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Review

Solid Phase Extraction Technique – Trends, Opportunities and Applications

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Abstract

Solid phase extraction (SPE) is an extraction method that uses a solid phase and a liquid phase to isolate one, or one type, of analyte from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantify the amount of analyte(s) in the sample. The general procedure is to load a solution onto the SPE phase, wash away undesired components, and then wash off the desired analytes with another solvent into a collection tube. Solid-phase extractions use the same type of stationary phases as are used in liquid chromatography columns. The stationary phase is contained in a glass or plastic column above a frit or glass wool. The column might have a frit on top of the stationary phase and might also have a stopcock to control the flow of solvent through the column. Commercial SPE cartridges have 1-10 ml capacities and are discarded after use. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantify the amount of analyte(s) in the sample. Solid phase extraction procedures are used not only to extract traces of organic compounds from environmental samples but also to remove the interfering components of the complex matrices in order to obtain a cleaner extract containing the analytes of interest. The SPE technique is widely applied for isolation of analytes from a liquid matrix and purified extracts.

This paper is a review of the literature regarding general information about SPE technique, new trends in SPE technique and its application.

Keywords: solid phase extraction, solid phase microextraction, selective sorbents, SPE application, new trends

Introduction

Solid phase extraction is the very popular technique currently available for rapid and selective sample preparation. The versatility of SPE allows use of this technique for many purposes, such as purification, trace enrichment, desalting, derivatisation and class fractionation. The last few years have been chracterized by a wide interest in this technique and many publications describing SPE methods have been published [1]. This period is connected with the intensive development of research procedures for novel types of sorptive materials and lasted from the late 1960s until the beginning of the 1980s. The introduction of a wide spectrum of sorptive materials into analytical procedures gave a new stimulus for the development of SPE methodology [2].

The principle of SPE is similar to that of liquid-liquid extraction (LLE), involving a partitioning of solutes between two phases. However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase. This sample treatment tech-

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nique enables the concentration and purification of analytes from solution by sorption on a solid sorbent and purification of extract after extraction. The general procedure is to load a solution onto the SPE solid phase, wash away undesired components, and then wash off the desired analytes with another solvent into a collection tube [3].

Mechanism of Solid Phase Extraction Process

The selection of an appropriate SPE extraction sorbent depends on understanding the mechanism(s) of interaction between the sorbent and analyte of interest. That understanding in turn depends on knowledge of the hydrophobic, polar and ionogenic properties of both the solute and the sorbent. The most common retention mechanisms in SPE are based on van der Waals forces ("non-polar interactions"), hydrogen bonding, dipole-dipole forces ("polar" interactions) and cation-anion interactions ("ionic" interactions).

Each sorbent offers a unique mix of these properties which can be applied to a wide variety of extraction problems. Four general theory interactions exist:

– Reversed phase involves a polar or moderately polar sample matrix (mobile phase) and a nonpolar stationary phase. The analyte of interest is typically mid- to nonpolar. Retention of organic analytes from polar solutions (e.g. water) onto these SPE materials is due primarily to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the sorbent surface. These nonpolar – nonpolar attractive forces are commonly called van der Waals forces or dispersion forces. The nonpolar solvent, which can disrupt the forces between the sorbent and compound, is used to elute an adsorbed compound from a reversed phase SPE tube or disk. The following materials are used as reversed phase: carbon-based media, polymer-based media, polymer-coated and bonded silica media. Carbon-based media consist of graphitic, non-porous carbon with a high attraction for organic polar and nonpolar compounds from both polar and nonpolar matrices. Retention of analytes is based primarily on the analyte's structure, rather than on interactions of functional groups on the analyte with the sorbent surface.

Polymer-based sorbents are styrene/divivinylbenzene materials. It is used for retaining hydrophobic compounds which contain some hydrophilic functionality, especially aromatics. Elution steps can be done with mid- and nonpolar solvents, because the polymeric packing is stable in almost all matrices. Polymer-coated and bonded silica media is a hydrophobic-bonded silica that is coated with a hydrophilic polymer. The pores in the polymer allow small, hydrophobic organic compounds of interest (e.g. drugs) to reach the bonded silica surface, while large interfering compounds (e.g. proteins) are shielded from the bonded silica by the polymer and are flushed through the SPE tube.

– Normal phase involve a polar analyte, a mid- to nonpolar matrix (e.g. acetone, chlorinated solvents and hexane) and a polar stationary phase. Retention of an analyte under normal phase conditions is primarily due to interactions between polar functional groups of the analyte and polar groups on the sorbent surface. These include hydrogen bonding, л- л interactions, among others. A compound adsorbed by these mechanisms is eluted by passing a solvent that disrupts the binding mechanism, usually a solvent that is more polar than the sample's matrix.

The bonded silicas have short alkyl chains with polar functional groups bonded to the surface. These silicas, because of their polar functional groups, are much more hydrophilic relatively to the bonded reversed phase silicas. As with typical normal phase silicas, these sorbents can be used to adsorb polar compounds from nonpolar matrices.

The polar adsorption material is modified silica gel commonly used as the base of all of the bonded phases. The functional groups that are involved in the adsorption of compounds from nonpolar matrices are the free hydroxyl group on the surface of the silica particles. That may be used to adsorb polar compounds from nonpolar matrices with subsequent elution of the compounds in an organic solvent more polar than the original sample matrix.

– Ion exchange SPE can be used for compounds that are in a solution. Anionic (negatively charged) compounds can be isolated on an aliphatic quaternary amine group that is bonded to the silica surface. Cationic (positively charged) compounds are isolated by using the silica with aliphatic sulfonic acid groups that are bonded to the surface. The primary retention mechanism of the compound is based mainly on the electrostatic attraction of the charged functional group in the compound to the charged group that is bonded to the silica surface [4].

The meaning of pH in SPE is very important. Solutions used in SPE procedures have a very broad pH range. Silica-based packings usually have a stable pH range of 2 to 7.5 and at pH levels above and below this range, the bonded phase can be hydrolized and cleaved off the silica surface, or the silica itself can be dissolved. In SPE, however, the solutions usually have a contact with the sorbent for a short period of time. The fact that SPE cartridges are disposable, and are meant to be use only once, allows one to use any pH to optimize retention or elution of analytes [5, 6]. Fig. 1 shows the method for selecting a mechanism to determine SPE procedure.

The Formats and Procedures in Solid Phase Extraction

In SPE technique four types of sorbent formats exist: free disks (which are generally 47 mm in diameter or the standard filtration size), disks in syringe barrels-cartridge (which vary in size from microsized disks in 1 ml syringes to a 6 ml syringe), a 96-well microtiter plate configuration that uses the 1-ml disk, and the SPE pipette tip.

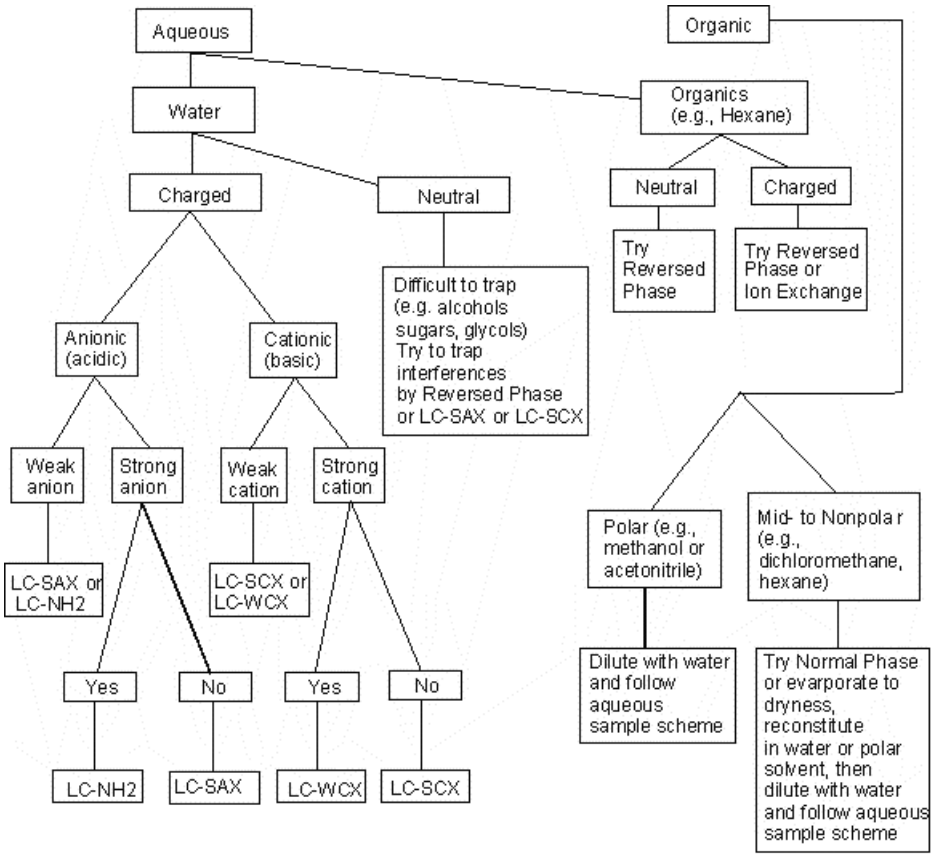

Fig. 1. Choice of SPE procedure.

Cartridges

The main format in SPE is the syringe-barrel and cartridge type. The SPE cartridge is a small plastic or glass open-ended container filled with adsorptive particles of various types and adsorption characteristics. The cartridge type is still the most popular format with typically 40-60 μm d_p packing material. Limitations of packed SPE conventional cartridges include restricted flow-rates and plugging of the top frit when handling water-containing suspensed solids such as surface water or wastewater. Therefore, the typical volume of sample is 500 ml if the sample has not been carefully filtered before. In the case of filtered sample the volume can be higher. SPE cartridges are available in a wide range of sizes, with volumes ranging from 1 ml to 50 ml. During selection for the optimum cartridge size for a particular application, the following factors must be considered: ability to retain all analytes, volume of original sample, and final volume of the purified sample after elution [5].

Many different types and amounts of sorbent are contained between two polyethylene or stainless steel frits in glass or polypropylene cartridges which have different column volumes. Cartridge design has certain disadvantages for water analysis. For example, the cross-sectional area is small, therefore sample processing rates are slow and the tolerance to blockage by particles and adsorbed matrix components is low, and channeling reduces the capacity to retain analytes. The cartridges are still the most frequently used, mainly because of the limited number of commercially available sorbent on disk [6].

The SPE extraction cartridge is particularly attractive for use in pesticide residue analytical methods, since it often eliminates the need for expensive and environmentally sensitive solvents. The SPE packing materials or cartridges are very often used for extraction of pesticides from a water matrix or for purificaton of pesticide extract. The pesticides can be adsorbed on the sorbent bed while the interferences pass through without retention. The pesticides are then eluted and carried forward to an appropriate determination step.

Discs

Another design which has become available in the last few years is the disks. Disk is a variation of the extraction cartridge. Discs consist of a 0.5 mm thick membrane where the adsorbent is immobilized in a web of microfibrils.

The sorbent (on polymer or silica) is embedded in a web of PTFE or glass fibre. Glass fibre disks are thicker and more rigid, providing higher flow-rates than with PTFE membranes. The sorbent particles embedded in the disks are smaller than those found in the cartridges (8 μm diameter rather than 40 μm). The short sample path and small particle size allow efficient trapping of analytes

with a relatively high flow-rate through the sorbent, as compared to the cartridges. The disks are primarily used to reduce analysis time when handling large volumes of aqueous environmental samples.

Empore is the first format of disks used. One of the disadvantages of using disks instead of cartridges is the decrease in the breakthrough volume, mainly for more polar compounds. For this reason, disks are used when there is a strong interaction between the analyte and the sorbent [8]. The typical Empore disk has a plate height of 0.1 mm, whereas a typical cartridge has a bed height of 1 cm.

The modification of extraction disks is cartridge disks. The extraction disk cartridges come in three diameters: 4 mm/1 ml, 7 mm/3 ml and 11 mm/6 ml. The sorbent layer is placed in the plastic barrel of the syringe cartridge and held in place by retainer rings. Disks have a large surface area per unit bed mass. This facilitates their use for passive sampling by immersing the disc in the sample as opposed to the conventional approach of passing the sample through the disc in a manner similar to filtration. Passive sampling is convenient for both field and laboratory applications, but has been little explored so far.

For small sample sizes, it is easier to miniaturize discs than cartridges, and several disc devices that contain only a few miligrams of sorbent are available[6].

Disks are available in several different diameters (4.6 mm, 47 mm and 93 mm), with the larger volumes allowing faster flow-rates. The most frequently used disk size is the 47 mm, which is suitable for standard methods (0.5-1 l water sample volumes) [5]. The construction of extraction disks allows relatively high flow-rates of samples through the bed, in comparison with cartridges filled with the same material because of the absence of channelling and the faster mass transfer provided by the smaller particle size. Membrane extraction disks which consist of the filter above the membrane hale also been developed. J.T. Baker has introduced new laminar disks known as Speedisks which consist of a thin bed of microparticles supported in a laminar structure in a preassembled disk. The prelocation of 1 l of surface water without any previous filtration takes less than 5 min [7, 8].

Disks have two distinct advantages over conventional SPE cartridges. Firstly, they often can be operated with smaller elution volumes and higher flow rates. The improved performance of the disk can be attributed to the small particle size (8-12 μm) of the sorbent embedded in the politetrafluoroethylene (PTFE or Teflon), compared to 40-80 μm in a conventional cartridge. The decrease in void volume and increase in surface area associated with the small particles promote partitioning. Hence, a smaller mass of sorbent is required to process a similar volume of sample, permitting the use of smaller volumes of solvent for elution. Secondly, the increase in density and uniformity of packing provided by the smaller particles mitigates breakthrough and channeling, which permits the use of higher flow rates and reduces extraction times [9, 10]. The disks are filled with the same chromatographic material as conventional cartridges. The chromatography is similar to that in the cartridges. The differences lay in particle size. For the same bed height between a disk and a cartridge, the disk has many more particles and a much more tortuous path of flow, which means that there is considerably more surface area available and the kinetics of sorption are much quicker for the disk than the cartridge. The Speedisks are most often used for isolation pesticides from high-volume water samples [11].

SPE Pipette Tips

The automation of SPE began to drive new formats to cope with the automated systems. Among different devices in SPE the new devices are pipette tips introduced in 1998. Pipette extraction represents a simple and rapid method of solid phase extraction. The solid phase sorbent is positioned inside a pipet tip, held in place by a screen and filter. Because the stationary phase is mixed with the sample, the conditioning step necessary for conventional solid-phase extraction is not required. The sample is subsequently sent to a waste container, and the stationary phase is washed only with 1 ml of water or buffer. After the washing step solvent is sent to waste and the adsorbed analytes are eluted by drawing up only 0.1- 0.3 ml of solvent, vortex mixing, and transferring the solution into GC or HPLC vial. The time-consuming concentration step is not required because of the small volume of solvent. The SPE pipette tips technique has some advantages: faster extraction time (1-2 min.), one extraction method for all analytes, clean extracts, less sample volume (200 μl) than typical SPE, less solvent volume (200 – 400 μl) and less solvent waste.

Several companies began to manufacture pipette tips, each with a different twist, e.g.: Millipore (Bedford, Massachusetts) introduced the ZipTip 4 pipette tip and EST Analytical's (Cincinnati, Ohio) introduced DPX disposable pipette extraction tips (provided a pipette tip designed to extract drugs of abuse from small volumes of urine or serum). In this configuration, the sorbent is placed loosely between two frits inside the pipette tip; sample is drawn and mixed with the stationary phase. The matrix is sent to waste, and the adsorbed drugs are eluted with the small amount of solvent and analyzed by GC-MS [10].

96-Well SPE Plates – New Trends in Solid Phase Extraction

The new format of SPE is 96-Well SPE Plates. The 96-well plate format is based on the standard 96-well microtiter plate format. Parallel sample processing allows 96 samples to be extracted in approximately one hour or less. Processing 96 samples simultaneously reduces handling errors and limits labour-input.

Each of the 96 wells has a small 1 or 2 ml SPE column with 3-10 mg of packing material. The packing material in an SPE cartridge is placed between the bottom frit or

membrane and the top frit. Types of 96-well SPE plates exist: fixed and flexible. The plates are called fixed because most of them have a fixed volume and fixed amount of sorbent. Compared with typical SPE cartridges, these small packed bed wells have different flow characteristics, masses and volumes, so they require some adjustment of the SPE conditions [12, 13]. These plates are rather costly and, given that a test may use only a few of the wells, laboratories must incur considerable expense in method development experiments.

The modification of Flexible 96-well SPE plates. The flexible well plates have removable small round or square plastic SPE cartridges that fit tightly into separate 96-hole base plates. Each individual cartridge has top and bottom frits and a stationary-phase packing, as used in the fixed 96-well SPE plate design. Users can place individual cartridges in as many positions as needed. The individual cartridges can contain different stationary phases or different amounts of the same phase. In some instances, different cartridge volumes (1 or 2 ml) are available and can be used for larger samples. A vacuum manifold is often used to pull liquids through a flexible well plate, and users can plug the unused holes of the base plate with plug strips if only a portion of the 96 wells is needed [14].

384-well SPE plates also exist and have the same external dimensions as the current 96-well SPE plates, so SPE wells would be very tightly sandwiched together.

An advantage of automated method development is that analysts can expect improved method precision and accuracy over manual SPE methods because they generally have better control over the sample and solvent manipulation in an automated environment.

New formats for SPE provide reduced bed masses, high-throughput capabilities and greater convenience for method development. A small mass of the bed allows faster method development, reduced solvent volume and shorter sample preparation times. The transfer of manual methods to automated methods has been improved by the advent of removable, flexible cartridges that can be used manually or in an automated environment.

Sorbents in SPE

The applicability of SPE is mainly determined by the sorbent used in the extraction column. Hydrophobic and hydrogen bonding energies are rather small compared with electrostatic interaction energies. Although it can be stated that a more selective extraction may be performed by using higher energy interactions. Nowadays a large number of sorbents are available, and the most frequently used group of sorbents are:

- chemically modified silica gel,
- polymer sorbents,
- graphitized or porous carbon.

Solid phase extraction is performed using either silica-based or organic resin-based sorbents, with suitable physical characteristics and chemical properties. The nature of the base material and the additional functional groups both affect the way that the sorbents are used.

The sorbents in all cases are three-dimensional polymeric materials which are manufactured under conditions designed to provide a very porous but rigid material with a high surface area.

Silica and Bonded Silica Sorbent

Silica gel can be used as a very successful adsorbing agent, as it does not swell or strain, has good mechanical strength and can undergo heat treatment. The surface of silica particles is heterogeneous, with a variety of different types of silanol groups present. Silica can be used as an SPE sorbent without further modification. To increase its applicability and the options available to the scientist for choosing the appropriate extraction mechanism, the surface of the silica material is usually modified by bonding a wide variety of functional groups to the surface. The nature of the functionality can be non-polar (e.g. C_{18}), polar (e.g NH_2), ionic or mixed-mode (C_8/cation exchange). With respect to the applied washing and elution solvents, the most frequently used groups of sorbents can be divided into the following categories: reversed-phased (RP), normal-phase (NP) and ion-exchange (IE) [15].

Bonded silica sorbents are manufactured by reacting an organosilane with the silica surface. The organosilanes that are used consist of a silicon atom bonded to an organic functional group like C_{18}, and 1-3 chlorine atoms. The two common types of bonding are monofunctional, where the organosilane has one chlorine atom, and trifunctional, where the organosilane has three chlorine atoms. Sorbents manufactured using monofunctional silanes tend to be less stable to extremes of pH because of the single point of attachment of the silane to the silica particle.

Bonded phases which can be used in normal phase fashion are formed when the R group of the silyl derivatizing agent is a cyano, amino or diol group. These bonded silicas are less retentive than silica gel toward very polar analytes and therefore permit extractions impossible to achieve with unbonded silica. As in normal phase adsorption chromatography, the sample is dissolved in a solvent of low solvent strength, allowing partitioning of the analyte onto the polar sorbent surface when the sample is passed through the column.

Reversed phase chromatography refers to any system in which the sorbent is less polar than the mobile phase or sample solution. Octadecyl, octyl, cyclohexyl, butyl, phenyl, and cyano substituted siloxanes can be used to extract nonpolar and small polar analytes. The octadecyl and octyl bonded silicas have been the universal extraction sorbents.

Most polar bonded silicas contain $–SO_3^-$ and $–N^+(CH_3)_3$ ionic functional groups. These phases are used for the extraction of acids and bases from aqueous solution according to the classic theories of ion exchange. The $–SO_3^-$ group is a strong anion exchanger (SCX) for the extrac-

tion of basic analytes from solution. The $-N^+(CH_3)_3$ group is a strong anion exchanger (SAX) for the adsorption of acids. To achieve optimum extraction conditions, the ion-exchanger sorbent and the analyte should be oppositely charged [16, 17]. Sorbents for solid phase extraction and separation mechanisms for solid phase separations are listed in Table 1.

Very popular and often used sorbents are: Florisil, Alumina and silica gel. Florisil (a magnesium silicate) is particularly suited to clean up of extracts from fatty foods because it retains some lipids preferentially. Florisil is very good for cleaning up extracts containing nonpolar pesticides, such as the chlorinated hydrocarbons; it produces very clean eluants, removes most interferences when eluted with nonpolar solvents. Alumina (Al_2O_3) can be substituted for Florisil for the clean up extracts of fatty foods. It is particularly useful for isolation of certain polar pesticides without losses in silica gel.

A simple solid-phase extraction (SPE) clean up method has been developed for the determination of organochlorine (OC) pesticides in vegetables. Pesticide residues were extracted with acetone and dichloromethane. Extracts were further cleaned up with an octadecyl, C_{18} SPE column. The pesticides retained in the column were eluted out with hexane and petroleum ether and quantitatively determined by gas chromatography (GC) using micro electron capture detector (μ-ECD) [18].

Polymer Sorbents

Modern porous polymer sorbents are generally copolymers of styrene and divinylobenzene. The porous polymers have moderate surface areas (< 600 m^2/g) or are bioporous and highly crosslinked with surface areas of 700-1200 m^2/g. The larger surface areas of the highly crosslinked polymers result in higher retention.

The most widely used polymeric sorbents are the styrene-divinylbenzene copolymers (PS-DVB) [17]. This sorbent overcame many of the limitations of bonded silicas. Polistyrene-divinylbenzene resin copolymer is a hydrophobic resin which has greater analyte retention, mainly for polar compounds, than their hydrophobic surface containing a relatively large number of active aromatic sites which allow π-π interactions with unsaturated analytes. Sorbent resins overcome many limitations of bonded silicas [7, 19].

The higher potential of PS-DVB resins, such as Amberlite XAD-type, over C_{18} silicas for trapping polar compounds was largely demonstrated but these sorbents were not available in prepacked cartridges because they required laborious purification steps before use [19].

XAD resins have been used to extract a variety of organic pollutants from water. XAD-1, XAD-2 and XAD-4 are PS-DVB with a highly hydrophobic character. XAD-7 and XAD-8 are acrylic ester resins with a higher affinity for polar solutes. XADs based on PS-DVB have some drawbacks including lack of selectivity, low breakthrough volumes for very polar compounds, and low sampling rate, and they require extensive cleaning before use.

Another widely used PS-DVB copolymer is the PLRP-S resin. It has been used in the extraction of organic pollutants such as pesticides from natural waters. PLRP-S has the same drawbacks as XADs: lack of selectivity and low breakthrough volumes for very polar compounds.

In recent years chemically modiefied resins used in the SPE of polar compounds from environmental waters have been developed. Into polymeric resins different polar functional group such as acetyl, hydroxymetyl, benzoyl have been introduced. These modiefied resins have excellent hydrophilicity and give higher recoveries than their unmodiefied analogues. This has been attributed to an increase in surface polarity which enables the aqueous sample to make better contact with the resin surface.

Graphitized or Porous Carbon

Active carbons are a sorbent with high specific microporous surface areas containing polar groups. Graphitized carbon blacks (GCBs) are obtained from heating carbon blacks at 2700-3000°C in an inert atmosphere. They are essentially non-specific and non-porous sorbents with a surface area of about 100 m^2/g. All carbon blacks contain various functional groups at the surface following the oxygen chemisorption. The surface framework of GCBs used in SPE was shown to be contaminated by oxygen complexes. These groups are able to interact so strongly with sufficiently acidic compounds that conventional solvent systems are not able to desorb them [20]. Because of the presence of positively charged chemical heterogenites on their surface, they can be considered to be both reversed-phase sorbents and anion exchangers. Some commercial GCBs, such as Carbopack, Carbograph 1 and Envi-Carb, have been used in the analysis of different analyte e.g.: phenolic compounds, pesticides and surfactants in water. The new GCB sorbents: Carbograph 4 and Carbograph 5, with surface areas greater than 100 m^2/g were used to determine different analytes in water and they yielded better recoveries for more polar compounds than Carbograph 1. Graphitized carbon black exhibits the advantage of simultaneous extraction of neutral, basic and acidic compounds. The versatility of this sorbent is likely due to its behaviour as both a non-specific (i.e. van der Waals interactions) and anion-exchange sorbent. Some drawbacks of the GCB sorbents are that they have excessive retention but this can be overcome by performing the elution in the backflush mode [20].

Porous graphitic carbon (PGC) was available in SPE cartridges at the end of the 1980s. The new carbon-based sorbent is a porous immobilized graphitic carbon in which the graphite is immobilized on a silica structure, and this is why PGC is more stable than GCB. PGC have average specific surface area of 120 m^2/g, uniform pore structure with mean pore diameter of 25 nm and a porosity of 75%. The use of PGC was investigated for the trace enrichment of polar phenolic compounds and compared with polymeric sorbents.

Table 1. Sorbents for solid phase extraction and separation mechanisms for solid phase separations.

Sorbent	Structure	Analyte type	Dissolving solvents	Elution solvents
Reversed Phase				
Octadecyl (C_{18}) Octyl (C_8) Ethyl (C_2) Cyclohexyl Phenyl	$-(CH_2)_{17}CH_3$ $-(CH_2)_7CH_3$ $-CH_2CH_3$ $-CH_2CH_2$-C_6H_{12} $-CH_2CH_2$-C_6H_6	Nonpolar	methanol/water, acetonitrile/water	For nonpolar analytes: hexane, chloroform For polar analytes: methanol
Normal Phase (bonded)				
Cyano (CN) Amino (NH_2) Diol (COHCOH)	$-(CH_2)_3CN$ $-(CH_2)_3NH_2$ $(CH_2)_3OCH_2CHOHCH_2OH$	Slightly- moderately polar - strongly polar	hexane, chloroform	methanol
Normal Phase (adsorption)				
Kieselguhr (Diatomaceous Earth) Silica gel Florisil Alumina (neutral)	-SiOH $-SiOH$ Mg_2SiO_3 Al_2O_3	Slightly- moderately polar - strongly polar	hexane, chloroform	methanol (dependent on type of analyte)
Ion Exchangers (anion and cation Exchange)				
Amino (NH_2) 1°, 2°- Amino (NH/NH_2) Quaternary Amine (N^+) Carboxylic acid (COOH) Propyl Sulfonic Acid (SO_2OH) Aromatic Sulfonic Acid ($ArSO_2OH$)	$-(CH_2)_3NH_2^+$ $^-(CH_2)_3NH^+CH_2CH_2NH_2$ $^-(CH_2)_3N^+(CH_3)_3$ $-(CH_2)_2COO^-$ $^-(CH_2)_3SO_2O^-$ $^-(CH_2)_3$-C_6H_6-SO_2O^-	Anion exchange - Ionic Acid Cation exchange - Ionic Base	Water or buffer (pH=pKa +2) Water or buffer (pH=pKa-2)	1) Buffer (pH=pKa +2) 2) pH where sorbent or analyte is neutral 3) Solvent with high ionic strength 1) Buffer (pH=pKa-2) 2) pH where sorbent or analyte is neutral 3) Solvent with high ionic strength

Carbon is unlike other adsorbents, as it has different elution characteristics due to its lipophilic nature. Sorbent absorbs preferentally nonpolar and high molecular weight pesticides. Carbon sorbents were used mainly for extracting different volatile compound from air and pestcides from liquid phase, e.g. a novel porous carbon sorbent CARB GR was used for isolation of dicarboxyimide fungicides residues from grape wines and clean-up of extract with subsequent capillary gas chromatography-flame ionization detection, electron-capture detection (ECD) and mass spectrometry-ion-trap detection (MS-ITD) analysis [21].

Selective Sorbents

Molecularly Imprinted Polymers

The molecularly imprinted polymers (MIPs) technique has become increasingly popular in recent years. Molecularly imprinted polymers are very stable and can withstand heating to temperatures higher than 120°C and treatment with organic solvents, strong acids and bases with only small losses in selectivity. Molecularly imprinted polymers are made by synthesizing highly crosslinked polymers in the presence of a template molecule. After removal of this molecule, the polymer can be used as a selective binding medium for the template (analyte) or structurally related compounds. The template and monomer are first mixed in order to form a stable prepolymerization complex in a selected solvent. Subsequently, the polymerization is initiated in the presence of a suitable cross-linker. After polymerization, the polymer is ground and sieved to an appropriate particle size, and the template is removed, leaving cavities complementary in shape, size and functionality. These cavities are able to selectivity rebind, in given conditions, the analyte (the template) from the complex mixture. The selective interactions between the template and the monomers are based upon hydrogen bonds and ionic and hydrophobic interactions. The preparation of MIPs is easy and inexpensive, and they can be easily adapted to different analytical purposes [2, 22]. MIPs are being used in an increasing number of applications as separation materials, as antibody/receptor binding site mimics in recognition and assay systems, as enzyme mimics for catalytic applications, and as recognition elements in biosensors as well as facilitated chemical synthesis [2].

To date, their most extensively investigated application has been the separation of materials in molecuraly imprinted solid phase extraction (MISPE). The polymers can be packed in disposable cartridges for off-line SPE or in columns for on-line SPE. Experimental parameters such as particle-size distribution, packing uniformity and flow characteristics affect recovery and reproducibility in the same manner as they do with conventional SPE materials. Only a few practical applications of MIPs for SPE have been demonstrated so far, most of which are for the isolation of drugs, pesticides, phenolic compounds and amino acids [23].

Molecularly imprinted polymers can provide a strong contribution to the selectivity and sensitivity of analytical methods. Theoretically, they can solve a given selectivity problem, reduce method development time, reduce analysis time and, possibly, allow the use of more conventional detectors [8].

Immunosorbents

Recently, new extraction sorbents involving reversible and selective antigen-antibody interactions, called immunosorbents (ISs), have been synthesized in order to trap structurally related pollutants. The immuno-extraction technique consists of using SPE cartridges filled with antibody materials bonded onto silica-based sorbents. Immunosorbents are prepared by covalently bonding a suitable antibody to an appropriate sorbent. In the immunosorbent, the antibody is immobilized onto a silica support and used as an affinity ligand to extract the target analyte, and other compounds with similar structures, from the aqueous sample. These sorbents are used in disposable cartridges or as short precolumns in coupled-column LC. The ideal support for an immunoaffinity sorbent is rigid and porous so that the flow rates of environmental sample can be high. Likewise, it should provide functional groups to enable appropriate coupling with a sufficient number of antibodies and be hydrophilic to prevent nonspecific interactions with the analytes and the sample matrix.

The immunosorbents are very interesting materials because of their high selectivity, which allows extraction, concentration and clean up from complex matrices in a single step, and from large sample volumes, when required. The high degree of molecular selectivity is because of the specificity of the antibody-antigen (analyte) spatial fitting and interactions. Cross reactivity, considered a disadvantage in the development of single-compound schemes, is advantageous for multiresidue methods.

Immunosorbents have been applied to determination of several pesticides off-line and on-line. Applied to the coupling of ISs with LC-APCI-MS detection is a powerfull technique for the determination and quantitation of polar pesticides in environmental matrices at the low ppt level without the need for additional clean-up steps. By using a small sample volume, very low detection limits can be reached due to the enhanced selectivity and high sensitivity [23-25]. These sorbents have been applied also for the clean up of river water samples and soil samples, followed by liquid chromatography with diode array detection (LC-DAD) [26] and have been applied in the analysis of triazines and phenylurea herbicides in different waters.

Solid Phase Extraction Process

The SPE process can provide samples that are in solution, free of interfering matrix components and concentrated enough for detection. Solid phase extraction is achieved through the interaction of three components: the sorbent, the analyte and the solvent. The analyte must be attracted more strongly to the sorbent than to the matrix. The best solid

phase extraction mechanism and procedures are defined by the characteristics of the analyte in the sample. The steps of the solid phase extraction process are shown in Fig. 2.

Sorbent selection depends on the analyte characteristics, the sample matrix and the analytical method. Column selection also depends on the impurities that must be separated from the analyte. If the analytes are polar, normal phase extraction is indicated. When the analytes are less polar, reverse phase separation is advised. The properties of sample impurities can often be exploited in analyte purification, for example in the case of meats and adipose tissue samples containing high fat, matrix components are very soluble in nonpolar solvents. Following nonpolar solvent extraction, the moderately polar analytes can be adsorbed on a polar adsorbent (e.g., silica gel, Florisil or Alumina) while fats pass through the column unretained [6].

For solvent selection the eluotropic strength of adsorption on silica ε° and the polarity index p' are very helpfull in the design of extraction procedures. The polarity index is an accurate measure of the polarity of the solvent for liquid-liquid extraction (measure of solvent's ability to interact as proton donor, proton aceptor or dipole). An eluotropic series arranges solvents in order of decreasing elution strength for solutes from a particular adsorbent. Selection of an appropriate ε° can be obtained from the plot of solvent strength for various binaries as a function of binary composition. Solvent strength does not vary linearly in this plot but provides a good approximation in developing new solid phase extraction methods. Binary mixtures with ε° greater than 0.5 generally should be considered first for the dissolution of nonpolar solutes prior to adsorption on nonpolar adsorbents (e.g., octyl and octadecyl bonded silicas). The choice of an eluting solvent is determined by the relationship of ε° and the polarity of the analyte. The high ε° of methanol (0.73), is the basis for its selection as an eluate for the removal of moderately polar and strongly polar analytes from polar adsorbents. Methanol is unique in its interaction with both nonpolar and polar groups. Methylene chloride (ε° = 0.32), frequently elutes nonpolar analytes effectively from nonpolar bonded phases. Although the principle "like dissolves like" underlies all experimental work, evaluation of the solvent is still more art than science [9].

When an analyte is extracted from a matrix by a sorbent, selective removal of the impurities can frequently be accomplished by changing the polarity of solvent.

The analyte and interferents separation in the SPE technique could be realized by the three following ways [1]:

– selective extraction where on sorbent bed during enrichment process only selected components are retained while the remaining compound (impurities) aren't retained on the bed;
– selective washing where the compound of interest and the impurities are retained on the sorbent bed when the sample passes through. The impurities are rinsed through with wash solutions that are strong enough to remove them, but weak enough to leave the compounds of interest behind;
– selective elution where the adsorbed compounds of interest are eluted in a solvent that leaves the strongly retained impurities behind.

Application of Solid Phase Extraction

Solid phase extraction procedures are used not only to extract traces of organic compound from environmental samples but also to remove the interfering components of the complex matrices in order to obtain a cleaner extract containing the analytes of interest.

Table 2 shows the application of solid phase extraction technique in analysis of different compounds in various matrices. There are only a few examples of the application of this technique for extraction or purification.

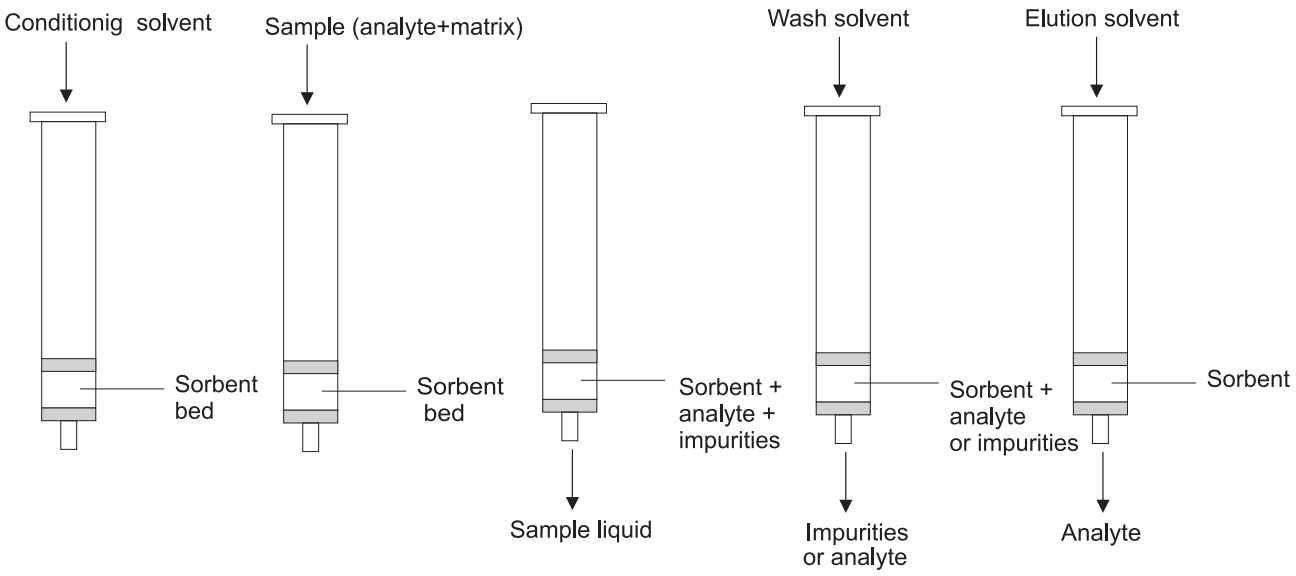

Fig. 2. Solid Phase Extraction steps

Table 2. Application of SPE as extraction and purification of extract technique.

Type of matrix	Type of analytes	Extraction Technique	Clean up technique	Final analysis	Literature
soil, sediments	PAHs, PCBs, pesticides	PLE	SPE (comparision different sorbent beds; C_{18}, Ph, NH_2, Diol, Al, EnviCarb+Al, CN+Al, C_{18}+Al, NH_2+Al Ph+Al, Diol+Al, Diol+C_{18}+Al, PH+C_{18} +Al.)	GC-MS	27
water	pesticides	C_{18}, ENV (styrene-divinylobenzene)	-	GC-MS	28
water	pesticides	SPE (GCB, XAD-4, Tenax GC)	-	GC-NPD, GC-ECD	29
river water	pesticides, triazines, organochlorine compounds	SPE (LiChrolut EN)	-	GC-ECD	30
drinking water	pesticides	SPE (C_{18})	-	HPLC-DAD	31
water	PCBs	SPE (47 mm disk C_{18}, n-pentane and n-heptane)	SPE (Florisil)	GC-ECD	32
water	herbicides, pesticides, PCBs	SPE (47 mm disk $C_{18,}$ ethyl acetate) dichloromethane) and (47 mm disk SDB, ethyl acetate) dichloromethane)	-	GC-NPD, GC-MS	33
soil	pesticides	sonication	SPE (C_{18}, C_8)	GC-ECD	34
soil	pesticides	sonication (acetone)	SPE (47-mm disk of C_8)	GC-ECD, GC-NPD	35
plant	triazine herbicides	LE (methanol)	SPE (SAX strong anion exchange)	LC-diode araary detector and UV detector	36
hops	fungicide	sonication	SPE (isolate aminopropyl)	GC-MSD	37
wheat, maize	pesticides organophosphorus	SFE, LE (acetone/methanol)	SPE (EnviCarb)	GC-FPD	38
fruits	pesticides	sonication	SPE: graphitized carbon (ENVI-Carb), polymer,	GC-MS	39
fruits, vegetables (apples, peas, green beans, oranges, raspberries, clementines, carrots, wheat)	pesticides	sonication (DCM/petroleum ether)	SPE on beds: GCB, PSA, GCB+NH_2, GCB+SAX, GCB+PSA, GCB+PSA+SAX	GC-FPD, GC-ITMS	40
fruit, vegetables	pesticides	MSPDE	SPE (GCB, C_{18})	GC-ECD	41
fruits, vegetables	pesticides	sonication (acetone)	SPE: cross-linked polystyrene divinylobenzene (LiChrolut EN)	GC-MS	42
vegetables	pesticides	LE (hexane)	SPE (silica gel)	GC-ECD	43
oranges, sweet potatoes, green beans	pesticides	SFE	SPE (octyldecylsilane, diol, Tenax, Porapak Q)	GC-ion trap-MS	44
apple, cabbage, grape, green pepper, plum and squash	pesticides	LE (methylene chloride/petroleum ether)	SPE (C_{18}, GCB, PSA, C_{18}- NH_2)	GC-ECD, GC-MS	45
tomatoes	pesticides organochlorine	sonication	SPE (Florisil)	GC-ECD	46

Table 2, continued

potatoes	pesticides	LE (light petroleum/dichloromethane)	SPE (C_8)	GC-FID, GC-NPD	47
apple	pesticides	LE (ethyl acetate)	SPE (polystyrene divinylobenzene, ENV+)	GC-ECD, GC-MS	48
spice powder	pesticides organochlorine	LE (n-hexan (dichloromethane)	SPE (silica, Florisil)	GC-ECD	49
butter fat, corn oil	pesticides	SFE	SPE (Florisil)	GC-ECD GC-ELCD	50
soya oil	pesticides, organochlorine compounds	Extrelut – 3, C_{18}, combination Extrelut – 3 and C_{18}	-	GC-ECD	51
fatty food (chickens, pork meats)	PCDD/Fs	Soxhlet extraction	SPE (C_{18}, Florisil)	GC-MS	52
fish meal, lard fat	PCBs	PLE (hexane)	SPE (silica gel impregnated with sulphuric acid (40% v/v))	GC-ECD	53
fish (plaice, flounder, sole, cod)	PCBs	Soxhlet extraction	SPE (Alumina)	GC-ECD	54
powdered milk	PCBs, PCDD/F	Soxhlet extraction	SPE: SiO_2-H_2SO_4, Florisil	HRGC-HRMS	55
human milk	DDT, DDE, hexachlorobenzen, hexachlorocyclohexane,pesticides	LE (hexane/acetone)	SPE (Florisil, Alumina, C_{18})	GC-ECD	56
cow milk, human milk	polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, PCBs	1. Soxhlet extraction (pentane/dichloromethane) 2. SPE (C_{18}) 3. Integrated extraction and clean up(C_{18}, HCDS and carbon column)	SPE (HCDS, carbon column)	GC-HRMS	57
human milk	pesticides	LE (hexane/acetone)	SPE (Alumina, Florisil, C_{18})	GC-ECD	58
honeybees	pesticides	SPE diatomaceous earth	GPC (BioBeads SX)	GC- NPD, GC-ECD	59
eggs	pesticides organochlorine	SFE, LE (acetonitrile)	SPE: C_{18}, Florisil	HRGC-ECD	60
eggs	pesticides	LLE (acetonitrile)	SPE (Florisil)	GC-ECD, GC-NPD	61
serum, human food	PCBs	Soxhlet extraction, LLE	SPE (Florisil, acid silica, BioBeads SX-3)	GC-MS	62
human blood	dioxins (PCDDs/Fs, PCBs)	LE (acetone/hexane, chloroform/methanol) and PLE (acetone/hexane, ethanol/hexane)	SPE (TS-ML-AC)	GC-MS	63
lanolin	pesticides	SPE (celite, hexane/dichloromethane)	SPE (silica gel)	GC-ECD	64

TS-ML-AC – Tandem simplified multilayer silica gel-activated carbon silica gel chromatography
PLE – Pressurized Liquid Extraction
LE – Liquid Extraction
SFE – Supercritical Fluid Extraction
MSPDE – Matrix Solid Phase Dispersion Extraction

Coupling Solid Phase Extraction with Various Techniques

SPE coupled to HPLC equipped with DAD, fluorescence, atomic emissions or mass spectrometric detection are the methods of choice for the trace level determination of pesticides. In general, the combination of SPE and LC is often used, because it is not necessary to remove all residual water from cartridges or disks, and elution solvents (e.g., methanol) are compatible with the final separation method [65]. On-line SPE-LC is based on the use of exchangeable precolumns with a length of 5-20 mm and a diameter of 2-4 mm, packed with a suitable sorbent and closed at both ends. When the sample is transferred from the precolumn to the separation column, by means of a suitable eluent, separation should start with a narrow peak profile in order to keep band broadening as small as possible. The most important operational parameters in an on-line column-swtching system are:

- the stationary phase, which provides additional selectivity by allowing two-dimensional separations,
- the eluent, also providing additional selectivity but causing compatibility problems in a number of cases,
- the flow direction, which is important for avoiding clogging of the capillaries and for increasing the robustness of the system and the time again providing additional selectivity by using a fixed time window. The advantages of on-line SPE are that the systems are fully automated, that the configuration is flexible and that the equipment can be exchanged [20].

An automated one-step supercritical solvent extraction and in-line clean up system was developed for organochlorine and organophosphorus pesticide residues contained in fats and fat samples. This procedure utilizes supercritical carbon dioxide modified with 3% acetonitryle at 27.58 MPa and 60°C to extract and separate the pesticide residues from the fat on a C_{18} bonded phase. The automated C_{18} system was based on using two C_{18} columns. One column is used in the extraction and in-line clean-up step while the other column is being flushed with solvent and nitrogen, thus preparing it for the next sample. This automated clean-up procedure should be considered as an alternative clean-up procedure for specific pesticide residues in fats and fatty samples because it simplifies the extraction and clean up step and reduces solvent consumption and hazardous waste [50].

On-line clean up and pressurized liquid extraction procedure for the determination of polychlorinated pesticides and PCB in food matrices was used. This method was based on a simultaneous extraction/clean up step requiring a minimum of sample handling. In this procedure acid silica gel was placed on the two bottom filter papers of the extraction cell. Next the matrix mixed with anhydrous sodium sulfate was placed on the bed' top in extraction cell. The extraction was performed on an ASE system in suitable conditions and hexane as solvent. This methodology gives increased possibilities of automation with no extra clean up step needed, leading to substantial time savings compared to classical methodologies [66].

Today combined extraction and clean up procedure utilizing selective PLE with on-line clean-up are very often applied. This combined extraction/clean up strategy has drastically decreased the time spent on sample handling. The first on-line clean up attempt ever in PLE was performed for the extraction of PCBs from fish, utilizing acidic Alumina in the extraction cell. In a later work several fat retainers (e.g., basic Alumina, Florisil) were tested on their fat retaining capability for the extraction PCBs [67, 68].

Conclusions

Solid phase extraction has appeared as alternative to liquid-liquid extraction owing to simplicity, low cost and easy automation. The SPE can be a powerful method for sample preparation and today a laboratory cannot do without it. Solid phase extraction is a widely used sample-preparation technique for isolation, concentration, clean-up and medium exchange. The development of more selective and more easy-to-use sorbents has resulted in simplified procedures and a diminution of the risk of errors.

SPE have many advantages in comparision with more traditional sample preparation techniques (e.g. liquid-liquid extraction), such as: high recoveries of the analyte, concentartion of analyte, highly purified extracts, ability to simultaneously extract analytes of wide polarity range, ease of automation, compability with instrumental analysis and reduction in organic solvent.

References

1. BIZIUK M., Pesticides, occurrence, determination, and disposal. WNT: Warsaw **2001** (In Polish).
2. RAWA-ADKONIS M., WOLSKA L., NAMIEŚNIK J. Modern techniques of extraction of organic from environmental matrices. Critic. Rev. Anal. Chem. **33**, 199, **2003.**
3. CAMEL V. Solid phase extraction of trace elements. Spectrochimica Acta B **58**, 1177, **2003.**
4. Supelco Bulletin, Sigma-Aldrich Co **910**, **1998**.
5. FONT G., MANES J., MOLTO J.C., PICO Y. Solid-phase extraction in multi-residue pesticide analysis of water. J. Chromatogr. A **642,** 135, **1993.**
6. SABIK H., JEANNOT R., RONDEAU B. Multiresidue methods using solid phase extraction techniques for monitoring priority pesticides, including triazines and degradation products, in ground water. J. Chromatogr. A **885**, 217, **2000.**
7. FRITZ J. S., Analytical Solid-Phase Extraction, Villey-VCH: New York **1999**
8. FARID A. E. Trends Anal. Chem. **20**, 649, **2001**
9. HENNION M.C., Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography. J Chromatogr. A **856**, 3, **1999.**
10. MASQUE N., MARCE R.M., BORRULL, F. New polymeric and other types of sorbents for solid-phase extraction of polar organic micropollutants from environmental water. Trends Anal. Chem. **6**, 384, **1998.**

11. TYSZKIEWICZ H., BIZIUK M., HUPKA J., ZALESKA A. Determination of organochlorine pesticides in water suspension by means of liquid-liquid extraction, solid phase extraction, solid phase microextraction and direct aqueous injection. Chem. Inż. Ekol. **8**, 935, **2001.**
12. FERRER I., BARCELO D. Validation of new solid-phase extraction materials for the selective enrichment of organic contaminants from environmental samples. Trend Anal. Chem. **3**, 180, **1999**.
13. MAJORS R.E. Trends in sample preparation. LC-GC Europe **19**, 2, **2001**.
14. WELLS D. A. Solid-phase extraction of acidic herbicides. LC-GC **17**, 600, **1999.**
15. YU J., WU C., XING J. Development of new solid-phase microextraction fibers by sol–gel technology for the determination of organophosphorus pesticide multiresidues in food. J. Chromatogr. A **1036**, 101, **2004.**
16. LINGEMAN H., HOEKSTRA-OUSSOREN S. J. F. Particle-loaded membranes for sample concentration and/or clean up in bioanalysis. J. Chromatogr. A **689**, 221, **1997**.
17. POOLE F.C. New trends in solid-phase extraction. Trends Anal. Chem. **22**, 362, **2003.**
18. FRANKIE J. P., ZEEUW R.A. Solid-phase extraction procedures in systematic toxicological analysis. J. Chromatogr. A **713**, 51, **1998.**
19. CHAI A., KUET L., SENG L. Solid phase extraction clean-up for the determination of organochlorine pesticides in vegetables. Malaysian J. Chem. **6**, 39, **2004**.
20. PICHON V. Solid-phase extraction for multiresidue analysis of organic contaminants in water. J. Chromatogr. A **885**, 195, **2000**.
21. HENNION M.C. Graphitized carbons for solid-phase extraction. J. Chromatogr. A **885**, 73, **2000.**
22. MATISOVA E., KAKALIKOVA L., LESKO J., DE ZEEUW J. Application of porous carbon for solid-phase extraction of dicarboxyimide fungicide residues from wines in combination with high-resolution capillary gas chromatography and gas chromatography-mass spectrometry. J. Chromatogr. A **754,** 445, **1996**.
23. PICHON V., CHEN L., HENNION M.C., DANIEL R., MARTEL A., LE GOFFIC F., ABIÁN J., BARCELÓ D. Preparation and evaluation of immunosorbents for selective trace enrichment of phenylurea and triazine herbicides in environmental waters. Anal. Chem. **67**, 2451, **1995.**
24. MARTIN-ESTEBAN A., FERNÁNDEZ P., STEVENSON D., CÁMARA C. Mixed immunosorbent for selective on-line trace enrichment and liquid chromatography of phenylurea herbicides in environmental waters. Analyst **122**, 1113, **1997.**
25. FERRER I., PICHON V., HENNION M.C., BARCELÓ D. New polymeric and other types of sorbents for solid – phase extraction of polar organics micropollutants from environmental water. J. Chromatogr A. **777**, 91. **1997.**
26. ENSING K., BERGGREN CH., MAJORS R.E. Selective sorbents for solid-phase extraction based on molecularly imprinted polymers. LC-GC North America **19**, 942, **2002.**
27. WELLS D. A. Accessory products for SPE using 96-well plates. LC-GC **17**, 808, **1999.**
28. MOTTALEB M.A., ABEDIN M.Z. Determination of chlorinated pesticides in soil by solid phase extraction-gas chromatography. Anal. Sci. **15**, 283, **1999**.
29. STAJNBAHER D., ZUPANCIC-KRALJ L. Multiresidue method for determination of 90 pesticides in fresh fruit and vegetables using solid-phase extraction and gas chromatography/mass spectrometry. J. Chromatogr. A **1015**, 185, **2003.**
30. BURKE E.R., HOLDEN A.J., SHAW I.C. A method to determine residue levels of persistent organochlorine pesticides in human milk from Indonesian women. Chemosphere **50**, 529, **2003**.
31. FOCANT J. F., PAUW E. D. Fast automated extraction and clean-up of biological fluids for polychlorinated dibenzo-*p*-dioxins, dibenzofurans and coplanar polychlorinated biphenyls analysis. J. Chromatogr. B **776**, 199, **2002**.
32. KITAMURA K., MOCHIZUKI A., CHOI J. W., TAKAZAWA Y., HASHIMOTO S., ITO Y., FUJIMINE Y., MORITA M. Optimization of a method for determining dioxin in whole blood samples based on solvent extraction and simplified cleanup. Analyst **129**, 315, **2004**.
33. WESTBOM R., THÖRNEBY L., ZORITA S., MATHIASSON L., BJÖRKLUND E. J. Development of a solid-phase extraction method for the determination of polychlorinated biphenyls in water. J. Chromatogr. A **1033**, 1, **2004**.
34. HOPPER M. Automated one-step supercritical fluid extraction and clean-up system for the analysis of pesticide residues in fatty matrices. J. Chromatogr. A **840,** 93, **1999.**
35. PIHLSTROM T., OSTERDAHL B. G. Analysis of pesticide residues in fruit and vegetables after cleanup with solid-phase extraction using ENV+ (Polystyrene-divinylbenzene) cartridges. J. Agric. Food Chem. **47**, 2549, **1999**.
36. COLUME A., CARDENAS S., GALLEGO M., VALCARCEL M. A solid phase extraction method for the screening and determination of pyrethroid metabolites and organochlorine pesticides in human urine. Rapid Communications In Mass Spectrometry **15,** 2007, **2001.**
37. HENGEL M.J., SHIBAMOTO T. Gas chromatographic – mass spectrometric method for the analysis of dimethomorph fungicide in dried hops. J Agric. Food Chem**. 48,** 5824, **2000.**
38. MANIRAKIZA P., COVACI A., SCHEPENS P. Single step clean up and GC-MS quantification of organochlorine pesticide residues in spice powder. Chromatographia **52**, 787, **2000.**
39. RAMOS L., ELJARRAT E., HERNANDEZ L. M., RIVERA J., GONZALEZ M. J. Comparative study of methodologies for the analysis of PCDDs and PDCFs in powdered full-fat milk, PCB, PCDD and PCDF levels in commercial samples from Spain. Chemosphere **38**, 2577, **1999**.
40. ESKILSSON S. C., BJÖRKLUND E. Analytical-scale microwave-assisted extraction. J. Chromatogr A. **902**, 227, **2000**.
41. DĄBROWSKA H., DĄBROWSKI Ł., BIZIUK M., GACA J., NAMIEŚNIK J. Solid-phase extraction clean-up of soil and sediment extracts for the determination of various types of pollutants in single run. J. Chromatogr. A **1003**, 29, **2003.**
42. WIGFIELD Y. Y., SELWYN J., KHAN S., MCDOWELL R. Comparison of supercritical fluid extraction and solvent extraction of twenty-two organochlorine pesticides from eggs. Chemosphere **32**, 841, **1996.**

43. JUNKER-BUCHHEIT A., WITZENBACHER M. Pesticide monitoring of drinking water with the help of solid-phase extraction and high-performance liquid chromatography. J. Chromatogr. A **737**, 67, **1996**.
44. LAWRENCE J. F., MENARD C., HENNION C., PICHON V., LEGOFFIC F., DURAND N. Evaluation of immunoaffinity chromatography as a replacement for organic solvent clean-up of plant extracts for the determination of triazine herbicides by liquid chromatography. J. Chromatogr. A **752**, 147, **1996.**
45. POLESE L., SANNOMIYA M., SANDER A. P. Extraction and clean-up procedure for analysis of organochlorine pesticide residues in ethoxylated lanolin. Il Farmaco **9-10**, 637, **2000**.
46. KIM Y., YANG, H., YOUNG L. S., KIM M. Levels of PCDDs and PCDFs in two kinds of fast foods in Korea. Chemosphere **43**, 851, **2001.**
47. ROSSI S., DALPERO A. P., GHINI S., COLOMBO R., SABATINI A. G., GIROTTI S. Multiresidual method for the gas chromatographic analysis of pesticides in honeybees cleaned by gel permeation chromatography. J. Chromatogr. A **905**, 223, **2001**.
48. SCHENCK F. J. Determination of organochlorine and organophosphorus pesticide residues in eggs using a solid phase extraction clean up. J. Agric. Food Chem. **12**, 6412, **2000**.
49. MORELLI-CARDOSO W. M. H., CARDOSO R. T. M., MELLO J. L., ABRANTES S., MENEZES K. M. P. Extraction and clean up method for the determination of twenty organochlorine pesticides residues in tomatoes by GLC-ECD. J. High Resol. Chromatogr. **22**, 619**, 1999**.
50. TORRES C.M., PICO Y., MANES J. Comparision of octadecylsilica and graphitized carbon black as material for solid-phase extraction of fungicide and insecticide residues from fruit and vegetables. J. Chromatogr. A **778**, 127, **1997**.
51. PLANAS C., SAULO J., CAIXACH J. Automated solid-phase extraction of priority and suspected endocrine disrupting pesticides and metabolites. Organohalogen compounds, sampling, clean up and separation **66**, 126, **2004.**
52. DOONG R.A., LEE C.Y. Determination of organochlorine pesticide residues in foods using solid-phase extraction clean-up cartridges. Analyst **124**, 1287, **1999.**
53. BENEY P. J., BREUER G. M., JACOBS G. H, LARABEE – ZIERATH D. Review, evaluation, and application of solid phase extraction methods. Hotline Solid Phase Extraction **6**, 1, **1996.**
54. HIROTAKA O., KAZUHIKO A., MASAHIRO O., SHIHJIRO H. Multiresidue analysis of pesticides in vegetables and fruits using two-layered column with graphitized carbon and water absorbent polymer. Analyst **126**, 1529, **2001.**
55. MUCCIO A., GENERALI T., BARBINI D.A., PELOSI P., AUSILI A., VERGORI F., GIROLIMETTI S. A new sample clean-up procedure based on ion-pairing on RP-SPE cartridges, for the determination of ionizable pesticides. J. Chromatogr. A **765**, 61, **1997**.
56. FONT G., MANES J., MOLTO J.C., PICO Y. Solid phase extraction in multi-residue pesticide analysis of water. J. Chromatogr A **642**, 135, **1993.**
57. AQUILAR C., BORRULL F., MARCE R. M. Determination of pesticides in environmental waters by solid-phase extraction and gas chromatography with electron-capture and mass spectrometry detection. J. Chromatogr. A **771**, 221, **1997**.
58. LECHOTAY S. J., VALVERDE-GARCIA A. Evaluation of different solid-phase traps for automated collection and clean-up in the analysis of multiple pesticides in fruits and vegetables after supercritical fluid extraction. J. Chromatogr. A **765,** 69, **1997**.
59. KEVIN N. T. N., SEAN H. W. P. Supercritical fluid extraction and quantitative determination of organophosphorus pesticide residues in wheat and maize using gas chromatography with flame photometric and mass spectrometric detection. J Chromatogr. A **907,** 247, **2001**.
60. SCHACHTERLE S., FEIGEL C. Pesticide residue analysis in fresh produce by gas chromatography-tandem mass spectrometry. J. Chromatogr. A **754**, 411, **1996**.
61. MARTÍNEZ E., PAZOS M., CARRO A., LLOMPART M., CELA R. Residues and trace elements – application of strategically designed sample composition to the rapid analytical screening of milk samples for polychlor. J. AOAC Intern. **86**, 846, **2003.**
62. REDONDO M. J., RUIZ M. J., BOLUDA R., FONT G. Optimization of a solid-phase extraction technique for the extraction of pesticides from soil samples. J. Chromatogr. A **719**, 69, **1996**.
63. SCHENK F. J., LECHOTAY S. J., VEGA V. Comparison of solid-phase extraction sorbents for cleanup in pesticide residue analysis of fresh fruits and vegetables. J. Sep. Sci. **25**, 883, **2002**.
64. JUAN C. Y., THOMAS G. O., SEMPLE K. T., JONES K.C. Methods for the analysis of PCBs in human food, faeces and serum. Chemosphere **9**, 1467, **1999**.
65. SPORRING S., BJŐRKLUND E. Selective pressurized liquid extraction of polychlorinated biphenyls from fat-containing food and feed samples Influence of cell dimensions, solvent type, temperature and flush volume. J. Chromatogr. A **1040**, 155, **2004.**
66. MULLER A., BJŐRKLUND E, VON HOLST CH. On-line of pressurized liquid extracts for the determination of polychlorinated biphenyls in feedingstuffs and food matrices using gas chromatography-mass spectrometry. J. Chromatogr. A **925**, 197, **2001**.
67. Application Note ASE 322. Dionex: Sunnyvale CA **1996**.
68. GÓMEZ-ARIZA J. L., BUJALANCE M., GIRÁLDEZ I., VELASCO A., MORALES E. Determination of polychlorinated biphenyls in biota samples using simultaneous pressurized liquid extraction and purification. J. Chromatogr. A **946**, 209, **2002.**