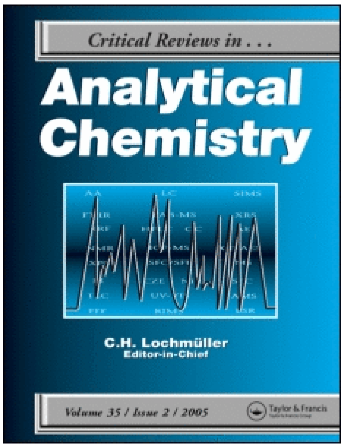

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On-Line Coupling of Solid Phase Extraction Sample Processing with High-Performance Liquid Chromatography

*Krystyna Pyrzynska and Ewa Pobozy**

Warsaw University, Chemistry Dept., Laboratory of FIA and Chromatography 1 Pasteur Str., PL-02-093 Warsaw, Poland

* tel. +48 22 822 02 11 ext. 398. fax. +48 22 822 35 32. www.chem.uw.edu.pl/people/KPyrzynska/

I. INTRODUCTION

The development of hyphenated measuring systems is one of the predominant trends in modern analytical chemistry. Special coupling of a flow sample pretreatment methods to other instrumentation may lead to a substational progress in many types of analysis. High-performance liquid chromatography is a mature technique commonly used in routine analysis. However, despite the performance of modern chromatographic systems, sample preparation is still the most tedious and time-consuming step. In the determination of traces of the analytes, especially in biological fluids, macromolecular compounds (e.g., proteins) have to be removed from a sample prior to HPLC analysis, because they could precipitate by the large amounts of organic solvents. Nonspecific or irreversible bounding on the surface of the chromatographic support could also occur, resulting in a limited lifetime of the column. Moreover, because analyte concentration is usually low, some kind of preconcentration and/or derivatization is often required to improve analyte detectability.

Solid-phase extraction (SPE) is an attractive approach to the preparation of environmental, food, and biological samples because it involves high enrichment factor, lower consumption of organic solvents, and better reproducibility than liquid-liquid extraction techniques. In addition, SPE could be performed on-line by direct connection to the chromatographic system; therefore, a fully automated technique could be utilized. Hyphenated on-line SPE-HPLC systems are designed to improve not only sensitivity and selectivity of determination but also the precision due to lower human participation. Thus, they represents a fast, modern, and reliable approach to trace analysis.

This article reports the application of several SPE systems for automated preparation of environmental, food, and biological samples prior to chromatographic analysis. New selective phases such as restricted access matrix, molecularly imprinted polymers, and immunosorbents are described. The review concludes with a selected list of recent applications.

II. ON-LINE COUPLING OF SPE WITH HPLC

The most commonly method used for on-line coupling of SPE unit with HPLC involves the application of small precolumn located in six-port high-pressure switching valve (Figure 1). An aliquot of sample is injected or aspirated from a relatively large volume. Then potentially interfering sample constituents are flushed into the waste. After preconcentration/separation step the retained analytes are eluted on-line via the switching valve onto the HPLC analytical column. This system is called the“column-switching method”. The solid sorbent packed in a precolumn must be pressure resistant, and the dimensions of a precolumn have to be as small as possible to avoid band broadening and a decrease of the efficiency of the analytical column. For the same reason, the backflush desorption mode has been used most often. The

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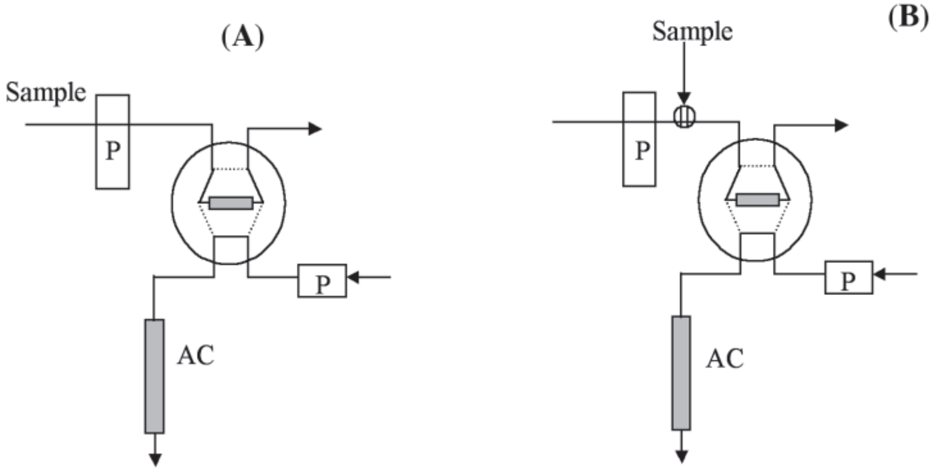

FIGURE 1. Typical configuration for on-line column-switching SPE sample processing with HPLC. (A) large volume of sample, (B) small sample volume.

most efficient system is obtained when both a precolumn and a analytical column are of the same nature. The systems with more than one switching valve as the interface were used for cleaning up and/or precolumn activation.[1]

To avoid the damage of precolumn, like in the case of immunoassay sorbents, by direct connection to the analytical column, it is necessary to use the additional trapping precolumn, most often C_{18} or polymers.[3-5] Such on-line system is also used when desorption from the first precolumn is achieved with an aqueous solution of low pH that requires a large volume of eluent.[2,4] The application of the same SPE columns connected parallel in the 10-port valve allows to obtain higher number of analysis cycle.[6] Timm et al.[7] used six identical precolumns filled with RP-Amide C_{16} and a special precolumn selector for determination of the active metabolites in plasma and urine samples. When a certain pressure limit was reached, the selector switched automatically to a fresh precolumn enabling routine overnight analysis without possible interruption of precolumn clogging.

For samples with very complex matrix it is sometimes necessary to use several different precolumns connected in series. An internal-surface reversed-phase precolumn was applied for plasma assay, while two precolumn packed with cyano and phenyl bonded silica were effective for urine samples.[8] The analyte fraction transferred from the precolumn was finally separated on an octadecyl analytical column. Three precolumns with different bonded silica, connected in the order C_8, silica-CN and silica-phenyl were included in a fully automated HPLC system for hydroxybenzeno[*a*]pyrene determination.[9,10] The direct transfer consisted in discarding the uninteresting parts of a sample initially eluted on a primarily column and in transferring the fraction of interest onto a next column with a minimal of overlapping interferences (Figure 2). A column-switching HPLC method applying several different precolumns for the simultaneously determination of uric acid and creatinine in human serum and urine was developed by Selhi et al.[11]

The fully automated systems where the analytes are extracted on-line with SPE precolumn and separated by HPLC are commercially available from Spark (PROSPECT),[12-14] Merck (OSP-2),[15,16] Gilson (ASPEC XL),[17,18] and the precolumns from Waters (Oasis).[19,20] The exchangeable cartridges packed with different type of sorbents could be applied for sample preparation. Those systems allow an increase in the amount of analyzed samples and are especially useful in clinical and pharmaceutical analysis as well as environmental monitoring. The new generation of Oasispoly(divinylbenzene-co-*N*-vinylpyrrolidone] material could be used to extract analytes with a broad range of polarities due to the adsorption capacity for both lipophilic and hydrophylic compounds.[21] Successfully combining of an on-line SPE-HPLC fractionation with a sensitive mutagenicity

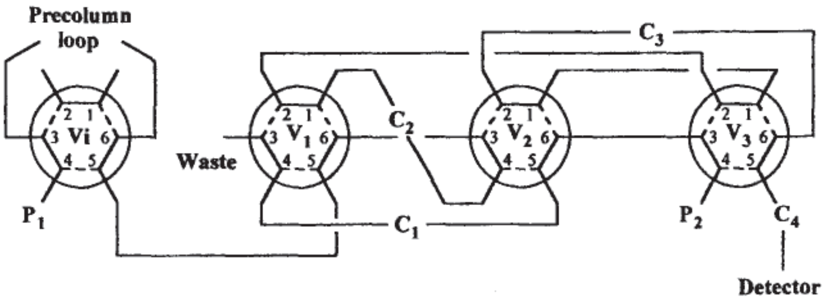

FIGURE 2. Column–switching HPLC system with several different precolumns. (From Ref. 10.)

test allowed assesing both genotoxic and toxic properties of (pro)mutagenic compounds.

The other way of on-line connection of SPE sample processing with HPLC analysis is the application of six-port valve as only an interface for switching arrangements. In such a system cleaning up/preconcentration of the analytes utilizing SPE can be realized in low- or high-pressure mode and precolumn is not placed in the valve. As a precolumn and analytical column are connected only for short time after valve switching, the portion of the eluate from the first one is transferred onto the analytical column where separation proceeds. The elution conditions and switching time are optimized to transfer a heart-cut fraction containing all analytes.[22-25] In this system it is possible to use a bigger size of precolumn. When the dimensions of both columns are comparable the method is called "column-coupling"[26] or "two-dimensional chromatography".[27,28] The precolumn eluate could be also trapped in sample loop of the interface valve.[29,30]

A coupled-column system, consisting of a combination of molecularly imprinted polymer (MIP) and a C_{18}-silica precolumn, was chosen to separate the enrichment from the purification procedure.[31] This system allowed getting very high extraction efficiency and selectivity for determination of triazine in the HPLC mode (Figure 3). Precolumn with a polyethylene powder was very useful for the deproteinization of human serum samples.[32] No further treatment, such as the addition of another agent and centrifugation, was necessary. A protein-free sample was then directly introduced into the chromatographic column. The proposed method was applied to the automated routine determination of serum cations.

Pasternyk et al.[26] proposed two precolumn for the determination of β-glucoronides in urine. They were connected with three six-port switching valves. Internal-surface reversed phase precolumn containing a hydrophobic inner phase, and a hydrophilic outer phase was used for the separation from biological matrix, while deconjugation was performed on the enzymatic precolumn.

The low-pressure on-line sample pretreatment unit utilizing alumina microcolumn was applied for determination of levodopa and carbidopa, derivatives of dopamine.[33] Despite the wide and substantially different plasma concentration ranges of those two components, the analysis could be carried out simultaneously. Similar continuous cleanup/preconcentration system coupled on-line with HPLC was applied for separation and determination of main liposoluble vitamins and several hydroxy metabolites of vitamin D_3 in clinical samples.[34-36]

III. DERIVATIZATION

Derivatization procedures are often used in combination with the HPLC method to improve the sensitivity for the analyte and/or to achieve a chiral separation. In this respect, different possibilities have been reported. For example, in some procedures the derivatization step is processed off-line and after some preparation steps, for example, solvent extraction, evaporation, dissolution in buffer, an aliquot of derivatizing mixture

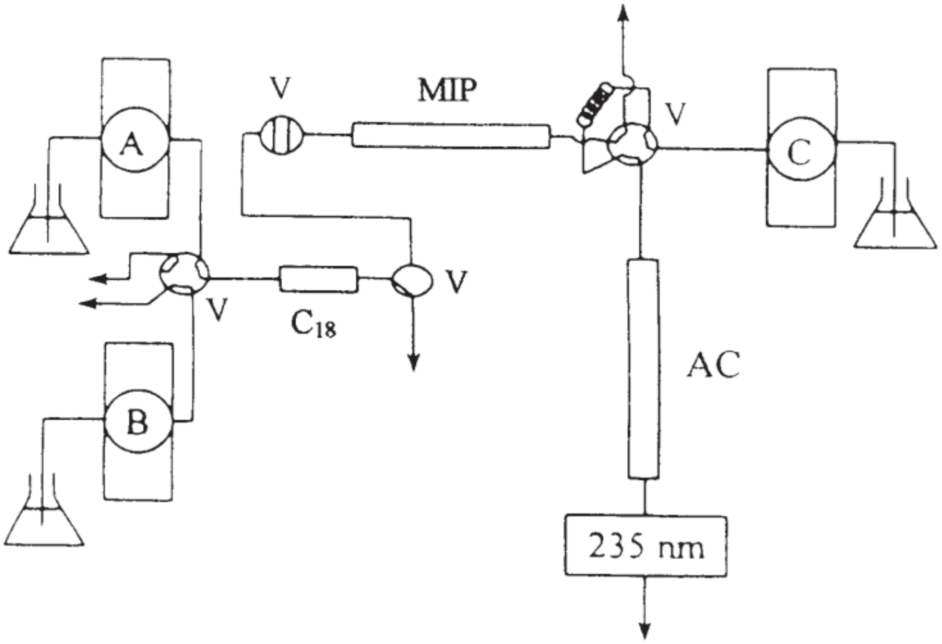

FIGURE 3. System configuration for column-coupling HPLC. A,B,C — pumps, V — switching valves, AC – analytical column. (From Ref. 31.)

is injected into a column-switching HPLC system. The sample is then purified on a SPE precolumn and finally transferred by the mobile phase to the analytical column.[37-41]

Other assays are based on the use of reagents attached to solid supports.[42,43] These materials effect sample clean-up and at the same time, or somewhat later, the reagent part of material is made to react with the analyte. However, the synthesis of the solid-phase reagent is often difficult, and its periodic regeneration is required to obtain a high yield, fewer side production, and reproducible results. Ideally, a sample clean-up and derivatization should be integrated in the same process.

Herráez-Hernández et al.[44,45] proposed a very simple procedure for on-line derivatization of drugs based on the employment of precolumns packed with conventional C_{18} materials. The precolumn was used to purify the sample and concentrated the analytes and, next, to retain the derivatives (the reagent mixture was injected into the precolumn immediately after sample cleaning). This method offers minimum sample handling and better control of parameters affecting the reaction rates, especially the reaction times. A fully automated system for the direct determination of organic as well as inorganic mercury species utilized preconcentration of mercury complexes formed on-line with ammonium pyrrolidine dithiocarbamate on a precolumn of bonded silica with octadecyl functional groups.[46,47] Retained mercury species were then eluted with a methanol-acetonitrile-water mixture and subjected to separation on the analytical column before determination by CVAAS.

IV. PACKING OF SPE MICROCOLUMN

A. Bonded-Phase Silica Sorbents

The most commonly used material for SPE precolumn is based on silica chemically modified with C_{18}, C_8 and to a lesser extent C_2 groups. In particular, bonded silica with octadecyl functional groups are the most widely used due to their great capacity.[19,41,46-51] The mechanism of retention on C_{18} silica is based on hydrophobic interaction, thus this SPE packing provides high recoveries for nonpolar analytes. The residual silanols, however, could play a significant role in the extraction scheme. The modified silica with a monofunctional silane or with a lower amount of alkyl chains bonded at the surface have been designed under the name C_{18}/OH or polar C_{18}. In addition to hydrophobic interactions, hydrogen bonding or ionic interactions can occur with polar analytes.[52] New types of alkyl silicas have been also prepared by bonding with an alkylchain reagent containing an embedded polar carbamate or amide functionality.[53-55] In this case, residual silanol groups are shielded from interaction with polar analytes.

Column-switching HPLC method with C_{18} precolumn was applied for the determination of organomercury species[46,47] as well as Ni(II), Cu(II), and Hg(II).[49] These metals were preconcentrated as complexes formed on-line with dithiocarbamates. An enrichment system employing C_{18} precolumn was proposed for the determination of Nb(V) and Ta(V) (as PAR-citrate ternary complexes) in geological samples.[41] Among different sorbents, C_{18} material gave the best performance for cleaning berry wine samples in the HPLC determination of flavonoides,[50] for preconcentration of polycyclic aromatic hydrocarbons, for continuous cleanup/preconcentration of hydroxyvitamin D_3 metabolites from human plasma,[34] determination of drug levels in pharmacokinetic studies,[14,56] retinoids in plasma,[57] and herbicidies in human serum.[58]

A fully automated procedure for the determination of pesticides in surface water has been proposed by Lacorte et al.[48] This modified system for the automated monitoring of organic pollutants (SAMOS) containing on-line coupling of sampling, filtration, preconcentration on C_{18} precolumn, and chromatographic separation could be operated unattended for at least 5 days.[59]

Cyanopropyl- and aminopropylsilica exhibit both polar and nonpolar interactions, and they have been used in continuous solid-phase cleanup/preconcentration of the analytes.[59,60] The advantage in using polar aminopropyl silica as an alternative to the nonpolar C_{18} precolumn for determination of several fat-soluble vitamins in clinical samples includes better detection and quantification limits.[35,36] An HPLC system with three SPE precolumn packed with C_8, cyano silica, and phenyl silica sorbents was applied to purification and concentration of 3-hydroxybenzeno[*a*]pyrene from hydrolyzed urine samples.[10] A combination of these sorbents guaranteed minimum interferences from the sample matrix.

B. Polymeric Sorbents

The most common polymers applied for solid-phase extraction are based on strryene-divinylbezene (like PRP-1 and PLRP-S). They exhibit higher capacity and better chemical stability (over the pH range 1 to 14) than bonded silicas. Due to specific Π-Π interactions, they are relatively selective for analytes with aromatic rings such as phenols.[13,19] Recent examples include the extraction of aromatic sulfonates,[12] pesticides,[61] heparin-binding proteins,[62] and nitro munitions from aquifer water samples.[63]

Most polymeric sorbents require pretreatment with methanol to make the hydrophobic surface more compatible with aqueous samples. The polystyrene resins with hydroxymethyl or acetyl substituents on the benzene rings give a more polar surface that functions well without a methanol pretreatment.[64] These substituents also increase the recoveries of polar analytes in SPE.

Another group of polystyrene supports very useful in enrichment of polar compounds are polymeric sorbents with a high specific area in the range 700 to 1200 m^2/g (e.g., LichrolutEN, Bond-Elut PPL, Hysphere-GP).[52] Their adsorption capacity and high breakthrough volumes are better then polymeric sorbents which usually have a specific surface area of 500 m^2/g. Due to stronger interactions, stronger eluent is necessary to transfer the preconcentrated analytes onto the analytical column.[65]

The introduction of sulfonic acid groups or amino groups into polystyrene resins provides a hydrophilic surface and ionic or ionizable analytes could be extracted by ion-exchange mechanism.[16,18,66,67] However, environmental samples often contain high amounts of inorganic ions that could overload the capacity of these sorbents. Thus, the selection of two-step extraction (combination of polymeric sorbent and ion-exchange resin) was recommended.[18]

The immobilization of β-cyclodextrin sulfate onto the hydrophilic vinyl-polymer gel allowed obtaining very selective solid support for extraction of heparin-binding proteins.[62] These modified polymers were applied in a column-switching HPLC system in analysis of biological fluids. The β-cyclodextrin sulfate-immobilized precolumn showed no loss of analytical performance over 2 years, during which about 400 samples were analyzed.

C. Restricted Access Matrix Sorbents

In recent years, special sorbents possessing restricted access properties have been developed to allow the direct injection of biological matrices into on-line SPE-HPLC systems. These sorbents, so-called restricted access materials (RAM), combine size exclusion of macromolecules (proteins) with the simultaneous enrichment of low-molecular-mass analytes by hydrophobic, ionic, or affinity interactions.[68] The topographical restriction is obtained either by a physical diffusion barrier (appropriate pore diameter < 8 nm) or by a chemical diffusion barrier (polymer network at the outer surface of the particulate).

The most popular RAM belongs to the group of internal surface reversed phase (ISRP) supports (Figure 4). The bonded reversed phase exclusively coverts the internal pore surface of a glyceryl-modified silica and the reversed-phase ligand is either C_4, C_8, or C_{18}. These sorbents are known as alkyl-diol silica (ADS), and they allow the extraction of a wide variety of components. The ADS sorbents packed in a precolumn have been applied mostly for drug analysis in biological fluids such as serum,[69] plasma,[69-74] and urine.[72] On-line column extraction utilizing these kinds of sorbents gave better results than the liquid extraction method and SPE with reversed-phase sorbents.[73] It was possible to inject 50 ml of plasma without changing precolumn parameters.

Ömnerfjord et al.[75] optimized alkyl-diol-silica RAM as a packing material in precolumn for the determination of triazines in environmental samples. This sorbent showed superior stability — more than 100 injections of highly complex samples containing humic substances could be done. Efficient sorption/desorption kinetics were found with medium-polar and nonpolar compounds; however, the retention properties were not sufficient for the most polar metabolites. RAM sorbent was also used in analysis of cow's milk to evaluate the residues of antibiotics.[76]

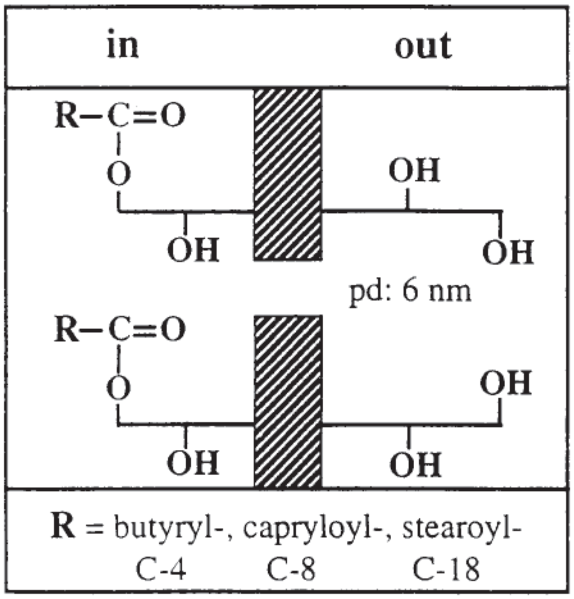

FIGURE 4. Topochemistry of an internal surface reversed phase precolumn packing (LiChrospher® ADS). (From Ref. 68.)

D. Immunosorbents

The main problem — how to developed of highly selective SPE sorbent allowing extraction, concentration, and clean-up in a single step — was resolved by using materials based on antigen-antibody interactions. Antibodies could be linked to an appropriate solid supports by covalent bonding, adsorption, or encapsulation to form an immunosorbent. It is important to choose a sorbent that is chemically and biologically inert, easily activated, and hydrophilic in order to avoid any nonspecific interactions. The most common approach involves the application of activated silica[77,78] or Sepharose, an agarose gel.[79] As antibodies are very selective toward the analyte to initiate the immune response, the obtained immunosorbent may extract and isolate this analyte from complex matrices in a single step. An antibody can also bind one or more analytes with the structure similar to the compound that has induced the immune response. This phenomena is so-called cross-reactivity of antibodies. It is usually negative feature for immunosorbents; however, they could be applied for single analyte and its metabolites or for a class of structurally related compounds.

The automated on-line immunosorbent phase extraction method was developed and validated for the analysis of triazine and phenylurea herbicides in environmental matrices such as sediments and natural waters.[80,81] Using 20 ml of sample volume very low detection limit (in the range of 0.001 to 0.005 μg/L) was obtained due to the enhanced selectivity. Other applications of immunosorbents deal with the determination of polycyclic aromatic hydrocarbons,[82] benzidine and related azo dyes in industrial effluents,[83] and pesticides degradation.[2-4] Figure 5 shows the performance of immunoaffinity extraction for the clean-up and isolation of okadaic acid in shellfish and algae compared with the results obtained using conventional twice-repeated liquid-liquid extraction.[77]

Martin-Esteban et al.[84] demonstrated that the use of a precolumn packed with yeast cells immobilized on silica gel allows for the simultaneous preconcentration of several pesticides with different chemical functionality from natural water samples. Humic and fulvic acid were not coextracted with the target compounds, which allowed the quantification by percolating only 25 ml of water sample.

E. Molecularly Imprinted Polymers

Molecular imprinting involves the formation of complex between a functional monomer and the template (target) molecule. Subsequent polymerization using a cross-linker, thus providing a rigid material. Then the template molecules are removed, and the resulting polymers have specific recognition sites allowing the binding of the template molecule. In molecularly imprinted polymer (MIP), the recognition is due to shape, size, and interactions (hydrogen, hydrophobic, and ionic) in likeness to immunosorbents. However, MIP could be prepared more easily and exhibit very good stability at high temperature, in a wide pH range, and in organic solvents.

Some problems occur with the application of molecularly imprinted polymers in SPE procedures.[52,85,86] One of them is the difficulty in removing all the template analyte, even after extensive washing. This can have serious implications when the polymer is to be used in the trace analysis. A possible method of circumventing the leakage problem is to apply a template analogue during the imprinting step rather than the template itself. Another problem is the difficulty in establishing quantitative and rapid desorption due to the high affinity of MIP for the analyte. Recent developments in the design of these sorbents have been reviewed.[86]

Molecularly imprinted polymers are an alternative to immunosorbents in SPE procedures for the clean-up and preconcentration of samples prior to analysis. However, their potential as materials for SPE is not yet fully explored, and there are only a few examples dealing with their application for on-line coupling to HPLC. Bjarnason et al.[29] performed the first on-line SPE of triazines from complex samples using two precolumns: C_{18}-silica and MIP. The triazines were selectively retained on the MIP precolumn, resulting in the relief of the matrix and improvement of the subsequent chromatogram. Recently, MIP precolumns

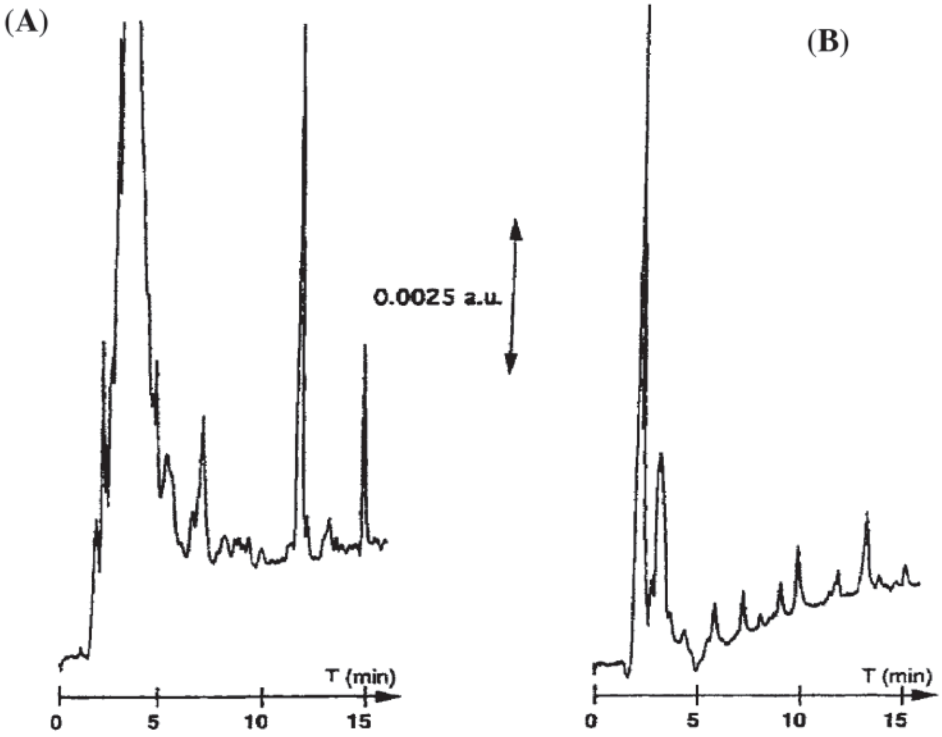

FIGURE 5. A comparison of analysis of mussel tissue reference material (MUS-2) cleaned by (A) liquid-liquid extraction and (B) immunosorbent. (From Ref. 77.)

have been used for extraction of 4-nitrophenol from environmental waters[87] and for the selective retention of stilbene-type estrogenic compounds.[88]

V. OPTIMIZATION OF SPE PROCEDURE

The main goal for application of SPE is to achieve extraction and clean-up of the sample in the same single step. This can be realized by a good selection of the type of sorbent or their combination. For this reason the properties of the analytes, nature of matrix, the required trace-level concentration, and the type of chromatography involved in the separation step should be taken into consideration.

Among the criteria used for the selection of SPE precolumn are the recovery of the compounds of interest, breakthrough volume, and the chromatographic performance (peak shape and width or asymmetry factor). The breakthrough volume represents the maximum sample volume that can be passed through the sorbent with a theoretical recovery of 100%. The measurement of this parameter has been usually performed by monitoring continuously or discretely UV signal at the outlet of a precolumn or can also be estimated using several models.[89,90] The value of breakthrough volume gives some information concerning the sorbent capacity for a given analyte. The shape of a curve after breakthrough corresponds to elution efficiency; the sharper the increase the smaller volume of eluent that is needed, and its means better sensitivity. A comparison of the breakthrough curves for chlorophenols (PCP) is shown in Figure 6. Using water-acetonitrile (70:30) at pH 2 much better elution efficiency was obtained for Hysphere Resin GP and Oasis than for Hysphere Resin SH, although the last sorbent exhibits the biggest breakthrough volume for PCP.[91]

The size of precolumn is another important parameter. The profile of the concentrated species transferred to the analytical column should be as narrow as possible to avoid band-broadening. Thus, the dimensions of the precolumn should

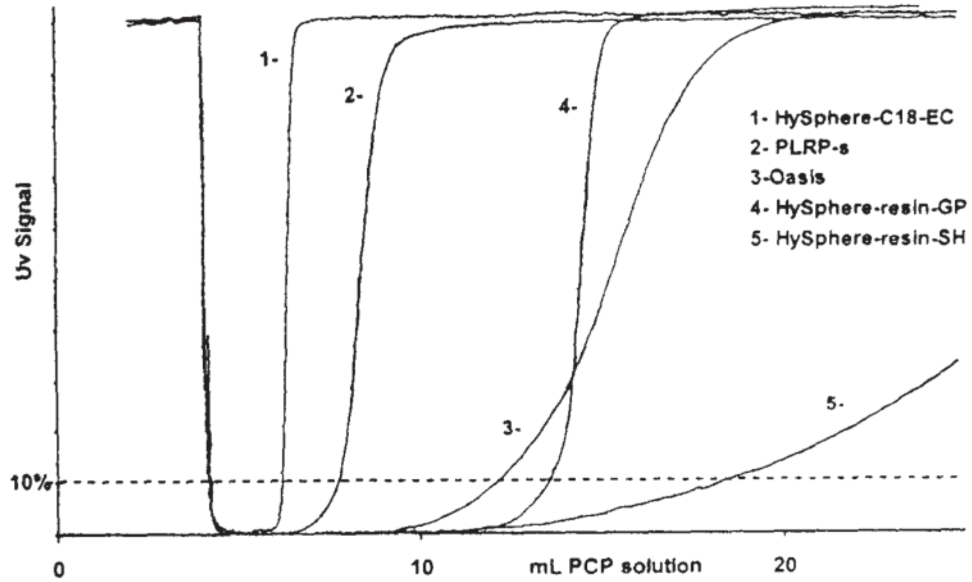

FIGURE 6. Breakthrugh curves for pentachlorophenol (20 mg/l, in 30% acetonitrile, pH 2) using different precolumns (10 × 2 mm); UV detection at 273 nm. (From Ref. [89].)

be and are typically 2 to 15 mm long and 1 to 4.6 mm i.d. for the classic 15- to 25-cm-long analytical columns.[89] The dimension of precolumn can be increasing without band-broadening when an appropriate methanol or acetonitrile gradient is applied.[90,91] The compatibility between the sorbents in precolumn and in the analytical column of HPLC system should also occur. The most effective system is ideally obtained when both are of the same nature. However, in practice the selection of sorbents in an analytical column is more limited. For aqueous samples a mobile phase in HPLC should be compatible with residual water, which will obligatorily transfer from a precolumn. Analytical columns packed with polymeric styrene-divinylbenzene or porous graphite carbon are less efficient in water-rich mobile phase but much more helpful in organic-rich phase.

A very important aspect of the performance of Hypsphere-GP and Hypshere-SH polymeric sorbents is the influence of sample volume on the analyte shape peak, especially for polar and moderately polar compounds.[13] For sample volumes up to 10 ml, the peak shape was very good for all tested substituted anilines and phenols. As the sample was increased up to 150 ml, the analyte peak shapes were gradually distorted.

The strategy of sample pretreatment using SPE is also guided by the method of detection and depends on the level of concentration required. Application of a simple detection mode, for example, diode array UV, requires more selective enrichment, while the use of specific quantification, such as fluorescence or electrochemical detection, can improve the limit of detection. The detection limit depends primarily on the detection mode and properties (i.e., spectral properties) of the analytes, but also on the type of sample matrix. A great difference (up to a factor of 10) was observed in the analysis of pesticides between the detection limits obtained in deionized water and those reached in ground or drinking water.

The bounded-phase silica generally are considered to be satisfactory for use in solid-phase extraction methods. However, the sorption properties of silicas vary with the percentage of carbon in the bonded phase and whether the sorbents are endcapped.[52] The application of a C_{18} silica with a high surface coverage of alkyl chain and residual silanols has been shown to increase the retention of some polar analytes by the addition of secondary interactions, mainly hydrogen bonding type. A significant improvement in recovery of chlorotriazine herbicide metabolites was obtained by McLaughlin and Johnson[92] applying partially nonendcapped C_{18} phase compared with the normal C_{18} phase apparently due to polar interactions. For the extraction of anilines and phenols from environmental aqueous samples, the silica-based and styrene-divinylbenzene polymeric sorbents, such as PRP-1 and PLRP-S, suffer from early breakthrough of the more polar compounds. To overcome this problem, new polymeric sorbents with high specific surface are (such as LiChrolut, BondElut, or Hypshere) have been introduced. These sorbents provide high recoveries, even for the most polar compounds. The observed analyte band-broadening can be minimized by using a suitable gradient that causes peak compression on the top of the analytical column and a good compromise between breakthrough volume and peak efficiency.[13,91] Moreover, for the enrichment of polar compounds, where the breakthrough volume is a critical parameter, some loss of efficiency should be accepted in order to achieve the desired detection limits. Renner et al.[51] proposed thermally assisted desorption after enrichment step to increase the efficiency of the subsequent on-line separation. This effect was particularly noticeable for compounds with values of retention factor above 5. Puig and Barceló[89] found that the differences in polymeric sorbents behavior should be related to the different physical-chemical characteristics of these materials such ad particle size, surface area, pore diameter, and volume as well as degree of cross-linking.

There is a great interest in providing selective extraction procedures that eliminate the co-extraction of matrix compounds. High selectivity has been obtained recently using new types of sorbents, such as molecularly imprinted polymers, immunosorbents, or restricted access materials. However, the preparation of antibody for each analyte is time consuming. The comparison of the results of an industrial textile effluent analysis obtained using precolumns packed with a nonselective polymeric sorbent and the antibenzidine immunosorbent is presented in Figure 7.[83]

VI. APPLICATION TO REAL SAMPLES

Among different extraction/preconcentration/clean-up procedures, solid-phase extraction is the preferred choice for the pretreatment of various kind of samples. This mainly stems from the availability of disposable extraction precolumns in a broad range of sorbents. The advantages of SPE include highly purified extracts, high analyte recoveries, effective concentration, the ability to extract analytes of varying polarities, easy automation, and reduced organic solvent consumption. Recent developments in the SPE area are in format, phases, high throughput purpose, and set up a new types of procedures.

In the environmental field, trends are to perform multiresidue extraction of many contaminants with a wide range of polarities at levels established by international organisations, whereas for biological samples trends are to performed analysis with high throughput. The 96-well plates, which appeared in 1997, are used for rapid sample clean-up in the pharmaceutical and biotechnological industries. The procedures are different depending on the field of application. Typical volumes for biological matrices are in the range of 1 to 5 ml for blood and plasma samples and up to a maximum of 50 ml for urine samples. Therefore, the breakthrough volume is not a basic parameter. In the case of environmental samples with the same on-line extraction, chromatographic separation and detection procedure the sample volume required to reach appropriate levels is typical 100 to 200 ml.

For analysis of biological fluids, the removal of proteins is the most important clean-up step because they can precipitate when in contact with solvents or buffer salts commonly used in mobile phases. RAM sorbents, which combine size-exclusion and reversed-phase mechanism, are well suited for the handling of biological samples as they prevent the access of matrix components, while the analytes of interest selective retain in the interior of the sorbent. Immunosorbents and molecular imprinted materials, which are based on molecular recognition, also allow achieving isolation and preconcentration in a single step. On-line SPE sample processing is very well suited for analysis of biological fluids such as urine[7-10,15,20-23,25-27,30,31,37,39,44-46,59,94-99,102] and plasma.[7,8, 15,16, 18,20,22, 33, 36,37, 56,57,69,70-73,94,96,100] The determination of various pharmaceutics and drugs of this kind of matrices is required for pharmacokinetic and bioavailability studies[7,8,15,16, 30,38, 45,56,

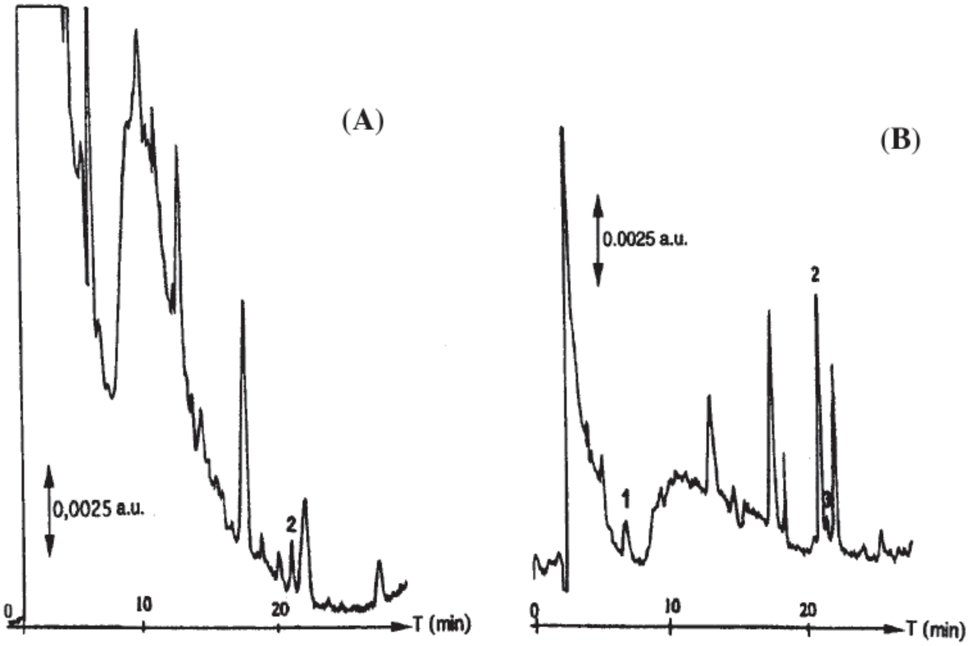

FIGURE 7. Analysis of 2.5 ml of textile effluent using a precolumn packed with (A) PRP-1 polystyrene sorbent and (B) the antibenzidine immunosorbent. Peaks identification: (1) benzidine, (2) 3,3'-dichlorobenzidine, (3) 4-aminoazobenzene. Analytical column: octadecyl Bakerbond ENV, 150 x 4.6 mm, acetonitrile gradient with 0.005 mol/l phosphate buffer at pH 7, UV detection at 287 nm. (From Ref. 83.)

[69-73,96-100,104] and for determining overdose or screening for drugs of abuse.[22,27,44,45,70,95, 102] Moreover, other analytes, such as herbicides,[31,58,101] flavonoids,[23,103,104] polycyclic aromatic hydrocarbons,[9,10] and mercury species[46] were determined in biological samples. The examples of application of on-line SPE for HPLC analysis in urine samples are presented in Table 1.

In the field of environmental analysis, the main goal is to detect an increasing number of contaminants. Pesticides and phenolic compounds as well as their transformation products are organic pollutants of great environmental importance. The determination of these contaminants has been increasing in recent years due to their toxicity, even at low concentrations. Humic and fulvic acids are commonly encountered in natural water samples, influencing the detectability of the analytes. This complex matrix does not allow obtaining appropriate limit of detection due to a broad peak at the beginning of the chromatogram and a noisy base line.[89,105] Masque et al.[61] found that the addition of a sulfite solution to natural water samples had a great effect on the initial humic and fulvic band.

Natural water samples also contain high amounts of inorganic ions that overload the capacity of ion-exchange sorbents. The chemical sample pretreatment based on the precipitation of calcium with oxalic acid and complexation of iron with EDTA is not efficient enough to remove inorganic cations. For analysis of chlorotriazines herbicides a two-step clean-up/preconcentration was proposed.[106] Water samples adjusted to pH 7 were pumped through the PRP-1 precolumn, where the solutes retained in their neutral form. Then they were transferred using small volume of water with acetonitrile at pH 1 to a second precolumn packed with a cation-exchange resin. The advantage of this two-step procedure is that the size of the PRP-1 precolumn can be increased in order to trap more polar analytes. The application of on-line SPE-HPLC systems for determination of different analytes in environmental waters are presented in Table 2.

TABLE 1
Application of SPE-HPLC Systems for Analysis of Urine Samples

Analyte	Sorbent	Analytical column (dimensions)	Detection method (LOD)	References
Pharmaceutics	LiChrospher 100 RP-18	Supelcosil LC-ABZ (150 x 4.6 mm)	UV (25 μg/l)	7
Pharmaceutics	silica-CN silica-phenyl	YMC-Pack C_{18} (150 x 4.6 mm)	UV (0.5 mg/l)	8
PAHs	C_8 or C_4, silica-CN, silica- phenyl	Vydac 201 TPB (200 x 3.2 mm)	Fluorescence (0.1-20 ng/l)	9,10
Drugs	Cation-exchange	Superspher 60 RP-B (250 x 4 mm)	Electrochemical (10 μg/l)	15,16
Pharmaceutics	Intertsil ODS-2	C_{18} (250 x 4.6 mm)	UV (0.5 mg/l)	20
Flavonoids	Zorbax 300SP-C3	Zorbax SB C18 (150 x 4.6 mm)	UV and MS (0.25 - 2.5 μg/l)	23
Nucleosides	Wakosil C_{18}	Tosoh TSK Gel ODS-120 (250 x 4.6 mm)	UV (50 pmol)	25
Antibiotics	Immunosorbent	Rexchrome C_8 (150 x 4.6 mm)	UV (0.36 mol/l)	26
Enanitiomers of drugs	RAM Lichrospher ADS	Chirobiotic T (250 x 4.6 mm)	Fluorescence (15 μg/l)	27
Pharmaceutics	Hypersil 5-CPS	Hypersil 5-ODS (250 x 4.6 mm)	UV (25 μg/l)	30

Herbicides	MIP	Hichrome C_{18} (250 x 4.6 mm)	UV (0.07 μg/l)	31
Pharmaceutics	Supelguard LC_8	Hypersil BDS C_8 (150 x 4.6 mm)	Fluorescence (50 μg/l)	37
Mevalonic acid	Devosil Ph-5	Devosil Ph-5 (150 x 4.6 mm)	Fluorescence (μg/l)	39
Amphetamines	Hypersil ODS C_{18}	Hypersil ODS C_{18} (250 x 4 mm)	Fluorescence (1 - 50 μg/l)	44
Amphetamines	Hypersil ODS C_{18}	LiChrospher RP 18 (125 x 4 mm)	Fluorescence (50 μg/l)	45
Mercury species	C_{18}	Zorbax C_{18} (250 x 4.6 mm)	CVAAS (5 - 10 μg/l)	46
Pharmaceutics	Silica-CN	C_{18} Ultrasphere (250 x 4.6 mm)	UV (0.1-20 mg/l)	59
Drugs	RAM	Hypersil ODS (200 x 3 mm)	MS (2 μg/l)	72

TABLE 2
Examples of the Application of SPE-HPLC System for Environmental Water Samples

Analyte	Sample volume (ml)	Sorbent	Analytical column (dimension)	Detection method (LOD)	Reference
Phenols	4 ml	PLRP-S	Spherisorb ODS-2 (250 x 4 mm)	Electrochemical (1 - 10 ng/l)	1
Pesticides	250 µl	Immunosorbent	Nucleosil Si-100 C_{18} (100 x 4.6 mm)	UV (0.1 µg/l)	2
Pesticides	45 ml	Immunosorbent	Spherisorb C_{18} (100 x 4.6 mm)	UV (6 - 10 ng/l)	3
Aromatic compounds	1 ml	Immunosorbent	C_{18} (100 x 3.2 mm)	MS (0.3 µg/l)	4
Aromatic sulphonates	15 ml	PLRP-S	Kromasil 100 C_{18} (250 x 4.6 mm)	MS (0.1 µg/l)	12
Aniline and phenols	50 ml	Hypsphere-GP Hypsphere-SH	Nucleosil 100-5 C_{18} (150 x 4.6 mm)	Electrochemical (0.05 - 0.2 µg/l)	13
Phenols	10-20 ml	Hypsphere-GP Hypsphere-SH	Kromasil C_{18} (250 x 4 mm)	MS	19
Herbicides	4 ml	PLRP	Inertsil ODS-2 (250 x 4.6 mm)	MS (0.03 µg/l)	40
Mercury species	10 ml	C_{18}	Bischoff C_{18} (53 x 4.5 mm)	CVAAS (0.4 ng/l)	47

Ni(II), Co(II), Hg(II)	2 ml	C_{18}	YWG-C_{18} (250 x 4.6 mm)	UV (0.16 - 1.1 μg/l)	49
PAHs	50 ml	C_{18} Zorbax ODS1	LiChrospher PAH (125 x 2 mm)	Fluorescence (pg/l)	51
Pesticides	50 ml	Lichrolut EN	C_{18} Superpher 60 RP (125 x 2 mm)	UV (below 100 ng/l)	
Munitions	10 ml	Porapak R_{DX}	Ultrasphere C_{18} (240 x 4.6 mm)	UV (0.1 μg/l)	63
Pesticides	100 ml	PLRP-S SDB-1	Supelco C_{18} (250 x 4.6 mm) Hypercarb (100 x 4.6 mm)	UV (0.1 μg/l)	65
Pesticides	20 ml	Immunosorbent	Shandon C_8 (250 x 4.6 mm)	MS (0.001 - 0.005 μg/l)	80
PAHs	20ml	Immunosorbent	PAH 16-Plus silica (250 x 3 mm)	Fluorescence (2 - 10 ng/l)	82
Azo dyes	25 ml	Immunosorbent	Bakerbond ENV C_{18} (150 x 4.6 mm)	UV (0.1 - 1 μg/l)	83

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