur, is known as the solvent's eluotropic strength. Charts of eluotropic series can be consulted to determine relative solvent strength, which is roughly equivalent to polarity. The eluotropic strength of elution solvents on a non-polar adsorbent (e.g. reversed-phase) increases in reverse order to that measured on polar sorbents such as silica or alumina. On reversed-phase sorbents, the eluting power increases as the solvent polarity decreases.

Many different solvents are used for elution of the analytes from sorbents in SPE. Elution by acetic acid, methanol, acetonitrile, acetone, ethyl acetate, diethyl ether, methyl-*tert*-butyl ether, methylene chloride, benzene and hexane, and aqueous buffers containing appropriate counterions have been reported. Miscible solvent mixtures produce elution solvents of intermediate eluotropic strength.

After candidate elution solvents are selected, an elution solvent screen is conducted. Elution solvents tested at a constant volume are compared for potential to elute the analytes of interest. When selecting a desorption solvent, the effect it will have upon contaminants adsorbed from the sample matrix must be considered. A control sample matrix should also be screened if possible. The solvent demonstrating the most desirable results in the elution screen is further examined for the variation of recovery as a function of the volume of eluting solvent. Generally, the elution solvent selected is the one for which the smallest volume produces acceptable recovery. However, elution with a larger volume of lower eluting strength solvent can have the advantage of leaving strongly retained contaminants on the sorbent as the analyte of interest is desorbed. Solvent selection must also be compatible with the analytical instrumentation used for final analysis.

The elution solvent screen may reveal that selective desorption is possible for an analysis. Selective desorption uses differences in the eluotropic strength of the elution solvents to produce serial desorption. Class separation or distinct fractionation of analytes may be possible if the chemical properties of the analytes differ enough that they respond differently to weak and strong elution solvents.

Elution rate The rate of elution can affect the SPE recovery of solutes from the sorbent. Particularly for highly hydrophobic compounds, there can be slow mass transfer from the stationary phase into the mobile phase. The problem can be overcome by reducing the flow rate during elution, even to the point of allowing elution to occur by gravity if necessary.

Sample concentration independence Finally, any protocol developed must be independent of sample concentration in the range of samples to be analysed. Care must be taken not to exceed the maximum loadability of the sorbent, but that is generally not a problem since SPE is primarily used for trace enrichment.

Applications

Early in SPE history, a series of simple yet ingenious experiments were developed by Bidlingmeyer and Warren that illustrate the principles of SPE. This author has used these experiments as practical demonstrations to introduce SPE method development to

Table 4 SPE isolation of food colours in grape drink

	A	*B*	*C*	*D*	*E*	*F*
Sorbent	C_{18}	C_{18}	C_{18}	Silica	Silica	Silica
Mechanism	Van der Waals isocratic separation	Van der Waals selective desorption	Van der Waals ion pairing/ silanol masking	Polar–dipole/ dipole isocratic separation	Polar–dipole/ dipole ion suppression isocratic separation	Polar–dipole/ dipole selective desorption
Pre-wash	1) IPA (70%) 2) Water	1) IPA (70%) 2) Water	1) IPA (70%) 2) Cetylpyridinium chloride	Water	Distilled white vinegar	Water
Retention	Drink mix[a]	Drink mix	Drink mix	Drink mix	Drink mix	Drink mix
Post-wash	Water	Water	Cetylpyridinium chloride	Water	Distilled white vinegar	Water
Elution	IPA (18%)	1) IPA (5%) 2) IPA (25%) 3) IPA (70%)	1) IPA (18%) 2) IPA (70%)	IPA (18%)	IPA (16% in vinegar)	1) Water 2) IPA (15%)

IPA, isopropyl alcohol.
[a]Mixture of FD&C Blue 1 and FD&C Red 40.

new users from elementary school students to adult analysts. These experiments are outlined here (**Table 4**) as practical applications of the foregoing discussion. They are a useful method development learning aid for novice users because:

1. they demonstrate the four-step process of SPE;
2. the dyes concentrated and separated in these experiments can be observed by the naked eye, clearly revealing extraction and recovery processes;
3. the stepwise recovery of dyes from the sorbent demonstrates the ability of SPE selectively to fractionate samples.

The experiments, A–F in **Table 4**, demonstrate the use of SPE to isolate food colours using inexpensive reagents. The analytes in these experiments are the dyes FD&C Blue 1 and FD&C Red 40 prepared in an aqueous mixture by dissolving grape-flavoured drink mix (Kool-Aid®), in distilled water. Other reagents necessary for these experiments include vinegar, rubbing alcohol and mouthwash. The original source (Bidlingmeyer and Warren) should be consulted for details.

The types of sorbents used for the experiments are a hydrophobic, reversed-phase sorbent, C_{18} (experiments A–C), and a polar sorbent, silica (experiments D–F). The same two analyte dyes are retained in each experiment, albeit via different mechanisms depending on the sorbent: by van der Waals forces on the C_{18} sorbent and polar dipole–dipole interactions with the silica sorbent.

On the reversed-phase sorbent (C_{18}), conditioning involves exposure of the sorbent to a water-miscible organic solvent followed by an aqueous wash (experiments A–C). The preparation of the sorbent surface to accept the analyte must be done in this order. Measuring exact amounts of solvents is not necessary during the pre-wash step. The sorbent is not allowed to dry between column preparation steps and the sample loading step. If it does dry out during column preparation, before the sample has begun to be loaded, the process should begin again. The silica sorbent is activated by a single aqueous pre-wash of water (experiments D and F) or distilled white vinegar (experiment E).

As the sample is loaded on to the sorbent, the organic dyes extracted are observed to become concentrated at the leading edge of the sorbent. After the sample is loaded, drying of the column is not crucial, and in fact it is useful in some analyses to dry the sorbent with vacuum before eluting the analytes.

FD&C Blue 1 (Brilliant Blue FCF, CAS # 3844-45-9) and FD&C Red 40 (Allura Red AC, CAS # 25956-17-6) are large dye molecules (formula weights of approximately 800 and 500, respectively) that have negatively charged sulfonate groups in aqueous solution. Although ionogenic, the molecular size and hydrophobicity of the dyes permit retention on the C_{18} sorbent even without pH control (experiments A and B).

Two of the experiments demonstrate the effect of matrix additives on retention (experiments C and E). In experiment E, distilled white vinegar is used for ion suppression by reducing the pH and its addition results in reversal of the elution order of the dyes. In experiment C, an ion-pairing reagent, cetylpyridinium chloride (Cepacol® mouthwash), is added as

a bulky, hydrophobic, positively charged counterion to pair with the negatively charged sulfonate groups of the dyes. In this case, cetylpyridinium chloride also behaves as a silanol masking agent.

Post-washes flush any remaining sample matrix from the interstitial pores of the sorbent, and in each experiment the post-wash solvent is the same as the last pre-wash solvent. Additives in the drink mix that are not retained (sugars, acids) will elute during sample loading and during the post-wash.

Elution is accomplished by varying the concentration of isopropyl alcohol (commercially available rubbing alcohol is approximately 70% isopropyl alcohol in water). The isopropyl alcohol is mixed with either water (experiments A, B, C, D or F) or vinegar (experiment E). Sample desorption volumes are measured for quantitative purposes. The volume recovered is always less than the volume added. The desorption can be done in one isocratic separation process (experiments A, D and E). Performed in stages, experiments B, C and F are examples of selective desorption. SPE columns generate around 20–200 theoretical plates and this is sufficient in many cases to produce a fractionation of components and chemical classes.

Future Developments

The history of SPE has already been marked by continuous advances in sorbents and the formats in which SPE sorbents are utilized. So, it's a fairly safe prediction that in the future there will be continued development of new SPE sorbents and modes of delivery. Along that trend, recent developments of molecularly imprinted sorbents for SPE and the 96-well plate format for SPE are gaining attention and are expected to be actively developed in the near future. Molecularly imprinted sorbents for SPE are polymeric phases formed with the analyte of interest as a print molecule. The template thus formed exhibits selectivity for the imprinted molecule. The 96-well microassay plate collection format is designed to utilize robotics for simultaneous extraction of 96 samples.

In addition to continued development of specialty-phase sorbents and formats, advances in performing more sophisticated chemistry associated with extraction are predicted. Applications of analyte derivatization in conjunction with SPE are already reported, with the chemical reaction occurring at the sorbent surface. Methods apply either to solid-supported reactants or solid-supported analytes. Selective adsorption schemes that utilize tandem chromatographic mode sequencing approaches will be used to solve complex multiclass/multiresidue extractions. More intricate mixed-mode adsorption mechanisms that mimic analyte–receptor 'lock-and-key' approaches to extraction are expected.

See also: **I/Extraction. II/Extraction:** Analytical Extractions; Solid-Phase Extraction; Solid-Phase Microextraction. **III/Airborne Samples: Solid-Phase Extraction. Bioanalytical Applications: Solid-Phase Extraction. Drugs of Abuse: Solid-Phase Extraction. Environmental Applications:** Solid-Phase Microextraction. **Herbicides:** Solid-Phase Extraction. **Immobilised Boronic Acids: Extraction. Immunoaffinity Extraction. Insecticides:** Solid-Phase Extraction. **Molecular Imprints for Solid-Phase Extraction. Solid-Phase Extraction with Cartridges. Solid-Phase Extraction with Discs. Solid-Phase Matrix Dispersion: Extraction. Solid-Phase Microextraction:** Biomedical Applications; Environmental Applications; Food Technology Applications; Overview. **Sorbent Selection for Solid-Phase Extraction. Appendix 2: Essential Guides to Method Development in Extraction.**

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