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Review

Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography

Marie-Claire Hennion*

Laboratoire Environnement et Chimie Analytique, Ecole Supérieure de Physique et de Chimie de Paris, 10 Rue Vauquelin, 75231 Paris cedex 05, France

Abstract

The objective of this review is to provide updated information about the most important features of the new solid-phase extraction (SPE) materials, their interaction mode and their potential for modern SPE. First, the recent developments are given in formats, phases, automation, high throughput purpose and set-up of new types of procedures. Emphasis is then placed on the large choice of sorbents for trapping analytes over a wide range of polarities, such as highly cross-linked copolymers, functionalized copolymers, graphitized carbons or some specific *n*-alkylsilicas. The method development is given which is based on prediction from liquid chromatographic retention data or solvation parameters in order to determine the main parameters of any sequence (type and amount of sorbent, sample volume which can be applied without loss of recovery, composition and volume of the clean-up solution, composition and volume of the desorption solution). Obtaining extracts free from matrix interferences in a few steps – one step when possible – is now included in the development of SPE procedure. New selective phases such as mixed-mode and restricted access matrix sorbents or emerging phases such as immunosorbents or molecularly imprinted polymers are reviewed. Selectivity obtained by combining two sorbents is described with the use of ion-exchange or ion-pair sorbents. Special attention is given to complete automation of the SPE sequence with its on-line coupling with liquid chromatography followed by various detection modes. This represents a fast, modern and reliable approach to trace analysis. Many examples illustrate the various features of modern SPE which are discussed in this review. They have been selected in both biological and environmental areas

Keywords: Reviews; Solid-phase extraction; Method development; Sorbents; Extraction methods

Contents

*Tel.: +33-1-4079-4651; fax: +33-1-4079-4776.
E-mail address: marie-claire.hennion@espci.fr (M-C. Hennion)

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1. Introduction

There can be no doubt that solid-phase extraction (SPE) is today the most popular sample preparation method. It is a very active area in the field of separation science and more than 50 companies currently make products for SPE [1–4]. But one should bear in mind that this trend is rather recent.

Disposable cartridges for solid-phase extraction have been introduced for more than 20 years [first cartridges in 1978, syringe-format types in 1979, precolumns for the on-line coupling with liquid chromatography (LC) in the early 1980s] yet, SPE development has been slow for many years. Liquid–liquid extraction (LLE) has remained the preferred technique for the preparation of liquid samples for several years especially in the environmental field. The increased development of SPE has occurred during the past five or six years, with many improvements in formats, automation and introduction of new phases. One reason was the pressure to decrease organic solvent usage in laboratories which has encouraged the requirement for solvent-free procedures and has greatly contributed to the growth of SPE at the expense of liquid–liquid extraction procedures [5]. The introduction of survey lists containing polar analytes such as some degradation products of organic micropollutants has also pointed out the need for alternative methods to LLE because many polar analytes are often partly soluble in water and cannot be extracted with good recoveries whatever the organic solvent selected [1,6]. At the same time, the availability of cleaner and more reproducible sorbents than in the past has also helped its increasing acceptance by regulatory agencies. Other reasons for the growing interest in SPE techniques are the large choice of sorbents with the capability for new ones of trapping polar analytes. New formats have been also introduced as for example the 96-well SPE plates and the microfibers for solid-phase microextraction. SPE is also an active area of research, as shown by the increasing number of publications describing new and more selective sorbents or procedures.

However, a key SPE problem remains the method development. The primary decision for analysts is the selection of the type of sorbent able to solve their trace-analysis problem. For many years *n*-alkylsilica has been the universal SPE sorbent, and one has forgotten that one important factor in the SPE process should be the solid-phase. Today, several types of sorbents have been introduced in the market, each one claiming ''universality'' or its better ability for trapping polar analytes. Let us cite highly cross-linked copolymers and their new functionalized form, graphitized carbons, as well as *n*-alkylsilicas which have evolved in the same direction since some of them have been especially designed for the extraction of polar analytes. Obtaining extracts free from matrix interferences in a few steps – one step when possible – has been recognized as an important goal and selectivity is now included in the development of the SPE procedure. It is clear that the more selective the SPE step is, the more sensitivity is obtained. Mixed-mode sorbents and restricted access matrix sorbents have been introduced in that way as well as the emerging selective sorbents such as immunosorbents or molecularly imprinted polymers.

A first objective of this review is to provide updated information about the most important features of these new SPE materials, their interaction mode and their potential for modern SPE.

SPE can be performed off-line, the sample preparation being separated from the subsequent chromatographic analysis, or on-line by direct connection to the chromatographic system [7–15]. On-line techniques do not require further handling of the samples between the trace-enrichment and the separation step and, therefore are highly suitable for fully automated techniques, which can be used on-site [16–20]. Hyphenation is the topic of this special issue and special attention will be given to on-line SPE–LC followed with various detection modes, which represents a fast, modern and reliable approach of trace analysis. Important advantages are a decrease of the risk of the sample contamination, the removal of analyte losses by evaporation and finally the transfer and analysis of the totality of the extracted species. In contrast to off-line SPE where only an aliquot of the extract is injected into the chromatograph, the analysis of the complete sample allows the sample volume to be dramatically reduced. It was surprising to see that gas chromatography (GC) has been the preferred method for analytical chemists for a long time but that on-line coupling of SPE with LC became the first robust on-line technique. This is explained by the good compatibility of the LC aqueous mobile phases with the SPE of biological or environmental samples which are mainly aqueous. On-line coupling of SPE with GC is more delicate because of the inherent incompatibility between the aqueous part of the SPE step and the dry part of the GC system. Much work has been done in this area and automatic devices have recently became avail-

able. This review will only consider on-line coupling of SPE with LC, the on-line coupling with GC being the topic of another paper of this issue.

2. Trends in solid-phase extraction

Recent developments in the SPE area are in format, phases, automation, high throughput purpose and set up of new types of procedures. In the environmental field, trends are to perform multiresidue extraction of many analytes with a wide range of polarities followed by a separation using LC in order to reduce the price and the time of environmental monitoring whereas in the clinical field trends are to perform analysis with high throughput. SPE is now accepted as the alternative sample preparation method to LLE in many US Environmental Protection Agency (EPA) methods for analysis of organic compounds in drinking water and wastewater.

2.1. Formats

The syringe-barrel and/or cartridge types are still the most popular format with typically 40–60 μm d_p packing materials. The design may vary in order to be robot-compatible. Reservoirs have been adapted in order to increase the sample volume. As a general rule, in addition to the use of cleaner phases, the manufacturers have made efforts to provide high-purity devices with low extractable contents using medical-grade polypropylene and polyethylene for the cartridge body and frits. Limitations of packed SPE conventional cartridges include restricted flow-rates and plugging of the top frit when handling water containing suspended solids such as surface water or wastewater. Therefore, the percolation of samples can take a long time for a typical volume of 500 ml if the sample has not been carefully filtered before. In order to avoid previous filtration and clogging, various approaches have been investigated to overcome the flow limitation. Depth filters containing diatomaceous earth have been available as accessories by some companies. The trends are now to integrate filters in the SPE cartridges (i.e., Envireluts from Bond-Elut). International Sorbent Technology (IST) has for instance equipped some cartridges with depth filters made of homogeneous cellular structures.

The second most popular format is the disk which allows higher flow-rates without channeling effects thanks to their large cross-sectional area and thin bed. However, the same limitations exist for the handling of samples containing suspended matter. Empore type disks became recently available with the sorbent trapped in a glass fiber matrix. They are thicker and more rigid thus providing faster flow-rate than PTFE disks and may require no supporting device [21]. Membrane extraction disks which consists in depth filter above the membrane have been also developed. J.T. Baker has introduced new laminar disks known as Speedisks which consist in a thin bed of microparticles supported in a laminar structure in a preassembled disk. The percolation of 1 l of surface water without any previous filtration takes less than 5 min [22].

The demand for high-throughput applications (combinatorial chemistry, drug screening, coupling with LC–MS) has been at the origin of the development of small-volume SPE with a packed bed lower than 50–100 mg. One format is the disc cartridge in which a SPE disc is placed in a SPE cartridge, allowing the use of automated systems. One of the newest formats of disc technology is the 96-well plate format which is used for rapid sample preparation and mainly for sample clean-up in the pharmaceutical and biotechnological industries [23]. The 96-well plates appeared in 1997 and are designed to fit automated plate handling system and are now sold by several manufacturers (Empore, Ansys Diagnostic, Whatman, Quiagen). They can be eluted with 100 to 200 μl of solvent. The SPE pipette tip developed by Ansys Diagnostic is an alternative to the 96-well plates and uses a conventional pipette tip that is fitted with a SPE disc. They are designed to be handled quickly by automated liquid-handling systems. The design of these formats was also made to reduce the void volume as well as the sorbent bed masses in order to have the minimum desorption volume.

Another small volume format is the microfiber which is immersed into the sample for sorption followed by direct desorption in the inlet of the gas chromatograph. Invented by Pawliszyn and his team in 1989, it has been introduced in the market by Supelco in 1992 and is known as solid-phase mi-

croextraction (SPME). This format has rapidly achieved some degree of popularity and examples can be found in reviews and some relevant publications [24–32]. This method involves the equilibrium sorption of analytes onto a small microfiber and is different from conventional SPE and will not be discussed in the present review.

2.2. Automation

Due to the sample volumes usually handled, the automation has been introduced differently for biological and environmental matrices. Typical volumes for biological matrices are in the range of 1 to 10 ml and automation has been optimized using cartridge formats with on-line coupling by automated injection of the extracts in the liquid or gas chromatograph. For environmental samples, typical volumes are 100–200 ml when SPE is coupled to LC and required specific devices.

Automation has contributed to the SPE development. The four individual steps of a typical SPE sequence – (i) conditioning of the sorbent, (ii) application of the sample, (iii) rinsing and cleaning of the sample, and (iv) desorption and recovery of the analytes to be separated – can be performed sequentially for up to 24 cartridges at the same time using extraction units working under positive or negative pressure. The whole sequence can also be easily automated with devices now available by several companies using any commercial cartridge or extraction disk formats. Examples are the ASPEC from Gilson, Microlab from Hamilton, AutoTrace and RapidTrace from Zymark. Possibility exists for some of these devices for automatic injection of an aliquot of the final extract into the chromatographic system. The complete automation also exists which couples on-line SPE and LC (Prospekt from Spark Holland, OSP-2 from Merck, ASPEC XL from Gilson). These apparatus improved productivity since the next sample is automatically prepared while the previous sample is being analyzed. Therefore, method development can easily be automated with various degrees of automation.

2.3. Sorbents

With regard to sorbent technology, many are now specified as specially made for broadening the polarity range. These include non-endcapped C_{18} silicas and monofunctional C_{18} silicas, the aim being to increase the number of non-modified silanol groups at the bonded silica surface in order to provide secondary polar interactions with solutes. The problem of the extraction of polar analytes in environmental water has been partly solved by the introduction of carbon-based sorbents and highly cross-linked styrene–divinylbenzene (PS–DVB) copolymers with high specific areas in the range 500 to 1200 m^2/g and high degree of purity. This is one of the most relevant developments of these last five years.

One limitation of both reversed-phase silica sorbents and many of the commercial polymeric sorbents is that they must be conditioned with a wetting solvent and not allowed to dry before the loading of an aqueous solvent. Functionalized polymeric sorbents have been shown to provide a better wettability and to increase the extraction recoveries of polar compounds. The new generation of polymers (Oasis from waters, Abselut from Varian) are designed to extract extensive spectrum of analytes, i.e., lipophilic, hydrophobic, acidic, basic and neutral with a single cartridge with a simplified procedure since no conditioning is required. But the universal procedure (apply 1 ml of sample, wash and elute) is limited to the handling of biological samples since the sample volume that is recommended is 1 ml.

Mixed-mode sorbents containing both non-polar and strong ion (cation and/or anion) exchange functional groups have been targeted for the extraction of basic drugs. New design of layered cartridges have been introduced by IST in order to optimize the desorption, or the fractionation of the analytes on a polarity basis, or the removal of matrix components. The development of such cartridges witnesses a new aspect of SPE, i.e., a better understanding by end-users of the chemistry involved in SPE processes.

Restricted-access packings combine size-exclusion and reversed-phase mechanisms and allow extraction and clean-up of samples in the same step. They are well suited for the handling of biological samples since they prevent the access of matrix components such as proteins while retaining the analytes of interest in the interior of the sorbent.

New selective sorbents are emerging which are based upon molecular recognition. A first approach uses antibodies which allows a high degree of molecular selectivity. Extraction, concentration and isolation are possible in a single step. Single analytes can be targeted but thanks to the cross-reactivity of antibodies, immunoextraction sorbents have been also designed to target group of structurally related analytes. A second approach deals with molecular imprinted materials, which avoids the inherent instability of biological materials. These two SPE materials are now emerging from laboratory studies and their development is expected in the near future.

2.4. Procedures

The procedures are different depending on the field of application. In many cases, the handling of biological samples differs from that of environmental samples, just because the sample volumes is limited to the low-ml order 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 for biologists whereas it is of importance for environmental chemists. One should also admit that in general the trace level is one- or two-orders lower in environmental chemistry because 1 ml is enough to solve many problems with biological samples whereas with the same on-line extraction, LC and detection procedure the sample volume required to reach many environmental levels is typically close to 100 ml. The trends are also to simplify the procedure for biological samples with the introduction of non-conditioned solid-phase extraction (NC-SPE) technology (Abselut from Varian).

In both fields trends are to achieve extraction and clean-up in the same step. This can be achieved by a better selection of the extraction sorbent or a combination of sorbents and/or the sample conditioning such as pH or ionic strength.

The concept of multiresidue extraction is becoming more and more relevant in environmental monitoring, including polar transformation products. The extraction is usually followed by a multiresidue separation of many compounds using reversed-phase liquid chromatography because of its suitability to separate compounds over a wide range of polarity without previous derivatization. When possible, clean-up is included in the SPE sequence, one key issue being the removal of fulvic and humic acids in environmental samples. Trends are also for a better use of SPE on site with further analysis in the laboratories, as shown by increasing studies on the stability of analytes once they are extracted on the solid-phase and the corresponding appropriate storage conditions.

3. Method development: the solid-phase extraction process and its similarities to liquid chromatography

When reviewing the literature on SPE methods, we can still often observe that some of them are poorly developed with little consideration to the chemistry involved in the process and are described as a largely empirical, labor intensive and time-consuming trial and error process [5,33,34]. It is certainly due in part to the fact that in analytical laboratories the sample preparation is not recognized as an important step in the whole analytical scheme and often given to the less trained chemist. But it is also due to the fact that the literature is rather poor which provides the necessary knowledge and guidelines for the selection of the extraction sorbents and other SPE relevant parameters.

Method development is related to the properties of the analytes of interest, the required trace-level concentration, the nature of the matrix, the type of chromatography involved in the separation step and the detection mode. To understand the extraction process, it is also necessary to understand the interactions between the analyte, matrix and sorbent.

Various models have been proposed from simple guidelines to sophisticated calculations. The parameters to be determined are indicated in the SPE sequence. They are the selection of the type and amount of sorbent, the determination of the sample volume which can be applied without loss in recovery – the so-called ‘‘safe sampling volume’’, the composition and volume of the washing or clean-up solution which can be applied without loss of analytes and finally, the composition and the volume of the elution or desorption solution.

A first approach for method development is the process which occurs during the extraction. Starting

from the knowledge of both the required sample volume for the trace-determination problem and the LC retention data of the analytes in the sample solvent, this approach provides information and guidelines for the selection of the sorbents. Other types of method development are based on more general models available for retention prediction in liquid chromatography such as the solvation parameters and system maps [35,36]. They can allow accurate computational calculations of the composition and volume of each step of the SPE sequence, providing that system maps and solvation parameters are available for all the sorbents and analytes, respectively. These two approaches are described because they are essentially complementary tools. They are both based on the analogy between SPE and LC, but one is based on the basic LC knowledge for rapid retention prediction whereas the second ones requires a depth knowledge of retention prediction models in LC.

3.1. Breakthrough volume and retention factor in the sample solvent

SPE isolates analytes in a sample by utilizing the principles of LC. The chemistry and principles are essentially identical for both off-line and on-line SPE. The processes involved are a frontal chromatography during the extraction step and a displacement chromatography during the desorption step. These two modes are well known and to a first approximation, SPE can be described as a simple chromatographic process, the sorbent being the stationary phase. The mobile phase is the obligatory solvent of the sample during the extraction step and an appropriate selected solvent during the desorption step. The choice of type of chromatography is first guided by the sample solvent. An aqueous matrix involves the selection of reversed-phase or ion-exchange LC, depending on the ability of analytes for ionization whereas a matrix in a non-aqueous organic solvent involves adsorption or normal-phase LC. Most of environmental and biological samples are aqueous. Non-aqueous matrices include liquid extracts from solid matrices which have to be re-extracted for clean-up. The main criteria for the selection of the type of chromatography depending on the analyte properties are well explained in the solid-phase application guides or books supplied by the manufacturers of SPE cartridges such as IST, Macheray Nagel, Varian, Gilson, Waters, SUPELCO).

The similarity between LC and SPE is now well-known. When a sample spiked with traces of a solute having and initial UV absorbance, A_0, is percolated through a SPE cartridge, a frontal or breakthrough curve can be observed, beginning at a volume, V_b usually defined at 1% of initial absorbance A_0 up to a volume, V_m defined at 99% of initial absorbance, where the effluent has the same composition as that of the spiked water sample (Fig. 1). Under ideal conditions, this curve has a bilogarithmic shape, the inflection point of which is the retention volume, V_r of the analyte. If the same SPE column is used in elution chromatography with water as mobile phase and with the same flow-rate, the injection of 10 or 20 μl of a concentrated solution of the same analyte will generate a peak detected at the same volume V_r.

3.2. Experimental measurement of breakthrough volumes

The recovery is defined as the ratio between the amount extracted and the amount applied. The breakthrough volume represents the maximum sample volume which can be applied with a theoretical 100% recovery and this explains why it has received much attention for measurements and prediction. However, the maximum amount that can be extracted corresponds to the application of a volume equal to V_m, but with a corresponding recovery lower than 100%.

Measurement of breakthrough volume has been performed by monitoring continuously or discretely the UV signal at the outlet of a precolumn or a cartridge [1,5,8,37–41]. However, these methods are time-consuming and reading V_b at the 1% level is neither easy nor accurate. Moreover, the sample should be spiked at a trace level in order not to overload the sorbent capacity, and the sorbent signal should be monitored at very low absorbances which may lead to problems with baseline stability or noise and analytes with poor UV properties. The more retained the analyte is, the larger volume the curve is spread over, because of the low plate number of cartridges or precolumns. For example, a break-

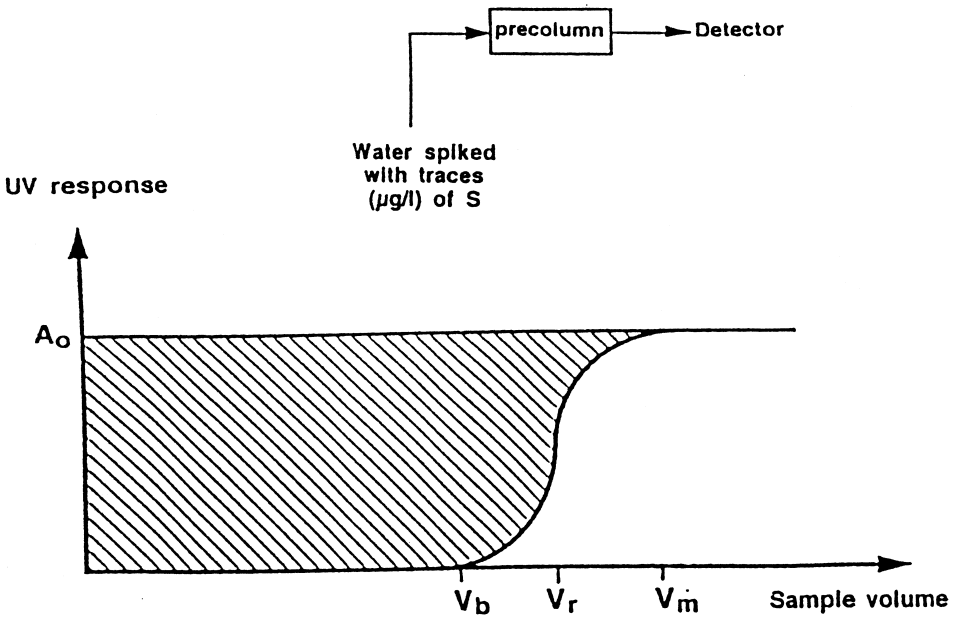

Fig. 1. Typical breakthrough curve obtained by recording the UV signal of the effluent of the precolumn. (The shaded area represents the maximum amount that can be preconcentrated.)

through curve was drawn for linuron using a 1 cm×0.2 cm I.D. precolumn packed with octadecylsilica and the front spread over 100 ml from a V_b value of 70 ml to a V_m value of 170 ml [37]. Another method has been developed for estimating breakthrough which consists of preconcentrating samples of increasing volumes, each containing the same amount of analytes and then measuring the peak-areas or heights eluted from the sorbent [10,37,42,43]. As the sample volume increases, the analytes concentration decreases, but the amount eluted remains constant provided breakthrough does not occur. When it occurs, the amount decreases. Another method was recently used for also estimating the V_b values under real conditions. Various sample volumes were spiked with several analytes and the curves in which the peak areas are plotted against the sample volume were constructed [44]. The point where the curve declines from linearity is considered to be the breakthrough volumes. An advantage of these two methods is that the V_b value of several compounds can be estimated simultaneously by preconcentration under the real experimental conditions of unknown samples via the whole off-line or on-line procedure.

3.3. Prediction of breakthrough values from retention factor values

Since experimental determination of the breakthrough volume is difficult, models have been developed for its prediction. According to the SPE–LC analogy represented in Fig. 1, V_b (at the 1% level) is mathematically related to the retention volume of the analytes with the sample solvent as mobile phase by the relation:

$$V_b = V_r - 2.3\sigma_v \quad (1)$$

where σ_v is the standard deviation depending on the axial dispersion along the bed of particles in the precolumn or cartridge. V_b is therefore controlled by retention and kinetic parameters [1,8,36,38,41]. The σ_v term can be calculated if the number of theoretical plates, N, of the precolumn or cartridge is known by the relation:

$$\sigma_v = (V_M/\sqrt{N})(1 + k_s) \quad (2)$$

where V_M is the void volume of the precolumn or the cartridge and k_s is the retention factor of the solute eluted by the solvent of the matrix (water for aqueous samples). V_M can be calculated from the

porosity of the sorbent (ϵ) and the geometric volume (V_c) of the precolumn or sorbent bed in the cartridge or disk ($V_M = \epsilon V_c$). With average values of density and porosity for the C_{18} silica used in cartridges, V_M is estimated as 0.12 ± 0.01 ml per 100 mg of sorbent. N can be directly measured with precolumns because the on-line set-up can allow the recording of breakthrough curve or of the elution peaks by direct injection onto a precolumn [37]. It is much more difficult to measure the efficiency of a SPE cartridge or that of an extraction disk, so that N has to be estimated. Miller and Poole [36] have studied the kinetic and retention properties of a SPE cartridge packed with 500 mg of C_{18} silica and they measured an average of 20 theoretical plates for a flow-rate of 5 ml/min.

A more general model and methodological approach has been described, the model for the extraction being based on frontal analysis as proposed by Lovkist and Jonsson [36,41,45–49]. The breakthrough volume is related to the properties of the cartridges by

$$\log V_b = \log QV_M + \log (1 + k_s) \quad (3)$$

where Q is the contribution of the kinetics properties of the sorbent bed to retention, V_M the hold-up volume for the sorbent bed and k_s the retention factor of the analyte with a mobile phase that corresponds to the sample solvent, wash solvent or elution solvent.

This mathematical definition is similar to that derived from Fig. 1, since combination of Eqs. (1) and (2) results in the following relation:

$$V_b = (1 + k_s)(1 - 2.3/\sqrt{N})V_M \quad (4)$$

The breakthrough curves can be easily been modeled according to the relations described in Eq. (3), provided that values are known for porosity of the sorbent, number of plates and amount of sorbent [5]. The recovery curves can easily be derived from the breakthrough curves [37]. These curves have been constructed with the hypothesis of an amount of 450 mg of sorbent, an average porosity of 0.70 and a number of plates of 20 and are shown in Fig. 2 [5]. The effect of the number of plates was shown to be negligible when modeling with the same conditions except a number of plates of 10 instead of 20. The only strong effect is the amount of sorbent, but the relation between V_b and V_M is straightforward in Eq. (4).

This model has been validated by experimental measurements of recoveries for several analytes using extraction disks packed with *n*-octadecylsilica or poly(styrene–divinylbenzene) (PS–DVB) sorbents [5]. The k_s values have been measured by LC using water as mobile phase or extrapolated from measurement in water–methanol mobile phase by the well known relation log k–methanol percent. A good agreement was obtained between experimental and modeled curves for oxamyl having log k_w values of 1.7 and 2.8 on C_{18} silica and PS–DVB sorbent and desethylatrazine with log k_w value of 3.5 on the PS–DVB sorbent. A great difference in sample volumes that can be percolated with a good recovery was observed when using a disk containing C_{18} silica or the PS–DVB copolymer. Taking a value of 90% for recovery, the corresponding sample volumes were, respectively, 30 and 300 ml for oxamyl on the two sorbents.

The recovery curves similar to those in Fig. 2 are more relevant that the breakthrough curves (not represented here). From a practical point of view, the analyst knows the detection limit of the separation–quantification system and the concentration limit to be reached in the unknown solutions to be determined. Then, it is easy to calculate the necessary sample volume for the required concentration limit. This curves allow a very rapid selection of the necessary k_s value for the analyte that the sorbent has to provide. They also indicate that breakthrough can be overloaded to a great extent, with small losses in recoveries, for compounds with high log k_s values. For example, a compound characterized by a log k_s value of 2.9 (calculated V_r value of 430 ml), has a breakthrough volume of 210 ml, but, the theoretical recovery value obtained with a sample volume of 500 ml is still around 85%. The US EPA methods accepts to validate extraction methods with recoveries in the range of 70 to 130%. Only compounds with log k_s lower than 2.5 will be extracted with recoveries lower than 50% with a 500-ml sample volume. It is worthwhile to note that when log k_s is lower than 2, the recovery decreases rapidly as soon as overloading of V_b occurs. As in practice a rapid approximation of V_b is required to appropriately

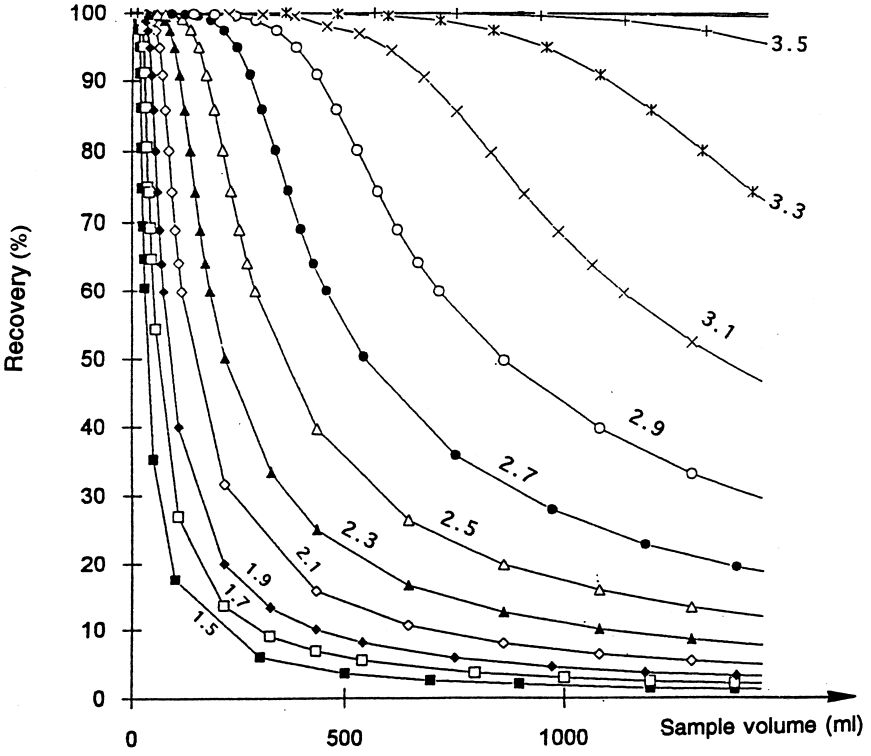

Fig. 2. Effect of the retention value log k of the analyte on the theoretical recovery curves versus sample volume. From Ref. [5]. (Log k reported on each curve. Prediction assuming 20 plates in the cartridge or disk, calculated with 450 mg of sorbent.)

select the sorbent, the approximation of V_b by V_r values was examined and it was shown that for log k_s values lower than 2, the difference between V_r and V_b is small because the front shape is sharp. The difference becomes important for log k_s above 2.5 because the breakthrough curves spread.

The highest the k_s value, the highest the sample volume can be applied without loss in recovery, which is often a required condition for the handling of environmental samples. For biological samples, the sample volume is small, so that obtaining high value of k_s is not as important as it is for environmental samples for the enrichment factor. However there can be an interest because a more efficient clean-up can be applied using a higher proportion of organic solvent in the washing solution.

3.4. Prediction models using solute descriptors

The description of the SPE process points out than predicting the SPE parameters is dictated by the k_s values. This explains why comparison of the sorbents have been made using k_s values for known solutes (or k_w values since many samples are aqueous) [10,50–56]. Obtaining the appropriate k_s values requires a good knowledge of the interactions between the analyte, matrix and sorbent. The k_s values are LC data and several methods exist for their extrapolation or prediction, depending on the retention mechanism.

3.4.1. Graphical extrapolation

Values of k_w can be estimated from chromatographic measurement with reversed-phase sorbents such as *n*-alkylsilicas, PS–DVB polymers and porous graphitic carbons [50] using analytical columns eluted with mobile phase composed of water–methanol mixtures. The advantage of this method is that experimental data are obtained rapidly by measuring the retention factor k of the analyte in methanol–water phases. Over a methanol content in the range 30 to 90%, the relationship is usually considered as

linear. A good agreement was observed between the V_r values for some chlorophenols, derived from experimental breakthrough curves, and the values calculated from extrapolated k_w values [38]. Differences were of the order of 10 to 20%. Then one can conclude that from rapid measurements with three or four mobile phases containing different methanol concentration, k_w, can be estimated by graphically extrapolating to zero methanol content. However, this relation is known not to be totally linear in water-rich mixtures and a better fit has been obtained with a quadratic relationship for some compounds [57]. It is possible to measure k_w directly by elution methods using a short microcolumn, and for the more apolar analytes the extrapolations are more rapid and more accurate because the experimental range for extrapolation is water-rich [58]. The shape of the curve log k–methanol % was investigated for various polar pesticides having different structures and functionalities. Most of the curves have shown that when a wide range of mobile phase was studied polar compounds did not give rise to linear variations. A very short analytical column has been used in order to obtain experimental data in water-enriched mobile phases. Extrapolation has been made from the usual range 40–60% of methanol and from quadratic relations with water-rich mobile phases. Results indicated that extrapolations from the usual range 40–60% of methanol were always lower than real values or those extrapolated from water-rich values [5].

3.4.2. Estimation from the octanol–water partition coefficient

Prediction of k_s values in reversed-phase chromatography using *n*-alkylsilicas was extensively studied in LC. Since the retention mechanism is primarily governed by hydrophobic interactions between the analyte and the carbonaceous moieties of the alkyl chains grafted at the silica surface, a relation has been observed between the retention factors of the analytes and their octanol–water partition coefficient (K_{ow}), which characterizes well the hydrophobicity of a compound and plays an important role in correlating phenomena of physico, chemical, biological and environmental interest [59–61]. A linear relation was found between the average log k_w values obtained with different C_{18}silicas using methanol–water as mobile phase and log K_{ow} for closely related compounds and even for compounds having different polarities and chemical properties [60]. Therefore, k_w values can be approximated without any additional measurements with log K_{ow} values. However, these values should be considered as approximate depending on the experimental procedure used for their determination. They can also be obtained now by computational approaches.

For very polar analytes, log K_{ow} was shown to be of limited help for predicting the SPE recoveries, especially for very polar analytes with log K_{ow} below 1.5. It can just serve as a first estimation, knowing that k_w thus predicted can be underestimated by a factor 10 to 50. For very polar analytes, a more rapid method is certainly to have in the laboratory a 10- or 5-cm long C_{18} column, and to extrapolate log k_w from k measurement in a methanol–water mixture containing as high as possible water content. This is can be easily performed with autosampler and HPLC devices.

For homologous analytes, a correlation between water solubility and k_s data has also been observed [62], but one must be aware that this approach is only valid for closely related analytes.

3.4.3. Prediction using the solvation parameter model

Poole and his group presented a general model for the prediction of the SPE experimental conditions from aqueous samples [36,41,45–48]. They started from the definition of the breakthrough volume given by Eq. (3). The values of Q and V_M were estimated from column values using the SPE sorbent as stationary phase. Then, the retention factor k_s was derived by calculation based on the Abraham's solvation parameter model [35] as a function of solvent composition from characteristic solute descriptors

$$\log k_s = c + mV_x + rR_2 + s\pi_2^H + a\sum a_2^H + b\sum b_2^H \quad (5)$$

where k_s is the retention factor for the solute at a defined sorbent and mobile phase composition. The solute descriptors are the characteristic molecular volume V_x and the excess molar refraction R_2. π_2^H is the solute's dipolarity/dipolarizability and Σa_2^H and

b_2^H the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively. According to the authors, these descriptors are available for more than 2000 compounds with others available through parameter estimates or by computational approach. They can also be measured by chromatographic systems using standardized methods.

The system constants are defined by their complementary interactions with the solute descriptors. The m constant reflects the differences in dispersion interaction in the solvated sorbent and the mobile phase. The capacity of the solvated sorbent to interact with solute n- or π-electron is referred by the *r* constant. Dipole–dipole and dipole-induced dipole interactions between the solvated sorbent and the mobile phase are represented by the *s* constant. The *a* and *b* constants are a measure of the difference in hydrogen-bond basicity and in hydrogen-bond acidity, respectively of the solvated sorbent and the mobile phase. The system constant can be obtained using multiple linear regression analysis of experimental log k_s values acquired for a group of varied solutes with known descriptors. A system map is a plot of the system constants as a function of solvent composition. Systems maps are available for several bonded silica sorbents (butylsiloxane-bonded, cyanopropylsiloxane, octadecylsiloxane-bonded, porous polymers and carbon in the reversed-phase mode) for several aqueous–organic solvent mixtures [34,45,63–68]. The advantage is that these maps are permanent once generated. An interesting application was published recently for optimizing the sample processing conditions for the isolation of estrogens from urine [34]. The solvation model was used to estimate retention factor and the corresponding breakthrough volumes for estrone, 17β-estradiol and estriol at all solvent composition from 1 to 80% organic solvent in water. The methanol was shown to provide higher V_b values. Any solvent composition where the breakthrough volume exceeds the required sample volume to provide sufficient analyte for quantification can be used for the analysis. Based on the breakthrough volume for the last retained analytes, 1 to 20% methanol could be used when the volume of hydrolyzed sample urine was fixed at 45 ml. Similarly, the system map was used to determine the composition and volume of the wash solvent which was selected to be as strong as possible within a safe margin of the lowest predicted breakthrough volume. Based on the breakthrough volume estimate for estriol at 14.4 ml, 6 ml of 40% (v/v) methanol was chosen. The same system map allowed to optimize the elution solution. The model was validated by the experimental determination of recoveries of the breakthrough volumes for three methanol–water compositions (70, 30 and 50% methanol) expected to yield breakthrough amenable to experimental determination. The influence of a complex matrix such as urine on the predicted conditions for the isolation of estrogens was achieved by preconcentration of 45 ml of spiked and non-spiked urine samples, applying the washing step with 6 ml of 40% methanol. Typical chromatograms are shown in Fig. 3a and b and one can see the efficiency of the washing step since there are very few interfering analytes. Acceptable recoveries were obtained up to a washing volume of 10 ml, as predicted.

This approach provides a framework for computer-assisted method development in SPE for aqueous samples. It is dependant of the availability of the system maps for the various sorbents and solvents as well as the descriptors of the analytes. We can expect this method development to be part of the software of the automated devices in the near future. But this method will be useful provided that analysts are still able to rapidly select the type of sorbent and this

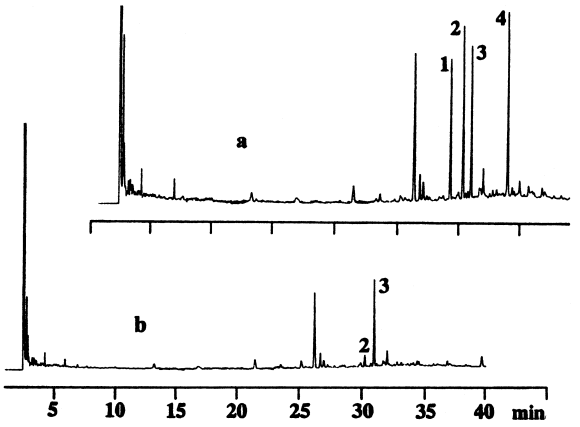

Fig. 3. Gas chromatographic separation of estrogens and equilenin (internal standard) as their trimethylsilyl ether derivatives isolated by SPE from (a) a spiked and (b) a blank non-pregnancy pooled urine sample. From Ref. [34].

Table 1
Energetic of interactions in solid-phase extraction

Interaction type	Energy (kcal/mol)
Dispersion	1–5
Dipole–induced dipole	2–7
Dipole–dipole	5–10
Hydrogen bonding	5–10
Ionic	50–200
Covalent	100–1000

objective requires to consider the chemical interactions involved in the process.

3.5. Importance of the binding energies for extraction and elution

A good knowledge of the interactions between analytes, matrix and sorbent is important for setting up an efficient extraction scheme. Binding interactions involved during the SPE process are the same as for LC and mainly includes hydrophobic interactions, hydrogen bonding and ionic interactions. The energies involved in these bonding vary considerably as reported in Table 1. Hydrophobic interactions defined as dipole–dipole, dipole–induced dipole and dispersive interactions have binding energies ranging from 1 to 10 kcal/mol (1 cal = 4.184 J). Hydrogen bonding involving polar groups have similar energies in the range 5 to 10 kcal/mol. Hydrophobic interactions are weak in comparison to ionic or electrostatic which are in the range 50 to 200 kcal/mol [8,33]. This difference in binding energies explains the important role of the residual silanol groups of reversed-phase silicas even if there are a very small number of silanol groups. It also explains why desorption is not always straightforward, because one has to keep in mind that a strong interaction can be attractive for the extraction step, but the more difficult to break. The effect of energetics of interactions will be discussed with the characteristics of the sorbents.

4. On-line coupling of solid-phase extraction to liquid chromatography

Off-line SPE utilizes disposable cartridges or disks containing 50 to 2000 mg of sorbent whereas on-line SPE–LC utilizes so-called precolumns which have obligatory small dimensions. A typical on-line arrangement is easy to perform in any laboratory using simple switching valves and commercial precolumns and their holders [8,10,16,17]. This system is also named column-switching liquid chromatography in the literature. Two automated devices are commercially available (Prospekt and OSP-2) which have the capability of using a fresh disposable precolumn for every sample. The trace-enrichment is carried out in a similar way to the off-line sequence using a solvent delivery unit which provides the solvent necessary to purge, wash and activate the precolumn and apply the required volume of sample. The main difference is in the desorption since the trapped compounds are eluted directly from the precolumn into the analytical column by a suitable mobile phase which also brings about the chromatographic separation.

4.1. Precolumn design and packings

Since the precolumn is part of the analytical column in the transfer and separation process, a first requirement is that it should be pressure-resistant. Precolumn holders are designed to receive stainless precolumns and disposable precolumns used in the two automated devices are made of stainless steel. The size of the precolumn is also of prime importance because the profile of the concentrated species transferred from the precolumn to the analytical column should be as narrow as possible at the beginning of the separation in order to avoid band-broadening. The quality of the coupling can be easily controlled by comparing chromatograms obtained by direct injection with those obtained by on-line preconcentration. Previous studies have reported that the dimensions of the precolumn should be adapted to those of the analytical column and are typically 2 to 15 mm long and 1 to 4.6 mm I.D. for a classical 15–25 cm long analytical column [8]. The use of a small precolumn is particularly important when the separation is carried out with an isocratic mobile phase [69]. The dimensions can be increased without band broadening when a gradient with a rapid increase in acetonitrile or methanol is used [17].

The packings should be LC-grade quality in order not to decrease the efficiency of the analytical

column. But since 5–10 μm packings do not allow high sampling rate, the trend now is to use 15–40 μm packings, and no decrease in efficiency was observed provided the precolumn dimensions should be low. Backflush-desorption should provide better peak shape than forward-desorption. However, in practice backflush-desorption may create clogging of the analytical column when real samples are analyzed. Since only a very slight difference is observed, forward-desorption is commonly used and then the precolumns act also as guard columns.

4.2. Consequence for method development

Although the basic principles of SPE are similar in off-line and on-line methods, there are two serious limitations of on-line SPE–LC systems for the method development. The first one is that they use small precolumns which contain a small amount of sorbent. In off-line SPE, there is the possibility of increasing the breakthrough volumes by increasing the amount of the sorbent up to 1 or 2 g without increasing the volume of the desorption solution too much. This is not possible in on-line techniques and typical amounts of sorbents in precolumns are in the range 20 to 100 mg, so that when analytes are poorly retained in the precolumn, the only solution is to select a more retentive sorbent.

The second limitation is that compatibility should occur between the sorbents in the precolumn and in the analytical column. The most efficient system is ideally obtained from a precolumn and an analytical column of the same nature [8]. For aqueous samples, the analytical system should use a mobile phase compatible with residual water which will obligatory transferred from the precolumn. In practice the selection of sorbents in the analytical column is more limited than those in the precolumns. When many compounds are to be separated over a wide range of polarity, their separation requires a highly efficient analytical columns with both water rich and organic-rich mobile phases. At present, only C_{18} silica columns meet this requirement. Analytical columns prepacked with polymeric styrene–divinylbenzene or porous graphitic carbon are very efficient in organic-rich mobile phase but less in water-rich. The compatibility of sorbents for precolumns and analytical columns is discussed in the next section for each SPE sorbent.

4.3. Reproducibility of the method with overloading of breakthrough volumes

In off-line SPE–LC, the recoveries should be known since quantitative analysis are made through external calibration curves obtained by direct injections, using the same experimental set-up. This is why it is recommended to have recoveries above 70%. With on-line systems, it is not advisable to carry out quantitative analysis by comparison with direct injections. First, the volume of many injection loops is specified to an average accuracy of 20% and calibration of a loop is a rather delicate and time-consuming operation. This does not have to be considered with off-line procedures because the same loop is used for both analysis of unknown extracts and construction of calibration curves. Secondly, slight but imperceptible band-broadening may occur.

For the above reasons, any quantitation method (calibration curves, standard addition, etc.) should be performed using the whole procedure, i.e., with the same experimental conditions (same types of precolumns, sample volume, analytical column, and on-line gradient elution) as selected for the analysis of unknown water samples. Therefore, it is not necessary to know the recovery of the extraction process for each analyte. When possible, it is better to handle a sample volume lower than the lowest breakthrough volume for more reproducible results. However, when multiresidue analyses are carried out, the sample volume is selected in order to detect most of the compounds at the required level. With a sample volume of 150 ml and using the Prospekt cartridges packed with PLRP-S, the recoveries of deisopropyl- and deethylatrazine are not 100% because breakthrough has occurred on PLRP-S, but it is possible to detect these compounds with reproducible results [20,70,71].

One advantage of automation in on-line preconcentration is that more reproducible results can be expected, provided the precolumns are packed with the same amount of sorbent and have the same efficiency from one experiment to another one. The overall reproducibility of the method includes both the reproducibility of the preconcentration and of the

LC system. The repeatability of peak-areas and heights obtained by direct-loop injections into the analytical column has been studied, using an acetonitrile gradient for the analytical separation. The relative standard deviation (RSD) was between 3 and 7%, and 3 and 5% for measurements of peak areas and peak heights, respectively [70]. In the same study, the reproducibility between cartridges was measured by preconcentrating 50 ml of LC-grade water spiked with 0.5 μg/l of pesticides, using a Prospekt system with a new precolumn packed with the PLRP-S copolymer in each run. The RSD was around 10% ($n=5$) for measurements of both peak areas and peak heights. RSD values below 10% have been also confirmed in other studies, thus indicating that the precolumns were packed under reproducible conditions. The flow-rate applied for the preconcentration varied from 1 to 5 ml/min and the same average 10% RSD was observed [72].

Validation of the automated on-line solid-phase extraction has been performed by participating in the Aquacheck inter-laboratory comparison study organized by the WRC (Medmenham, UK) where more conventional sample preparation methods and gas chromatographic determination were being used. The overall RSD between values obtained by the authors and the average value obtained by 14 or 15 other laboratories varied between 1.6 and 36% for atrazine and organophosphorus pesticides in finished drinking waters at levels ranging from 0.02 to 0.2 μg/l [73–76].

4.4. Potential for on-site monitoring

In the framework of the Rhine Basin programme, an automated LC monitoring system (SAMOS-LC for System for Automated Monitoring of Organic compounds in Surface waters) has been studied extensively. The procedure includes the loading of 100–150 ml of surface water onto PLRP-S precolumns of a Prospekt device at 5 ml/min. The on-line analysis was carried out using a C_{18} analytical column with an acetonitrile gradient at pH 3. The data were automatically evaluated, with the production of a report for compounds present at, or above, a certain concentration level between 1 and 3 μg/l [72]. The reproducibility of the retention times with a set of 25 to 30 pesticides was excellent with a RSD value of 0.2–1.5% ($n=20$). At an analyte concentration of 1 μg/l, the RSD of peak areas was in the range 1–15%, with a new precolumn in each run. The highest RSD were observed only for analytes eluting between 12 and 25 min and were explained partly by matrix interferences and partly by breakthrough of the more basic compounds on the PLRP-S cartridges [77]. The SAMOS system was made to act as an early warning system for use in the field. The robustness of the system was studied in two laboratories during five- and seven-month periods. No major problem was encountered for over 1000 analyses, apart from the exchange of a deuterium lamp and clogging of the preconcentration system with non-filtered waters. The system was recently optimized by a new filtration system, the attachment of refrigerated flasks and the use of two precolumns packed with different sorbents [78]. The clogging was reduced permitted an unattended operation for at least five days.

4.5. Potential for in-site sampling: analyte stability and storage on precolumns

The sample handling, transport and storage of samples can be greatly improved because of the small volume of the precolumns. Transport from the sampling site with storage at cool temperature such as −20°C is difficult for water samples in glass bottles. The in-the-field sampling and analyses in laboratory can also be advantageous for monitoring in remote areas. The use of a SPE cartridge and disk have been shown to be an alternative to storage of original samples or for analysis of samples collected in remote sites [79–85]. Several studies have examined the stability of analytes sorbed on disposable precolumns depending on the time storage, temperature, type of sorbent, precolumn, presence of water in the cartridge or not during the storage and influence of the pH and sample matrix. The stability of 19 organophosphorous pesticides was investigated on precolumns packed with C_{18} and although acceptable stability was observed for most of the compound tested, some poor recoveries were observed for unstable organophosphorus such as fenamiphos and fonophos [86]. One can expect a better storage using polymeric sorbents which do not have acidic properties such silanol groups at the C_{18} silica

surface. An improvement was observed using polymeric cartridges and fenamiphos was stable for one week at room temperature and fonophos for one month at 4°C and at room temperature [87]. Polymeric cartridges were also used for stabilization of polar phenolic compounds allowing their storage for two months at −20°C [88].

The stability of representatives of different groups of polar pesticides sorbed from water samples onto a polymeric sorbent was confirmed during a seven-week period at room temperature or at −4°C [89]. The differences between the stability when stored on wet or dry sorbent were negligible. The pH value of the water samples had a significant influence on stability of only those of compounds that had acido–basic properties.

5. Performances and limitations of common and emerging solid-phase extraction sorbents

5.1. Chemically bonded reversed-phase silicas

5.1.1. Characteristics of available C_{18} and C_8 silicas for solid-phase extraction

For many years most of the off-line SPE procedures for the handling of biological and environmental aqueous samples have been achieved using C_{18} silicas and to a less extent C_8 silica. First C_{18} silica cartridges were packed with the same stationary phases as those in LC columns except that the granulometry was larger. Their evolution followed those of LC with the objective of having more hydrophobic phases containing a minimum amount of residual silanol groups. Then, after demonstration of the potential of residual silanols for some additional interactions in the SPE process, several C_{18} silicas have been specifically designed for SPE.

A first requirement to SPE C_{18} silica is to provide high recoveries, not only for apolar analytes but also for polar ones. No special problem occur for the extraction of moderately and non-polar analytes. The approximate relation which correlates the retention factor in water and the hydrophobicity of analytes measured by the octanol–water partition coefficient indicates that extrapolated values of log k_w and log K_{ow} are very close [7]. Therefore, a simple reading of Fig. 2 indicates that 500 ml of aqueous samples can be applied onto a 500-mg cartridge with recoveries higher than 95% for every analyte with log K_{ow} above 3. The requirement of SPE silica providing high retention of analytes is for the extraction of polar analytes and when a large volume is required. It is well established that in LC, retention depends on the number of C_{18} chains bonded at the surface of the silica. In order to have a maximum amount of octadecyl chains at the silica surface, a first requirement is to modify bare silica which is very porous. Almost every SPE C_{18} silica are made from silica with high specific surface areas in the range of 500 to 600 m^2/g, which is not the case for LC stationary phases. Starting from such porous silica, every manufacturer of SPE products now provides several types of modified silicas. Some are available with a high percentage of carbon loading corresponding to a maximum surface coverage by the alkyl moieties. But their properties can differ depending whether mono- or trifunctional alkylsilanes have been used for the bonding and whether the bonding was followed by endcapping or not. There are also light loaded alkyl silicas with lower percentages of carbon which in general contain higher amounts of unmodified silanol groups. The characteristics of some common SPE octadecyl and octyl silicas are given in Table 2.

In LC, in order to obtain a better efficiency and a totally apolar material, the trends are to minimize the number of residual silanol groups of the original silica, and for this purpose, a trifunctionnal silane is used for bonding the *n*-alkyl chains and an endcapping is carried out with trimethylsilane after bonding [90–95]. The reason is that the chemical reaction with a trichlorooctadecylsilane involves several silanol groups per molecule of silane whereas reaction with monochlorosilane involves only one silanol group per molecule. However, the occurrence of residual silanol is unavoidable and extensive studies have pointed out that silica modified with trichlorosilanes and with a high degree of endcapping still contains some residual silanol groups [96–98]. Various chromatographic tests have been established to detect their occurrence and their effect [99–103]. New types of alkyl silicas have been also introduced by bonding an alkyl chain containing an embedded polar carbamate or amide functionality.

Table 2
Characteristics of some common commercial SPE C_{18} and C_8 silica sorbents (porosity, mean particle diameter, functionality of the silane used for bonding, end-capping and percent carbon content)

Sorbent	Manufacturer	Porosity (Å)	d_p (μm)	Silane function	End-capping	% C (average)
Bond-Elut C_{18}	Varian	60	40	Tri	Yes	18
Bond-Elut C_{18}/OH	Varian	60	40	n.a.[a]	No	13.5
Bond-Elut C_8	Varian	60	40	n.a.	Yes	12.5
Bakerbond C_{18}	J.T. Baker	60	40	Tri	Yes	17–18
Bakerbond C_{18}-Polar Plus	J.T. Baker	60	40	Tri	No	16–17
Bakerbond C_{18}-light	J.T. Baker	60	40	Tri	No	12–13
Bakerbond C_8	J.T. Baker	60	40	Tri	Yes	14
Isolute C_{18} (EC)	IST	55	70	Tri	Yes	18
Isolute C_{18}	IST	55	70	Tri	No	16
Isolute MF C_{18}	IST	55	70	Mono	No	16
Isolute C_8 (EC)	IST	55	70	Tri	Yes	12
Isolute C_8	IST	55	70	Tri	No	12
Sep-Pak C_{18} t	Waters	125	37–55	Tri	Yes	17
Sep-Pak C_{18}	Waters	125	37–55	Mono	Yes	12
Sep-Pak C_8	Waters	125	37–55	Mono	Yes	9
Chromabond C_{18} ec	Machery–Nagel	60	45/100	n.a.	Yes	14
Chromabond C_{18}	Machery–Nagel	60	45/100	n.a.	Yes	14
Chromabond C_8	Machery–Nagel	60	45	n.a.	No	8
DSC C_{18}	Supelco	70	50	Tri	Yes	18

[a] n.a. = Not available in data supplied by manufacturers.

The silanol groups are shielded from interaction with polar analytes [5,104–106].

A first difference between LC and SPE is in the contact with the modified silica. In LC, the mobile phase contains an organic solvent which is adsorbed to the stationary phase and ensures a good contact between the solute and the hydrophobic solid. This is the reason for recommending the addition of a small proportion of organic solvent (0.5%) in the sample before large volumes are applied. It was also observed that the contact between some polar analytes and a totally hydrophobic C_{18} silica during the SPE process was better when no endcapping is performed, or with a C_{18} silica which has been prepared using a monofunctional silane. As in LC, residual silanols can play a significant role in the extraction scheme, and modified silica using a monofunctional silane or with a lower amount of alkyl chains bonded at the surface have been designed for trapping polar analytes, since in addition to hydrophobic interactions, hydrogen bonding or ionic interactions can occur with polar analytes. The characteristics of various C_{18}SPE cartridges specifically ''designed'' for trapping polar analytes (often named C_{18}/OH or polar C_{18}) have been also reported in Table 2.

Highly energetic ionic interactions (see Table 1) can occur when silanol groups are ionized and when analytes are positively charged. The pK_a of a silanol group is not easy to determined because it depends on the experimental surrounding conditions. However, it is considered that at pH 2 it is uncharged and above 2 it becomes increasingly dissociated. The effect of the negative charge of the silanol groups can unambiguously be observed above pH 4. Many drugs contain amino groups or nitrogen and can be protonated depending on the pH. Even if the number of silanol groups is very low, these interactions are much stronger than hydrophobic ones and there are one of the most difficult to disrupt. This possibility for mixed-retention mechanism can be avoided by

experimental conditions where ionic interactions cannot occur. The pH can be selected so that either the silanol group or the analytes should be uncharged. The residual silanols can be masked by using a competing base such as triethylamine, ammonium acetate and other additives [33,107–109]. The mixed-retention mechanism can be also enhanced, but in most instances, mixed-mode sorbents which contains ion-exchange groups and alkyl chains are preferred.

Different problems are encountered in the analysis of biological matrices. First, the sample volume is usually low, typically 1 ml for plasma, and the risk is very unlikely to overload the breakthrough volume. Martin et al. have studied the effect of carbon loading on the extraction properties of C_{18} bonded silica used for the solid-phase extraction of acidic and basic analytes. A range of C_{18} sorbents containing from 5 to 22% of carbon, prepared with or without endcapping was evaluated for the extraction of two model acidic and basic analytes (anisidic acid and propanolol) from 1 ml of buffered plasma [110]. High carbon loadings (18 to 22%) were associated with poor extraction properties with losses during the sample application and wash. Recovery of extracted analytes was also inefficient, suggesting poor mass properties. The best SPE phases for the two analytes were those of intermediate carbon loading (15%). That is consistent with the characteristics of the C_{18} phases all of them having an average carbon loading between 16 and 17%. Significant effects of endcapping were only observed for the basic propanolol. No effect of the endcapping was observed for the acidic analytes.

5.1.2. Limitations of C_{18} silicas for trapping polar analytes from large sample volume

Using a C_{18} silica with a high surface coverage of alkyl chains and residual silanols has been shown to increase the retention of some polar analytes by the addition of secondary interactions, mainly hydrogen bonding type between silanol groups and polar groups. But in contrast to ionic interactions that can occur with ionized silanol groups, the binding energy of hydrogen bonding is comparable to hydrophobic interactions. So, one can expect a difference in behavior between phases having light carbon loading and those with high loading of carbons, but prepared using monofunctional silane or not end-capped. In the latter one hydrophobic interactions will be the highest possible and secondary interactions will be added whereas in the former one, hydrophobic interactions will be lower. This is illustrated by the two following examples. The recoveries obtained for a set of polar carbamates with an on-line system have been compared using precolumns of the same size but prepacked with two hydrophobic C_{18} sorbents (with high surface coverage, prepared using trifunctional silanes and end-capped) and one C_{18}/OH [5]. Some of the results are given in Table 3, showing that lower recoveries are obtained for every carbamate with the C_{18}/OH sorbent. Since the carbon content of C_{18}/OH is lower (13.5%) than that of the two C_{18} (18%), the recoveries are lower for the C_{18}/OH phase whereas they are comparable for the two C_{18} sorbents. These results are easily explained by the fact that the increase in secondary hydrogen bonding interactions is not able to overcome the decrease in hydrophobic interactions due to the lower amount of alkyl chains at the silica surface. Another study have compared recoveries obtained for polar priority phenols using an on-line system, and recoveries were found higher with the C_{18} silica from IST prepared using monofunctional silanes (C_{18} MF) that with that prepared using the trifunctional silane [111]. As examples, using a 100-ml sample and 10×2 mm I.D. precolumns, recoveries were 25 and 33% for 4-methylphenol and 4-nitrophenol with the standard C_{18} and 54 and 56%, respectively using C_{18} MF. Since the carbon content of the two

Table 3
Comparison of recoveries obtained for polar carbamates using different extraction sorbents in the precolumn (two standard C_{18} silicas, one specifically designed for polar compounds and a PS–DVB copolymer)[a]

Compound	Recovery (%)			
	a	b	c	d
Aldicarb sulfone	9	18	16	29
Oxamyl	12	25	22	49
Methomyl	9	19	16	39
Aldicarb	50	102	97	89
Carbofuran	102	98	106	94

[a] Sample volume 25 ml; precolumn size: 10×2 mm I.D., (a) C_{18}/OH from Varian, (b) standard C_{18} from J.T. Baker, (c) standard C_{18} from Varian, (d) PLRP-S PS–DVB from Polymer Labs.

sorbents are similar, the increase in recoveries is due to the additional hydrogen bonding interactions. Another study reported slightly higher recoveries for polar metabolites of atrazine using C_{18} polar plus [112]. However, one must have in mind that such an increase is small in comparison to the increase that can be obtained when using a PS–DVB copolymer. In the same study, using same size of precolumns, the breakthrough volumes of trichlorophenols were 50 ml using C_{18} silica, 70 ml using C_{18} MF and above 200 ml using the PS–DVB polymer PLRP-S.

Although C_{18} SPE sorbents with both a high carbon loading plus some residual silanols are best suited, they are not amenable to extract polar compounds from large sample volumes. Since there is no real definition of what is a polar analytes and a large volume, there is a general consensus among the manufacturers to provide data for phenol ($\log K_{ow} = 1.5$) and deisopropylatrazine (DIA), a polar metabolite of atrazine ($\log K_{ow} = 1.1$). Using a conventional SPE cartridge with 500 mg of packing, polar analytes with octanol–water partition coefficient $\log K_{ow}$ lower than 1.5 will be poorly extracted unless the sample volume is low. For example, the recovery of an analyte with a $\log k_w$ of 1.7 was predicted (and measured experimentally) at 70% with a sample volume of 30 ml using an extraction disk containing 450 mg of C_{18} sorbent. In contrast to phenol, DIA shows a quadratic relationship between its retention factor in log units and the methanol % of the mobile phase, so that its $\log k_w$ value was measured at 2.3, much higher than its $\log K_{ow}$ value. Its extraction recovery using a C_{18} disk from a 500-ml sample was measured to 21% whereas in the same condition that of phenol is lower than 5%.

In on-line techniques, breakthrough volumes are low due to the small size of the precolumns. On conventional 10 mm×2 mm I.D. precolumns, the breakthrough volumes of phenol and benzene are around 1 ml and 4 ml, respectively [7]. Therefore C_{18} silica is rarely used for multiresidue environmental analyses including polar analytes.

5.1.3. Other modified silicas

Among modified silicas, the selection of C_{18} silicas provides the greatest retention, but this is accompanied by the highest trapping of interferences. Therefore, if analytes can still be retained by silicas providing lower retention, less amount of interferences will be trapped. For highly hydrophobic analytes, ethylsilica (C_2) are available with carbon content in the range 3 to 6%, most of them being end-capped.

Other reversed-phase chemically bonded silica include cyclohexyl and phenyl phases. With the latter some increase in the retention of aromatic analytes has been observed [111]. Selective electron-donor- and electron-acceptor-bonded silica packings materials have been prepared for hydrophobic contaminants such as polychlorodibenzo-*p*-dioxins or planar and nonplanar polychlorobiphenyl congeners [113].

Cyanopropylsilica and to a less extent aminopropylsilicas are polar phases that exhibits both polar and non-polar interactions. They are available in endcapped and non-endcapped forms. Typical carbon loadings of cyanopropylsilicas are between 8 to 9%, so that hydrophobic interactions are not negligible. therefore, they can be used as reversed-phase sorbents. Di Corcia and Marchetti used such a column as a confirmation column for pesticide analysis [114]. Using a C_{18} silica column they could achieved the separation of 71 base–neutral pesticides over a wide range of polarity. The same mixture analyzed with a cyanopropyl modified silica column also shows a good separation, with the approximate same retention order although some characteristic inversion of retention order can be observed due to polar interactions.

Aminopropyl silica can also be used with aqueous matrices, especially in the biological field where the sample volume is low. For example, it was shown to be a good alternative for the continuous clean-up and preconcentration of vitamin D3 metabolites in plasma. Comparison with the use of a C_{18} sorbent indicated better detection and quantification limits [115].

5.1.4. On-line applications

Many on-line applications using C_{18} sorbents deals with biological samples, since the limitations for polar analytes hardly occurs with low volumes of samples. Some relevant examples showing that C_{18} is still the predominant form of SPE can be found in a recent review dealing with theory and methodology of antibiotic extraction from biomatrices [116]. Automated methods often use C_{18} cartridges, the solid-

phase extraction sequence being performed using an ASPEC XL device and in a second step, in order to avoid time-consuming evaporation and reconstitution steps, the solid-phase extraction is coupled on-line to a trace-enrichment system for further purification and concentration of the sample extract. Such an approach can be totally automated using pipetting robots for transfer between the two steps, as described for the determination of the glucocorticosteroid drug budesonide in plasma samples at the p*M* levels [117].

The strategy of the pharmaceutical industry is to reduce the time needed for research and development of a new drug, and this has induced high-throughput techniques such as combinatorial chemistry. So more compounds become available for further investigation which have led analytical laboratories to develop high throughput technology. LC with tandem MS is now well implemented in routine bioanalysis, allowing more rapid separations because the resolution has not to be optimized, and therefore, the need for more rapid sample preparation methods is becoming a priority. The automated high-throughput SPE system using microtiter solid-phase technology and a pipetting robot was implemented and validated using as model the determination of cimetidine in plasma [118]. The sample clean-up was performed using Microlute extraction plates in the 96-well format, each well packed with 50 mg of C_{18} silica. Separation was performed by LC with UV detection. Validation results on linearity, specificity, precision, accuracy and stability were found to be adequate. Good correlation was obtained between results from manual and automated methods. The average sample preparation time for a technician decreased from approximately 4 min per sample to 0.6 min.

Using on-line SPE–LC systems, a higher degree of automation with a lower amount of sample and low detection limits is usually obtained. A simple on-line laboratory set-up was automated for the simultaneous determination of aspirin and salicylic acid from plasma samples which required a 10-port switching valve [119]. The SPE column was a biocompatible polyether ether ketone (PEEK) precolumn (10×4.3 mm) prepacked with 30 μm Hypersil C_{18} silica. The sample was obtained by diluting an aliquot of fresh chilled plasma sample within 10 min of centrifugation with the same volume of a 0.2 *M* phosphoric acid solution. Then a 200-μl sample volume was loaded via a loop onto the extraction column. By turning the valve, the sample is injected onto the extraction column and during that time endogeneous components were removed to waste by the wash solution. The precolumn could be used 50 times without clogging because the on-line clean-up was possible after the sample application by a washing with acidified water before backflush elution. A typical chromatogram is represented in Fig. 4. Chromatograms correspond to the analysis of the blood of a healthy volunteer 45 min and 5 h after he was given 600 mg of aspirin. The method was shown to be simple, more rapid and robust that current methodology, and requiring only 300 μl of plasma and little handling by the analyst. Thanks to the loop injection, the method was reproducible and did not require the use an internal standard.

The on-line SPE and LC analysis of a novel potent retinoid and retinol in plasma and tissue was described which coupled an autosampler to the automatic SPE unit OSP-2 on-line with LC–UV and fluorescence detection [120]. Other examples can be found in the application books from the two manufacturers of on-line devices OSP-2 (Merck) and Prospekt (Spark Holland).

Since the sample volumes are low, the sorbent in the precolumn can have low carbon content depending on the polarity of the analytes. Using the Prospekt, cartridges packed with C_2, CN and C_8 have been compared when coupled to a C_8 analytical column for the determination of fluvastatin and its enantiomers in blood plasma. The C_2 precolumn was shown to provide the best preconcentration and clean-up procedure in the same step [121].

Some relevant applications have been also described in the environmental field for the on-line monitoring of moderately polar analyte. The methodology was validated from inter-laboratory studies for the determination of organophosphorus pesticides [74]. For more polar analytes, it is necessary to lower the sample volume in order to limit their breakthrough volumes and to use more sensitive detection modes such as mass spectrometry or fluorescence. In the SAMOS approach developed by Brinkman and co-workers, an early warning system was built combining an on-line trace-enrichment on a 10×3 mm precolumn prepacked with C_{18} silica coupled

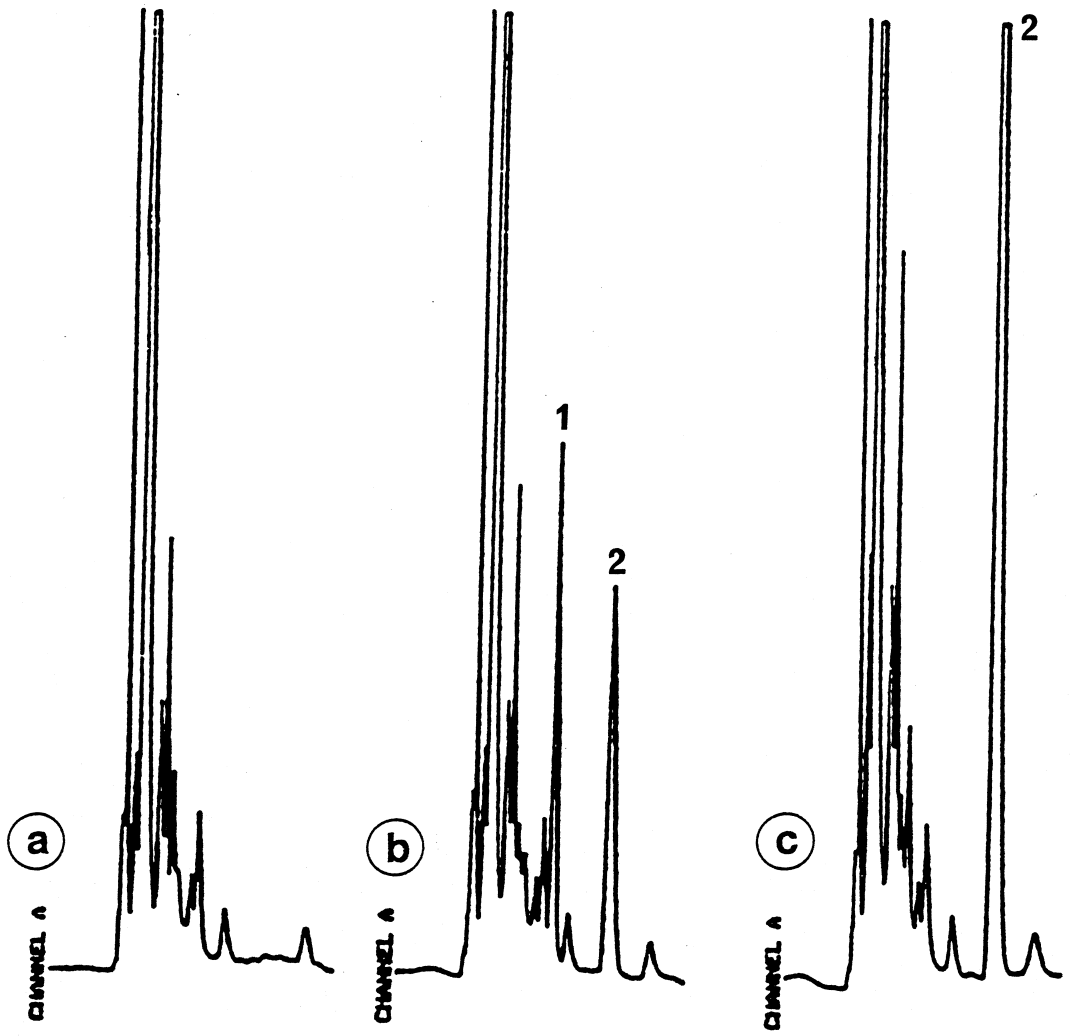

Fig. 4. Chromatograms of plasma from a volunteer before and after administration of 600 mg of aspirin (a) plasma immediately prior to administration, (b) plasma sample taken 13 min after administration, concentration of aspirin = 2.55 μg/ml and concentration of salicylic acid = 3.43 μg/l (peak 2) and (c) plasma sample taken 5 h after administration, concentration of salicylic acid = 13.98 μg/l. (Retention times of peaks 1 and 2 are 11.5±0.2 and 15.6±0.3 min.) From Ref. [119].

with a reversed-phase column liquid chromatography–thermospray mass spectrometry (LC–TSP-MS) [122]. When optimized MS conditions and 50-ml water samples were used, the detection limits of the pollutants tested were in the 2–90 ng/l range with time schedule selected-ion monitoring. Fig. 5 shows the full scan chromatogram corresponding to the on-line trace-enrichment for 50 ml of River Rhine water spiked with a mixture of 21 pesticides at 1 μg/l.

Fifteen moderately polar pesticides could be monitored in drinking water below the 0.1 μg/l level using on-line SPE with a C_{18} precolumn followed by LC–MS with atmospheric pressure chemical ionization and particle beam mass spectrometry [123].

On-column trace enrichment and LC using a single short (20 mm length) column was optimized for the rapid simultaneous identification and quantification of a wide range of organic microcontaminants in environmental water samples [124]. Several C_{18}

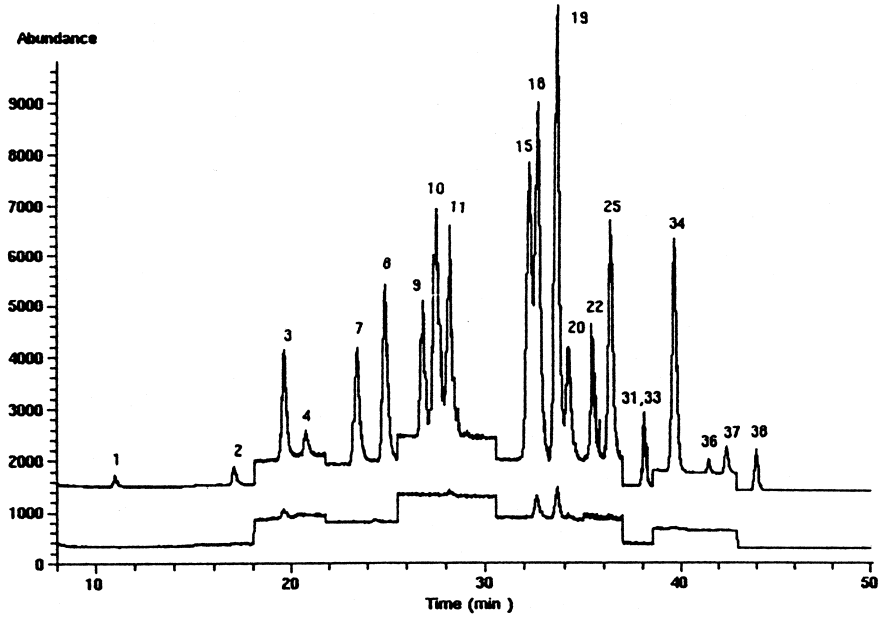

Fig. 5. On-line trace-enrichment reversed-phase LC–TSP-MS (PI mode) trace for 50 ml of (bottom) River Rhine water and (top) River Rhine water spiked with a mixture of 21 pesticides at the 1 μg/l level. Column 250×4.6 mm I.D. containing 5 μm C_{18}-bonded silica; precolumn 10×3 mm I.D. packed with C_{18} silica. Linear methanol–0.1 *M* ammonium acetate gradient. Peaks: 1=aldicarb sulfone; 2=1-(3-chloro-4-hydroxyphenyl)-3,3-dimethylurea; 3=dimethoate; 4=desmethylmetoxuron; 7=monomethylmetoxuron; 8,=metoxuron; 9=cyanazine; 10=monuron; 11=simazine; 15=atraton; 18=atrazine; 19=isoproturon; 20=diuron; 22=azinphos-methyl; 25=propazine; 31=chlorobromuron; 32=terbutryne; 34=prometryn; 36=parathion-ethyl; 37=diazinon; 38=disulfoton. From Ref. [122].

bonded silica packings materials provided good results in terms of analyte recoveries, peak capacity, linearity of response and precision. With 15-ml samples, detection limits of 0.1–0.4 μg/l and 0.5–1.1 μg/l were achieved for tap and surface water, respectively. The applicability of the system was demonstrated by the screening of real environmental samples and provisional identification of unknown using LC combined with tandem MS. The same column could be re-used more than 40 to 50 times [125,126]. A similar system was combined with tandem mass spectrometry for the rapid study of pesticide degradation [127].

Special attention should be given to very hydrophobic analytes such as polycyclic aromatic hydrocarbons (PAHs) and organochlorine or pyrethroid pesticides for example. Their trace level determination in water is rather difficult as their concentration in water is extremely low due to their low solubility. They tend to adsorb everywhere, on tubes, walls and containers, so that losses often occur during sampling and storage, even when plastic and glass are avoided. In order to avoid these sorption problems, methods which can increase their solubility are required, which is achieved by adding organic solvents to the sample or surfactants. The chromatogram in Fig. 6 corresponds to the LC–fluorescence chromatogram after the on-line trace enrichment of 10 ml of surface water spiked with 10 ng/l of each of the 16 EPA-priority PAHs and containing $3\cdot10^{-4}$ *M* Brij-35 using a 10×3 mm C_{18} precolumn [128]. The addition of the Brij solution was shown to better solubilize the more hydrophobic PAHs that the addition of 25% of methanol. Since it is well known that the major part of the PAHs present in surface water, it was also found that sample containing suspended solid could be analyzed because the micelles could be used to resolubilize PAHs adsorbed on suspended solids. A similar micelle-mediated sample preparation was applied for

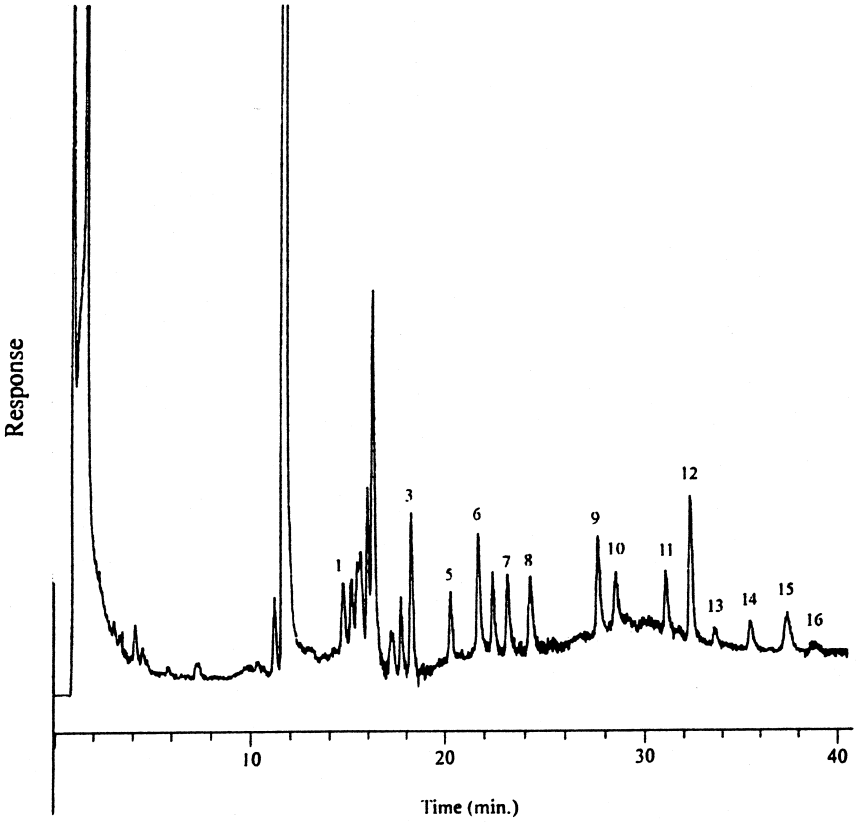

Fig. 6. On-line trace enrichment–LC–fluorescence chromatogram of 10-ml surface water sample containing 20 mg/l suspended solids, spiked at the 10 ng/l level with the 16 priority PAHs and containing $3 \cdot 10^{-4}$ *M* Brij-35, using a C_{18} silica precolumn. From Ref. [128].

the automated on-line determination of pyrethroid insecticides in surface water followed by LC with UV diode array detection (DAD) [129].

5.2. Apolar poly(styrene–divinylbenzene) copolymer sorbents

The capability of PS–DVB resins, such as Amberlite XAD-type, for trapping more polar compounds than C_{18} silicas was known at the early stage of SPE development. However, they were not available in prepacked cartridges because they required a laborious purification step before use. The first commercially available PS–DVB polymers for SPE were the LC-grade PRP-1 and PLRP-S in disposable precolumns designed for on-line preconcentration techniques. Then PS–DVB sorbents have been available in extraction disks for off-line applications. One advantage over C_{18} silicas is their stability over the pH range 1–14. This was clearly demonstrated when comparing blanks obtained after percolating high volumes through C_{18} and PS–DVB disks at acidic pH [130].

5.2.1. Polymeric sorbents with high specific areas

Resins with high specific surface areas in the range 700 to 1200 m^2/g, are now available in disposable cartridges. The characteristic of commercially available copolymers for LC and SPE are reported in Table 4. The manufacturers provide recoveries of phenol and deisopropylatrazine for comparison with C_{18} silicas, these recoveries being 100% for sample volume of 1 l and using only 200 mg of sorbent, thus show a much higher retention

Table 4
Characteristics of commercially available apolar copolymers used as LC and SPE sorbents

Sorbent	Manufacturer	Structure	Porosity (Å)	Average d_p (μm)	Surface area (m^2/g)
Bond-Elut ENV	Varian	PS–DVB	450	125	500
Bond-Elut PPL	Varian	Funct. PS–DVB	300	125	700
Abselut	Varian	(Dual funct)	100	65–80	500–650
SDB	J.T. Baker	PS–DVB–EVB[b]	300	40–120	1060
Speedisk-DVB	J.T. Baker	PS–DVB	150	n.a.[a]	700
Empore disk	J.T. Baker	PS–DVB	n.a.	6.8	350
LiChrolut EN	Merck	PS–DVB	80	40–120	1200
Isolute ENV+	IST	PS–DVB	100	90	1000
Envichrom P	Supelco	PS–DVB	140	80–160	900
Chromabond HR-P	Machery–Nagel	PS–DVB	n.a.	50–100	1200
Porapak RDX	Waters	PS–DVB–NVP[b]	55	120	550
OASIS HLB	Waters	PS–DVB–NVP	55	30 and 60	800
PRP-1	Hamilton	PS–DVB	75	5 and 10	415
PLRPS	Polymer Labs.	PS–DVB	100	15 and 60	550
Hysphere-1	Spark Holland	PS–DVB	n.a.	5–20	>1000

[a] n.a. = Not available in data supplied by manufacturers.
[b] EVB: ethylvinylbenzene, NVP: *N*-vinylpyrrolidone.

than C_{18} silica. However, these polymers are not available in analytical columns, because they do not possess all the properties required for a LC stationary phase. Therefore very few chromatographic data have been reported up to now, and those reported have been obtained by laboratory-packing analytical columns with polymer packings coming from SPE cartridges [5].

There are more numerous studies providing LC data for LC-grade PS–DVB, which are PRP-1 and PLRP-S, characterized by specific surface area lower than or around 500 m^2/g. Retention behavior of analytes on PRP-1 or PLRP-S sorbent has been studied and compared to retention obtained with C_{18} silicas. First, it was shown that log k_w could be also extrapolated from the relation log k_w–methanol content [10,50]. For a set of many organic compounds, the results indicated that solutes are about 10- to 40-times more retained by PRP-1 than by C_{18} silicas. The highest difference was for benzene derivatives substituted by nitro groups having a strong electron-withdrawing effect and the smallest for hydroxy group showing an electron-donating effect. The difference in retention was also dependant on the hydrophobicity. The more apolar the analytes are, the larger the difference in retention. Another approach has been described using the solvation parameters models [48]. Comparison with C_{18} silica indicated that significantly larger breakthrough volumes were obtained on the porous sorbent for polar analytes. The fit with the solvation parameters models for the retention indicated that the porous polymer sorbent competes more effectively with water in dipole-type interaction as hydrogen-bond acid than C_{18}. The influence of the solvent effect was closely investigated [65]. The authors concluded that retention resulted from a combination of adsorption and partitioning and was influenced by the equilibrium adsorption of organic solvent by the polymer from the mobile phase. The hydrophobic mechanism was confirmed and all polar interactions such as dipole-type and hydrogen bond formation were shown to be more favorable in the mobile phase and to reduce retention.

The effect of the specific surface area is important as shown in Table 5. In order to estimate log k_w values in water-rich mobile phases, 5-cm and a 3-cm long columns were, respectively, laboratory-packed with one of those high-specific-area PS–DVB polymer and with a stacking of PS–DVB polymer disks [51]. Data on C_{18} silica has also been reported for comparison. The retention factors are similar for PRP-1 and SDB disks, but the specific surface area are not very different (415 and 350 m^2/g respective-

Table 5
Comparison of log k_w values obtained with C_{18} silicas, various PS–DVB copolymers with different specific surface areas, and porous graphitic carbons (from Ref. [5])

Compound	Log K_{ow}	Log k_w[a]				
		C_{18}	PS–DVB (415)	PS–DVB (350)	PS–DVB (1060)	PGC
Cyanuric acid[b]	−0.2	<0.5	<0.5	nd	<0.5	2.6±0.1
Ammeline[b]	−1.2	<0.5	<0.5	nd	<0.5	2.4±0.2
Ammelide[b]	−0.7	<0.5	<0.5	nd	<0.5	2.5±0.2
Hydroxy-DIA	−0.1	1.0±0.1	1.0±0.1	nd	1.8±0.1	3.0±0.2
Hydroxy-DEA	0.2	1.5±0.1	1.8±0.1	nd	2.3±0.2	2.8±0.2
DEDIA	0	1.3±0.1	1.2±0.1	nd	nd	2.8±0.1
Deisopropylatrazine (DIA)	1.2	2.3±0.1	3.1±0.1	3.2±0.2	4.4±0.3	>3.5
Deethylatrazine (DEA)	1.4	2.7±0.1	3.5±0.3	3.5±0.2	4.8±0.3	3.2±0.2
Simazine	2.3	3.4±0.1	>4	4.1±0.2	5.9±0.3	>4
2-Chlorophenol	2.4	2.9±0.1	>4	3.6±0.2		>4
Oxamyl		1.7±0.1	nd	2.8±0.2	4.1±0.3	nd
Aldicarb	1.4	2.5±0.1	nd	4±0.2	5.3±0.3	nd
Carbendazim	1.5		nd	nd	5.7±0.3	>4
Chloridazon		2.3±0.1	nd	3.8±0.2		>4

[a] Log k_w values extrapolated from the relationship log k–percentage of methanol.
[b] Cyanuric acid: 2,4,6-trihydroxy-1,3,5-triazine, ammeline: 2,4-diamino-6-hydroxy-1,3,5-triazine; ammelide: 2-amino-4,6-dihydroxy-1,3,5-triazine.

ly) and are higher that those observed with C_{18} silica. With highly cross-linked PS–DVB, there is a large increase in retention, since the difference is between 1.3 and 1.8 log units, indicating that this polymer has 20–60-fold more retention power towards polar analytes than polymers with lower specific areas have. Comparison with C_{18} silica is higher than 100-fold. Similar values of k_w for deisopropylatrazine and deethylatrazine have been extrapolated using SDB, EnviChrom P and Isolute ENV+. These highly cross-linked PS–DVB sorbents are the sorbents to be selected for the extraction of very polar analytes when large sample volumes are required and many examples can be found in the recent literature [1,22,53,111,131–140]. Table 6 shows examples of high recoveries obtained from 1-l samples. The retention order is similar to that obtained with C_{18} silica and the higher the hydrophobicity of the molecule is, the higher retention. But, there is a limit in polarity for extraction of compounds [141]. In Table 4, one can see that log k_w values are lower than 2 for the highly polar degradation products of atrazine. Using a 200-mg PS–DVB cartridge, the recovery for deethyldeisopropylatrazine (2,4-diamino-6-chloro-1,3,5-triazine) was measured to 20±8% with a 500 ml sample volume.

5.2.2. Effect on the functionalization on retention

Chemically modified polymeric resins have been also introduced in recent years. First, modified resins were PS–DVB impregnated in membranes and higher recoveries were obtained for phenolic compounds when using acetyl-modified PS–DVB [142]. The sulfonation of PS–DVB resins was also shown to

Table 6
Recoveries (%) of extraction obtained for polar pesticides in water samples spiked at 0.1 μg/l on a 47-mm C_{18} disk (450 mg of sorbent, J.T. Baker, sample 500 ml), a 47-mm PS–DVB disk (PS–DVB, J.T. Baker, 450 mg of sorbent, sample 1 l) and on a 200-mg PS–DVB cartridge (J.T. Baker, sample 1 l) (from Ref. [51])

Solute	Log K_{ow}	C_{18} disk	PS–DVB (350)	PS–DVB (1060)
Oxamyl	na	<3	27	82
Deisopropylatrazine	1.1	21	53	92
Deethylatrazine	1.5	58	93	100
Carbendazim	1.56	62	84	88
Aldicarb	1.1–1.5	69	72	90
Simazine	1.96	95	90	94

provide a better contact with aqueous samples and to increase solute retention [143,144].

The high capacity resins have been also chemically modified by various chemical groups [145–148]. Table 7 reports extraction recoveries for polar analytes using precolumns of the same dimension (10× 3 mm I.D.) and packed with various modified or non-modified PS–DVB. The first three columns list non-modified PS–DVB and one can see the effect of an increase in the specific area on recoveries for the polar phenolics. Bond-Elut PPL is a functionalized PS–DVB and although the specific surface area is moderately high, recovery for phenol is acceptable. The effect of the introduction of a hydrophilic – either acetyl or α-carboxybenzoyl – moiety in the PS–DVB resins is an increase in recoveries for phenols. This is the result of an increase in the surface polarity which allows better contact with the resins and the aqueous samples.

5.2.3. Possibility of introducing sample clean-up during the sample percolation using highly cross-linked poly(styrene–divinylbenzene) sorbents

When moderately polar and hydrophobic analytes are to be determined, there can be an interest in adding a small percentage of organic solvent in the sample before percolation. A first effect is to avoid the adsorption of the more hydrophobic analytes on the containers and tubes. But the main advantage is that all the polar interferences are not extracted, because the addition of 10% of methanol causes a large decrease in k_s values. The breakthrough volume will not be estimated from log k_w values but from log k values obtained with that percentage of organic solvent. Selecting a polymer other than C_{18} silica can be advantageous when moderately polar are to be determined. Since the k_s values are much higher, moderately polar analytes will still be correctly recovered [22]. But one must be aware that the more polar analytes will be lost with the polar interferences.

The pH adjustment of the sample is an another factor that can be modified. Polar acidic analytes can be analyzed in contaminated surface-water samples, only provided that humic and fulvic acids are removed. When a C_{18} cartridge is used for the extraction and concentration of acidic herbicides, the sample has to be acidified at pH 2 or 3 and the co-extraction of humic and fulvic acids does not allow detection limits below the 0.5 μg/l level in surface water although the same procedure gave detection limits in the low −0.1 μg/l level in drinking-water samples. A clean-up procedure using a Florisil cartridge can be performed but with sometimes practical problems such as the dissolution the dry extract in pure hexane or isooctane. It is often necessary to add a small amount of moderately polar solvent, but then there is a risk of loss of the non-polar analytes which are eluted first in adsorption chromatography [149].

Recent work has shown that ionic organic compounds are well retained by highly cross-linked PS–DVB owing to interactions between the PS–DVB matrix and the organic part of the compounds [51]. This is of great interest for the analysis of acidic pesticides (ionisation constants in the range 3–6) which can be extracted under their ionic form from surface waters at pH 7–8 with good recoveries using

Table 7
Recovery obtained using different sorbents and preconcentrating 100 ml (values gathered from Refs. [145–147], see Table 5 for the characteristics of the sorbents)

Compound	PLRP-S (550)	LiChrolut EN (1200)	Hyspere-1 (1000)	Bond-Elut PPL (700)	Amberchrom[a]	Amberchrom[a] (a)	Amberchrom[a] (b)
Catechol	<0.1	17	nd	nd	3.5	4	nd
Orcinol	<0.1	60	nd	nd	11	30	nd
Phenol	5	60	73	43	18	50	41
4-NP	41	81	83	86	83	73	81
2,4-DNP	75	78	86	87	84	76	81
Oxamyl	nd	nd	82	82	nd	nd	74
Methomyl	nd	nd	77	77	nd	nd	71

[a] Amberchrom from Tosohaas, (Montgomeryville, PA, USA) particle size, 50–100 μm, pore size 110–175 Å modified by (a) acetyl groups and (b) α-carboxybenzoyl moiety.

500-ml samples. Then, most of the polar compounds cannot be determined due to a large matrix peak obtained at the beginning of the chromatogram when surface water samples are analyzed. This matrix peak is due to fulvic and humic acids which are co-extracted and co-eluted. Using PS–DVB, humic and fulvic acids are not recovered from the polymer as shown in Fig. 7. Therefore, polar analytes can be determined at trace level samples can be analyzed at pH 7 because there is a clear baseline at the beginning of the chromatogram. A consequence of the high retention of acidic pesticides in their ionic form, together with the absence of retention of humic and fulvic interferences, was the remarkable possibility of determining acidic and neutral pesticides in contaminated surface water samples without any clean-up, at the low 0.1 μg/l [51].

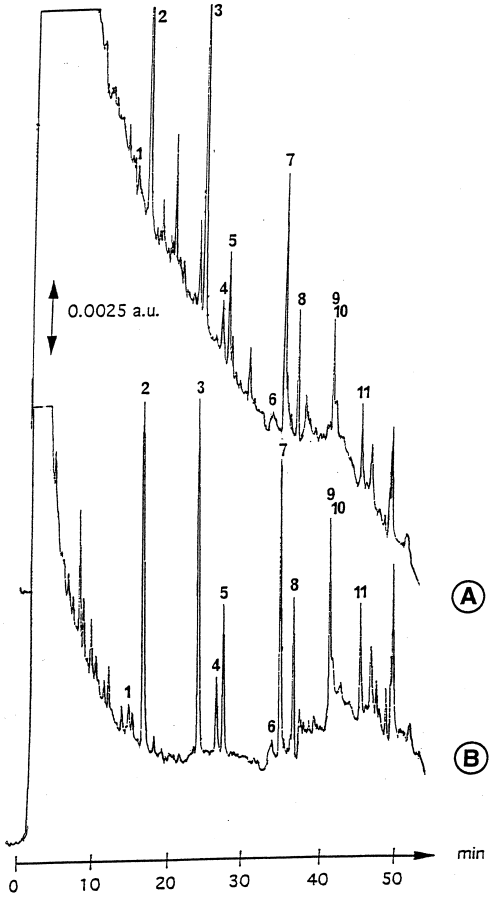

Fig. 7. Effect of the sample pH on the co-extraction of humic and fulvic acids. Preconcentration of 250 ml of drinking water spiked with 0.5 μg/l of each analyte (A) sample adjusted to pH 3 and (B) sample adjusted to pH 6. From Ref. [22]. (Preconcentration using Speedisk DVB, analytical column: Bakerbond Narrow Pore C_{18} silica, 25 cm×4.6 mm I.D.; acetonitrile gradient with $5 \cdot 10^{-3}$ *M* phosphate buffer at pH 3. Peaks: 1=aldicarb; 2=simazine; 3=atrazine; 4=isoproturon; 5=diuron; 6=ioxynil; 7=terbutylazine; 8=linuron; 9=flusilazole; 10=alachlor; 11=dinoterb. UV detection at 220 nm.)

5.2.4. New hydrophilc–lipophilic polymers: towards a "unique" method for the handling of biological samples

One limitation of both reversed-phase silica and polymer sorbents is that they must be conditioned with a wetting solvent and remain wetted before sample application. Two new patented sorbents have been recently introduced in the market. They are both presented as the "universal" extraction sorbent since they are capable to extract acidic, basic and neutral compounds whether polar or non-polar. One is the so-called hydrophilic–liphophilic balanced sorbent (Oasis HLB from Waters) and is a copolymer of divinylbenzene and *N*-vinylpyrrolidone. Retention of some analytes measured on a column packed with this polymer was compared with that obtained on C_{18} silicas, showing a large increase of k_w values [148]. For instance the log k_w value for catechol is around 2.5 whereas it is 1.1 on C_{18} silica. It was measured to 1.6 using PRP-1. The increase in retention is explained by both the specific area of 800 m^2/g and the occurrence of the pyrrolidone group in the polymer which is an hydrogen acceptor. More work should be done to look at the limitation in the extraction of polar analytes as it was done for other highly cross-linked PS–DVB (see Table 5), but environmental application with high sample volumes (>100 ml) have been presented by the manufacturer.

The structure of second new patented sorbent (Abselut from Varian) is not yet known, but its surface area is in the range 500 to 650, so that one can expect a similar behavior. It is designed for NC-SPE and for simultaneous extraction of acidic and basic analytes.

Extraction of drugs from plasma has been reported using an Oasis cartridge [150]. High and reproducible recoveries were obtained even though the cartridge were run dry for the determination of tetracyclic antibiotics in spiked serum. One ml of spiked

serum was acidified to a final concentration of 2% phosphoric acid, then vortex-mixed for 10 s before being loaded onto the cartridge, and then washed with 1 ml of 5% methanol. This procedure was able to remove all the matrix analytes as shown in Fig. 8 since the chromatogram corresponding to the spiked serum and the standard solution are very similar. Such works were at the origin of the ''universal procedure'' which consists of applying 1-ml of sample, washing with 1 ml of 5% methanol before eluting with 1 ml of methanol. Since the sorbent combines a high specific surface area with some polar hydrogen groups, it is easy to explain why there in no breakthrough of analytes with 1 ml of samples whatever the organic analyte, being polar, neutral or ionized, even when 5% methanol is added for the washing step. The Oasis sorbent is available in the 96-well plate format and therefore the method which has been developed on the cartridge with 1 ml of liquid at each step (conditioning, sample loading, washing and elution) is directly transferable to the plate format.

5.2.5. On-line applications

PLRP-S and PRP-1 prepacked in precolumns have been available for many years and coupled on-line with conventional analytical C_{18} column in numerous multiresidue applications. References can be found in reviews and book chapters [16,17] and some relevant applications have been recently reported [151–154]. A selective analysis of the herbicide glyphosate and its degradation product aminomethylphosphonic acid in water was described by on-line SPE–LC–electrospray ionization mass spectrometry [155]. Derivatization of the products were achieved directly in the native aqueous sample before the SPE and the whole method was fully automated, allowing detection in the concentration range of 0.05 to 3 μl in various types of water.

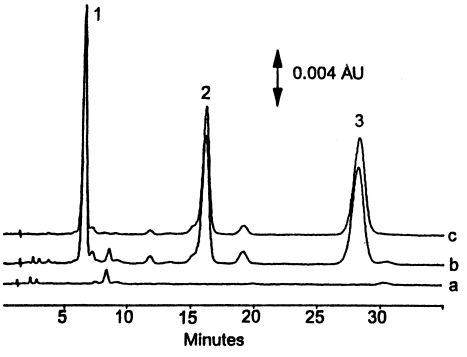

Fig. 8. Representative chromatograms for (curve a) the serum blank, (curve b) the serum matrix spiked with 2.5 μg/l of minocycline (peak 1), 2.5 μg/l of tetracycline (peak 2) and 5 μg/l of demeclocycline (peak 3) as the internal standard and (curve c) standard solution. Mobile phase: 0.1 TFA, 2% acetonitrile, 7 *M* methanol and water. UV detection at 350 nm. Extraction from 1 ml of acidified serum by an Oasis cartridge and washing with 1 ml of 5% methanol. From Ref. [150].

Usually, no visible or just a slight band broadening is observed when using an appropriate gradient for the transfer, because the difference in retention of analytes between these two PS–DVB sorbents with low surface area and C_{18} is not too large. A typical multiresidue on-line analysis of pesticides in drinking water is shown in Fig. 9 using a commercial 10×2 mm precolumn prepacked with PLRP-S and the Prospekt system coupled to LC and UV diode array detection. From a sample volume of 150 ml, although breakthrough has occurred, there is no problem for identifying deethylatrazine at a concentration of 0.09±0.01 μg/l [16]. Detection limits are lower than 0.1 μg/l for all the analytes, so that drinking water monitoring is possible with such an on-line system. The same analysis performed with 150 ml of contaminated surface water show a larger (around five-times higher) hump corresponding to the interfering humic or fulvic acids. For many analytes the detection limits is in the range 0.05 to 0.3 μg/l using UV diode array detection.

Table 7 shows the large increase obtained in analyte retention when using PS–DVB polymers with higher specific areas. Some studies have compared the breakthrough volumes of polar analytes using 10×2 mm I.D. cartridges as those used in the Prospekt automatic device. For deisopropylatrazine, these values are 4 ml when precolumns are prepacked with C_{18} silica, 25 ml with PLRP-S and above 300 ml with Hysphere-1, Isolut ENV+ and LiChrolut EN [17,87]. Therefore there is a real interest in using these highly cross-linked polymers in on-line devices for very polar analytes. But then the composition of the mobile phase and the gradient used for the separation and desorption are very important. This is illustrated in Figs. 10 and 11 involving the separation of polar pesticides

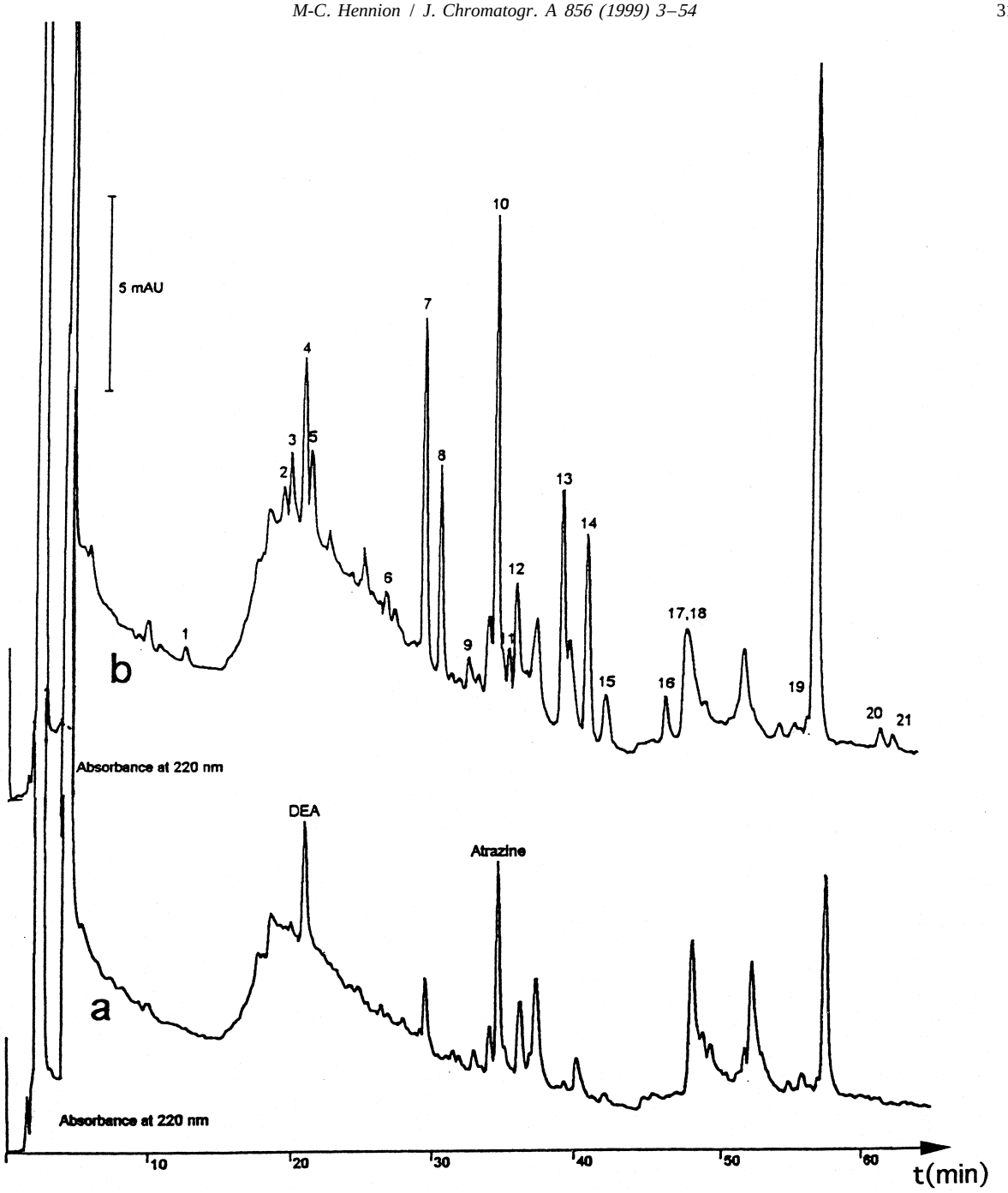

Fig. 9. On-line analysis of (a) 150 ml of a drinking water sample and (b) 150 ml of the same sample spiked with 0.1 μg/l of a mixture of 21 pesticides using the Prospekt system. Precolumn: 1 cm×0.2 cm I.D. packed with PLRP-S from Polymer Labs.; C_{18} analytical column ODS-80TM, 25 cm×0.46 cm I.D., water–acetonitrile (ACN) gradient: 15% ACN from 0 to 10 min, 50% at 33 min, 50% at 40 min, 48% at 48 min, 70% at 70 min; UV DAD, chromatogram at 220 nm. Peaks: 1=deisopropylatrazine, 2=metamitron, 3=hydroxy-atrazine, 4=deethylatrazine, 5=chloridazon, 6=aldicarb, 7=simazine, 8=deethylterbutylazine, 9=carbofuran, 10=atrazine, 11=isoproturon, 12=diuron, 13=propazine, 14=terbutylazine, 15=linuron, 16=terbuconazole, 17=alachlor, 18=metolachlor, 19=fexoxaprop-*p*-ethyl, 20=pendimethaline, 21=trifluraline. From Ref. [16].

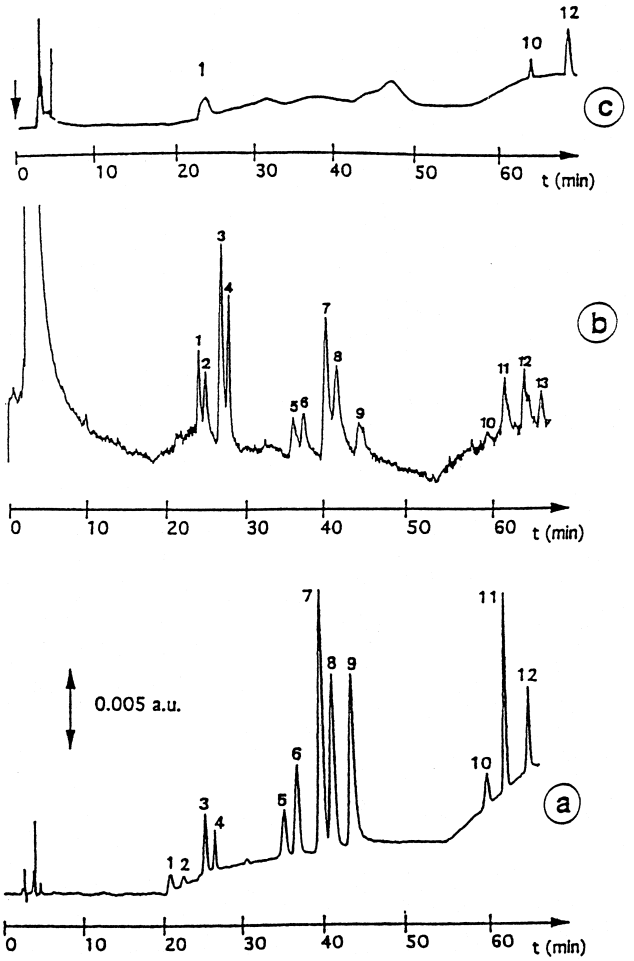

Fig. 10. Chromatograms obtained by on-line trace-enrichment of 100 ml of water spiked with 1.5 μg/l of each pesticide using a precolumn packed with (a) PLRP-S, (b) PS–DVB from J.T. Baker and (c) Hypercarb PGC. From Ref. [17]. (C_{18} analytical column Supelcosil LC-18-DB, 250×4.6 mm I.D.; acetonitrile gradient with a $5 \cdot 10^{-3}$ *M* phosphate buffer, 5% acetonitrile from 0 to 15 min, 10% at 20 min, 15% from 40 min to 50 min; solutes: 1=oxamyl; 2=methomyl; 3=deisopropylatrazine; 4=monocrotophos; 5=fenuron; 6=metamitron; 7=deethylatrazine; 8=chloridazon; 9=carbendazim; 10=aldicarb; 11=aminocarb; 12=metribuzin.)

[53,146,156]. Fig. 10 uses precolumns packed with PLRP-S, a PS–DVB from J.T. Baker (1060 m^2/g) and the porous graphitic carbon Hypercarb [17]. Fig. 11 uses precolumns prepacked by Hysphere-1 (>1000 m^2/g), Bond-Elut PPL (700 m^2/g) and Carbopack [145]. Fig. 5 contains several very polar analytes and the gradient allowing their separation starts with 5% acetonitrile, is 15% at 50 min and 30% at 60 min. This mobile phase does not contain enough acetonitrile to allow a good desorption of the analytes from the PS–DVB precolumn towards the analytical column and there is a large band broadening. Backflush desorption only slightly improved the transfer. In Fig. 11, the slope of the gradient is higher because there are few polar analytes mixed with moderately polar analytes as shown by the gradient which is linear from 20% acetonitrile to 40% in 20 min and 100% at 25 min. Therefore, there is no general rule for the apparent compatibility between sorbents and one has to be careful. This mainly depends on the shape of the gradient of mobile phase. A study dealing with the separation of polar carbamates described a bad transfer using a precolumn packed with LiChrolut EN (1200 m^2/g)

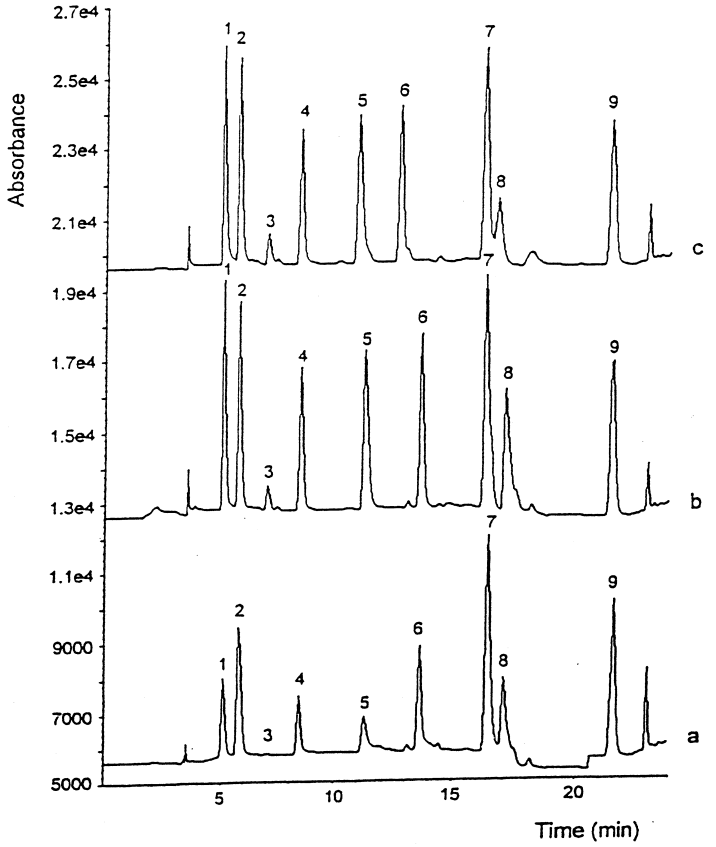

Fig. 11. Chromatograms obtained by on-line trace-enrichment of 100 ml of standard solution of 2 μg/l using a precolumn (a) Carbopack, (b) Bond-Elut PPL and (c) Hysphere. From Ref. [145]. (C_{18} analytical column Spherisorb ODS, 250×4.6 mm I.D.; linear gradient from 20% acetonitrile to 40% in 20 min; solutes: 1=oxamyl; 2=methomyl; 3=phenol; 4=nitrophenol; 5=2,4-nitrophenol; 6 bentazone; 7=simazine; 8=MCPA; 9=atrazine).

[157] whereas another study showed a good transfer for priority phenols [158].

In order to prevent the peak broadening due to the different nature of the analytical column and the precolumn adsorbent, the on-line coupling device can be modified by performing the desorption in the backflush mode, using only the organic solvent of the mobile phase, and then, via a third pump adding water, so that both solvents are mixed before reaching the analytical column [116,159,160].

Rapid on-column enrichment and clean-up for the direct analysis of pharmaceutical in plasma using a single polymeric PS–DVB column was reported [161]. The coupling of mass spectrometry with LC often results in enhanced selectivity and sensitivity compared to UV detection and this can remove the need for complete resolution of the analyte from endogenous materials in the matrix. The use of large-particle-size stationary phases and the possibility to use large porosity column end frits allow an on-line analysis approach to be used for the direct analysis of pharmaceuticals in biological matrices with extremely high throughput. An aliquot of plasma was mixed with an equal volume of standard and 50 μl were injected on a 50×1 mm column packed with the Oasis HLB (Waters) polymer. Endogenous materials were separated from the pharmaceutical of interest (new isoquinoline) and eluted from the column with a mobile phase of 100% aqueous formic acid (0.1%) for 0.2 min at 4 ml/min. During that time the eluent was diverted to waste to avoid soiling the mass spectrometer. For the remain-

der of the analysis, the eluent was split with 3.6 ml/min directed to the waste and 0.4 ml/min to the MS system. Over the period 0.2 to 0.8 min, the solvent composition was rapidly changed from 100% formic acid to 95% acetonitrile–5% formic acid (0.1%) and held at 95% acetonitrile from 0. 8 to 0.9 min and returned to 100% by 1 min. With a period of 0.2 min for re-equilibration, the total analysis time was 1.2 min, providing a theoretical throughput of 50 samples per hour. The turbulent flow chromatographic mode was optimized in term of gradient profile, system configuration and optimal injection volume for maximum throughput and robustness. The extraction/analysis single column was changed after approximately 100 injections due to its deterioration in term of broadened peak shapes and back pressure.

5.2.6. Extraction of analytes over a wide range of polarity: some practical considerations

It is always a challenge to extract as much as possible analytes in one run in order to decrease the cost and the time of the analysis in the environmental field. However, the probability is high to have in the mixture analytes with different polarities, water solubility, ionisation properties and volatility, especially when metabolites and degradation products are taken into accounts. But, as far as high cross-linked PS–DVB sorbents have now the capability of trapping both very polar, non-polar and ionised organic analytes, the challenge may be possible and is very attractive. First, it depends on the polarity of the most polar analytes and one has to check if these ones are extracted with good recoveries with the required sample volume to be handled. For the hydrophobic ones, in theory, there is no problem of breakthrough.

However, if the extraction can be predicted, very often practical problems coming from the physico–chemistry are encountered. A first one occurs during the sample percolation, because recoveries of hydrophobic analytes with very low water solubility are low unless a certain percentage of organic solvent or surfactant is added in the sample. Since this loss is not linked to the concentration of analytes but to the size of tubes and flask, it may be not visible when analytes are to detected in the range of 10 μg/l, but it is at the concentration of 0.1 μg/l [25]. But if the addition of an organic solvent solve the problem of the hydrophobic ones, it decreases the breakthrough volumes of the more polar ones which can be then poorly recovered using this procedure. The same problem is encountered with on-line preconcentration, and is visible by the lower recoveries of the most hydrophobic compounds.

Another problem is in the reconstitution of the extract. When very polar and non-polar analytes are together, complete solubilization of the extracts is often impossible: addition of water is required for the more polar ones, whereas very hydrophobic analytes can only be dissolved a non-polar organic solvent.

Therefore, the range of polarity and water solubility should be carefully checked for performing a good multiresidue analysis. Sometimes, it is quicker to split the list of analytes to be determined in two, polar and moderately polar on one hand and non-polar with the addition of organic solvent (providing also some degree of clean-up) on the other hand.

5.3. Carbon-based sorbents

The most widely used carbon-based SPE are graphitized carbon blacks (GCBs) obtained by heating carbon blacks at high temperature (2700–3000°C). The first available GCBs were non-porous with a low specific surface area (Carbopack B or ENVI-Carb SPE from Supelco, Carbograph from Alltech). However, their higher efficiency over C_{18} silica for trapping polar pesticides has been extensively shown by the group of Di Corcia et al. [114,162–166]. Carbograph 4 was introduced with a surface area of 210 m^2/g [166]. Every carbonaceous sorbent made from carbon blacks contains various functional groups at the surface following the oxygen chemisorption. Taking advantage of the positively charged active centers at the GCB surface multiresidue methods for pesticide analysis gave been performed which involved a fractionation between neutral and basic pesticides on one hand and acidic on the other hand [163,167–169]. Detection limits of 5 ng/l were obtained for the determination of 15 post-emergence herbicides from the preconcentration of of 4 l of drinking water using a reversible extraction cartridge packed with 0.5 g of Carbograph 4 [170]. This property was also exploited for the extraction of benzene and naphthalene sulfonate and was shown to be more efficient that conventional

ion-pair extraction on C_{18} silicas [171]. Carbograph 4 was used for the extraction and identification using LC–MS of biotransformation products of alcohol ethoxylate surfactants [172,173]. Carbon-based membrane extraction disks are also available and were used for the determination of *N*-nitrosodimethylamine at the ng/l level in ground water [174].

5.3.1. Potential for trapping very polar analytes

Graphitized carbon blacks are not sufficiently pressure resistant to be used in liquid chromatography so that no data indicating the LC behavior of solutes are available. Porous graphitic carbon (PGC) is available in SPE cartridges (Hypersep PGC) and is similar to the LC-grade Hypercarb, which appeared at the end of the 1980s [175]. It is characterized by a highly homogeneous and ordered structure and by a specific area around 120 m^2/g. Owing to its crystalline structure made of large graphitic sheets held together by weak Van der Waals forces, it is often presented as a more retentive reversed-phase sorbent than C_{18} silica, but the retention mechanism was shown to be very different from that observed on C_{18} silicas or PS–DVB polymers [176]. Compounds are retained by both hydrophobic and electronic interactions, so that non-polar analytes, but also very polar and water-soluble analytes were shown to be retained in water [50,177–184]. For example, the retention of morphine and its metabolites was recently shown to be very strong especially for the ionized compounds [185]. Prediction of retention data is difficult. High retention are usually obtained for planar molecules containing several polar groups with delocalized electronic charges via π-bonds and lone pairs of electrons. The potential of PGC for extracting very polar compounds was shown in Table 5 for dealkylated and hydroxylated degradation products of atrazine down to cyanuric acid whereas the limitation of both C_{18} silica and polymer are clearly shown for the very polar ammeline, ammelide and cyanuric acid with log k_w values lower than 0.5 whereas they are higher than 2 with PGC. Using a 200-mg PGC cartridge, recoveries were above 90% with the handling of 250 ml of water sample for all the metabolites except the three more polar ones for which a 500-mg cartridge was required to obtained similar recoveries [52]. Di Corcia et al. have described the ultratrace determination of atrazine and its six major degradation products using SPE with Carbograph 4 followed by LC–electrospray MS [186]. When polar phenols only included chlorophenols and higher chlorinated ones, comparison between GCB and highly cross-linked polymer gave similar results [187].

Since no guide can be given for log k_w prediction, The only rapid and easy mean is to inject the polar analyte of interest onto an available analytical column of PGC with a methanol–water mobile phase and to estimate log k_w values via the relation log k–methanol content.

Problems of elution are often mentioned [188]. Owing to the different retention mechanism, acetonitrile and methanol can be inefficient and it is preferable to use methylene chloride or tetrahydrofuran (THF). This was demonstrated by LC measurements showing that several analytes are still strongly retained with pure methanol or acetonitrile as mobile phase [189]. It is also better to perform desorption in the backflush way compared to the percolation way. Cartridges allowing percolation and desorption in the opposite way are now available.

5.3.2. On-line applications

Figs. 10 and 11 have shown that the on-line coupling of high cross-linked polymers with C_{18} silica analytical columns depended strongly on the gradient applied for the separation and consequently on the number of very polar analytes to be determined in the mixture. In the same figures, the on-line coupling of precolumns packed with Carbograph and Hypercarb have been reported and the same conclusions can be drawn. The selective on-line SPE and LC determination of diquat, paraquat and difenzoquat from environmental water was accomplished using the automated on-line device OSP-2 with a precolumn laboratory packed with Carbograph and a Hypercarb analytical column [190]. Sometimes, the on-line transfer in the backflush way was shown to improved the coupling [157]. When possible, the replacement of the C_{18} analytical column by an Hypercarb analytical column can solve the problem. Such a PGC column has been also coupled on-line with precolumns packed with high cross-linked polymer [53,157].

Fig. 9 shows a mutiresidue on-line analysis of

drinking water using the on-line coupling PLRP-S precolumn–C_{18} analytical column. It is obvious in this figure that the recoveries for deisopropylatrazine (DIA, peak 1) is low due to breakthrough of this polar metabolite. In a long-term survey of ground water, an on-line monitoring of the polar metabolites was performed using the on-line coupling PGC precolumn–PGC analytical column [20]. Fig. 12 shows the advantage of such a system since deisopropylatrazine is eluted after deethylatrazine and can be easily delayed to 40 min in the chromatogram, after the interfering compounds. The breakthrough volume of deisopropylatrazine on PGC is higher than 100 ml so that detection limits using 100-ml samples are in the low 0.1 μg/l.

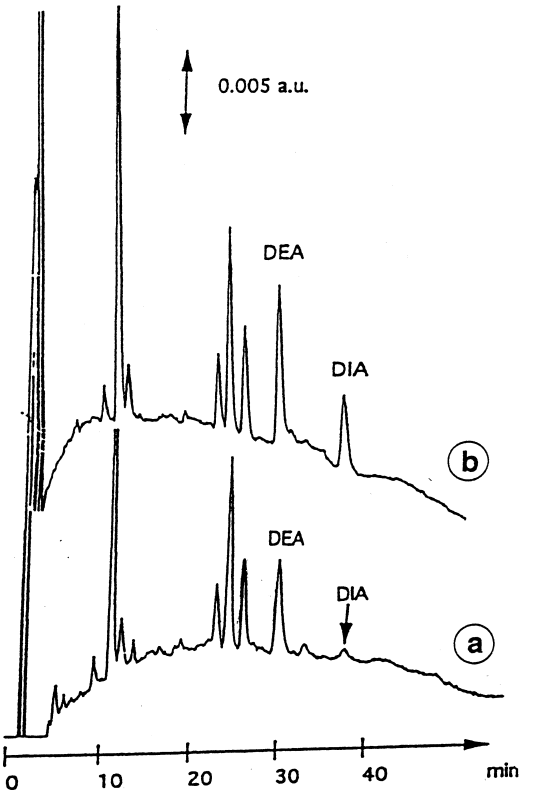

Fig. 12. On-line preconcentration of 100 ml of ground water (a) non-spiked and (b) spiked with 0.5 μg/l of DEA and DIA using the on-line coupling PGC precolumn–PGC analytical column. (a) 100 ml of non-spiked LC-grade water and (b) spiked with 0.2 μg/l of deethylatrazine (DEA) and deisopropylatrazine (DIA). Hypercarb column from Shandon, 10 cm×0.46 cm I.D., acetonitrile gradient with a $5 \cdot 10^{-3}$ *M* phosphate buffer at pH 7, 15% to 35% acetonitrile from 0 to 40 min; flow-rate: 1 ml/min, UV detection at 220 nm. From Ref. [16].

5.4. Ion-pair and ion-exchange sorbents

5.4.1. Off-line and on-line direct use

Ionic or ionizable analytes can be extracted by ion-pair and ion-exchange sorbents. Silica-based have the inherent disadvantages over polymers of being limited to the pH range 3 to 9 and having a lower capacity. Cation exchangers include weak carboxylic acid and strong aromatic or non-aromatic sulfonic acid groups. Weak anion-exchange groups are made of primary or secondary amino groups whereas strong anion exchangers are quaternary amine forms. They are available in disposable cartridges, precolumns and disks. The method development is easy for ionizable analytes because retention occurs for a sample pH allowing the analyte to be in its ionic form whereas desorption in its neutral form. If the analytes are ionic over the whole pH range, then desorption occurs by using a solution of appropriate ionic strength, according to the basic principles of ion-exchange chromatography.

The main problem encountered when environmental are handled comes from the fact that they contain high amounts of inorganic ions which overload the capacity of these sorbents. Cation exchangers have been used for the on-line determination of aniline derivatives after a chemical sample pretreatment based on precipitation of calcium with oxalic acid and complexation of iron with EDTA [191]. However, the pretreatment was not enough efficient for their determination at the sub μg/l level. The method was applied to the preconcentration of the pesticide aminotriazole which is polar and water-soluble, and not retained on C_{18} silica or polymers [192,193]. Since it is ionizable, a sulfonic acid-type of resin-based cation exchanger was used. The breakthrough volume on a precolumn (10 mm×2 mm I.D.) was measured as 150±10 ml with LC-grade water spiked with aminotriazole. With drinking water samples, the breakthrough volume was below 5 ml. After the chemical pretreatment to remove inorganic anions, the recovery with a 30 ml sample of spiked drinking water was 18% as a result of the competition between the remaining trace inorganic ions and organic ions, in favor of the inorganic ions.

When the organic ions of interest are more hydrophobic, then additional interactions occur with the matrix of the ion exchanger sorbent, so that the competition is in favor of the organic ions. One example is in the direct concentration of triazines at low pH using cation-exchange cartridges. Other common examples have been described for the trace-enrichment of linear alkylbenzenesulfonates (LASs) and benzene- and naphthalene sulfonates including their hydroxy- and amino-substituted derivatives [194–197]. Ion-pair extraction is usually selected with a C_8 or a C_{18} sorbent with either tetramethyl-, tetrabutyl- or cethyltrimethylammonium as ion-pair reagent. An on-line trace-enrichment was developed for the determination of aromatic sulfonic acids using ion-pair extraction on a PLRP-S precolumn coupled to a PLRP-S analytical column [194]. Pre-concentration from 30 ml of surface water allows detection at the low to sub μg/l, with good recoveries for most of the sulfonates except for the amino-substituted. Interferences from humic acids were reduced by the addition of sodium chloride prior extraction, but this affected the recovery of the most polar analytes. A fully automatic determination of aromatic sulfonates in aqueous samples was set-up using the SAMOS-LC system [195]. The methods combined ion-pair on-line extraction and ion-pair chromatography, both using C_{18} materials. The method was shown to be suitable for benzene, naphthalene, anthraquinone and stilbene sulfonates with detection limits in the sub-μg/l range. The method has been applied to waste water, river water, bank filtrate and water from different steps of drinking water production. Fig. 13 shows the fate of some naphthalene disulfonates during the several steps of the treatment of an industrial effluent in a water work, indicating thus a high stability of the naphthalene-1,5-disulfonate to biodegradation and ozonation.

Applications dealing with anion exchangers are less numerous. Trifluoroacetic acid was quantitatively recovered from most environmental waters by an extraction procedure using an anion-exchange Empore disk [198]. But, in saline samples where the

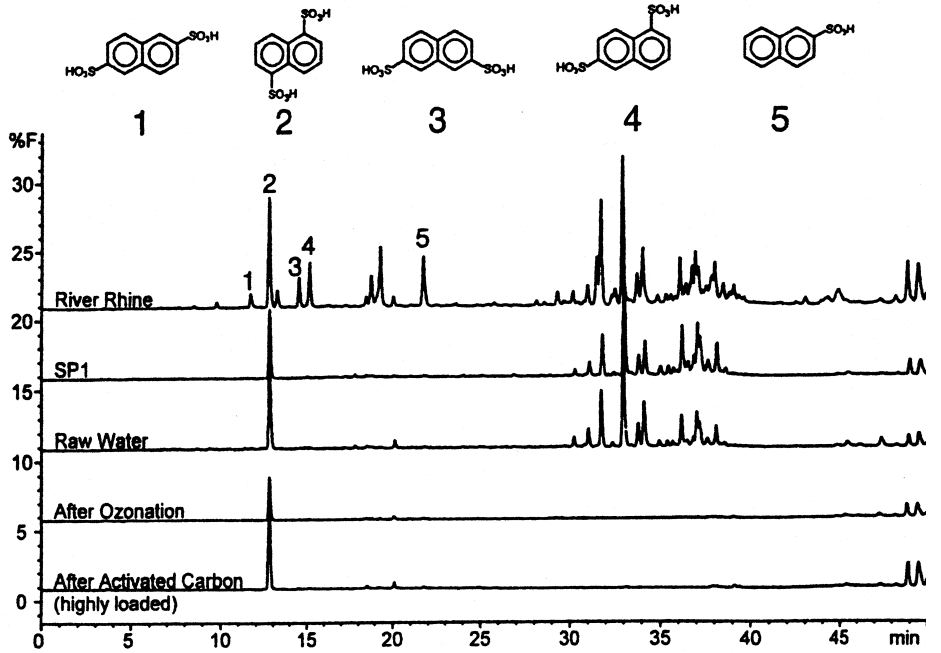

Fig. 13. Chromatograms of water samples from several steps of water treatment in a water works (fluorescence detection, wavelength combination $\lambda_{ex}=230$ nm, $\lambda_{em}=340$ nm). On-line enrichment of 50 ml using a 10×4 mm I.D. precolumn packed with C_{18} silica and operating in ion-pair mode by addition of 1 m*M* of tetrabultylammonium in the sample at pH 6.5. Ion-pair analytical separation using a 250 mm×4.6 I.D. ODS-Hypersil analytical column. From Ref. [195].

presence of competing anions interfered, a liquid–liquid extraction clean-up was necessary. Using the same type of disks, another study described the extraction of the negatively charged pesticide dacthal and its metabolites in ground water [199,200]. These results indicate a lower effect of interferences from the inorganic anions in natural water as compared to the effect of inorganic cations.

5.4.2. Use in combination with a reversed-phase sorbent for ionizable analytes in order to avoid overloading by inorganic ions

The direct percolation of samples through the ion exchanger can be avoided for analytes ionizable in the range of pH 1 to 13 by using a two-trap system which was first described by Nielen et al. [201]. The basis of this system is that compounds are retained on non-polar sorbents when they are in their neutral form, but not when they are in their ionic form. Polymeric sorbents are preferred over C_{18} silicas because of their larger pH range. In the tandem on-line system, the compounds are first trapped by a polymeric precolumn in their molecular form by appropriate pH adjustment while inorganic ions are directed to the waste. Then, the non-polar precolumn is coupled to an ion exchanger precolumn in series and only ionized analytes are desorbed from the sorbent and transfer to the ion exchanger by applying a few ml of deionized solution at pH adjusted in order to have solutes in their ionic form. Ionic solutes are then re-preconcentrated on the ion exchanger, which is subsequently on-line desorbed. This two-step extraction is selective since only ionic species are transferred. It was applied to chlorotriazines and their hydroxylated derivatives and was successfully applied to the determination of aniline derivatives at the 0.1 μg/l level in surface water [202,203]. The procedure using anion exchangers has been applied to the trace determination of phenols and phenoxyacid herbicides [201,204,205].

5.4.3. Use in combination with a reversed-phase sorbent in order to fractionate neutral and ionizable analytes or to remove neutral interferences

Fractionation between neutral and ionic analytes is performed by using a non-polar sorbent and an ion-pair or ion exchanger in series. The first on-line pre-fractionation in polarity groups (non-polar, moderately polar and ionic compounds) was performed by Nielen et al. using three precolumns in series, the first one packed with C_{18} silica the second with PRP-1 copolymer and the third one with a cation exchanger [191]. The sample was chemically pretreated in order to remove inorganic cations and the sample was adjusted at pH 3. Each precolumn was separately eluted by a continuous buffer–methanol gradient and separated on one C_{18} analytical column. This fractionation was applied to the analysis of waste water.

An on-line system using a C_8 silica precolumn and an ion-pair-loaded polymer precolumn in series enabled the fully on-line identification and trace-determination of 50 acidic, neutral and basic pollutants in surface water [206]. Different ion-pairing reagents were added to simultaneously enrich neutral analytes as well as permanently charged analytes like sulfonic acids and weak acids and bases. With 100 ml sample volumes, the limit of detection was in the low μg/l.

When looking at ionic analytes alone, the first neutral precolumn or cartridge can simply act as a clean-up since neutral interferences are left on the non-polar sorbents.

The coupling a first cartridge of LiChrolut and a second one packed with a strong anion-exchange (SAX) material was used for the extraction of glyphosate and its main metabolite aminophosphoric acid from water [207]. A C_{18} minicolumn was coupled to an anion exchanger for the determination of linear alkylbenzene sulfonates in water [208]. The same objectives occur when coupling a C_{18} cartridge to a graphitized carbon black for the determination of benzene and naphthalene sulfonates since it was shown that graphitized carbon black contains positively charged oxonium groups and can act as an anion exchanger [171,209].

5.5. Mixed-mode sorbents

Mixed-mode retention mechanisms have been observed during the solid-phase extraction of basic drugs using reversed-phase silica containing residual silanols at sample pH where both entities are ionized. Drugs usually contain functional groups and many of

them can be anionic, cationic or zwitterions depending on the sample pH. Around 80% of drugs contain nitrogen in a form that may be readily protonated [210]. This feature of drugs has been exploited to make sorbents that contain reversed-phase alkyl chains and cation exchanger bonded on the same solid. The most popular cartridge or disk formats contain octyl chains and cation-exchange groups (Bond-Elut Certify I and II from Varian, Isolute-Confirm HAX/HCX from IST, Spec form Ansys, and Narc-1 and 2 From J.T. Baker).

Mixed-mode sorbents allow the extraction and the clean-up of biological matrices (urine, blood or plasma) in the same sequence [211]. First, the sorbent is equilibrated, the cation exchanger being in the hydrogen form or sodium form if urine is analysed. The sample pH is such that the analytes are in their neutral form or negatively charged, but the amino group should be uncharged. Analytes of interest and other matrix compounds are then retained by hydrophobic interactions. After washing with buffer or water, the next step is an acidic step which has the effect of protonating the aminogroup of the drug which will be bound by ionic interactions. Since the ionic interaction is much stronger than the hydrophobic one (see Table 1), a washing step using methanol does not break the ionic interaction, but only the hydrophobic ones. That is the clean-up step, since matrix interferences are eluted from the cartridge. Finally, the desorption occurs with a basic solution in order to break the ionic interaction. Several applications are given in the application books from manufacturers. Discs and cartridges have been compared for the SPE of methadone from serum [212]. From serum solution several matrix effects were still encountered due to the lower capacity of discs, but overall, discs presented several mechanical and economical advantages

A PS–DVB-based mixed-mode resin that contains C_{18} and sulfonated cation-exchange groups was also shown to be efficient for isolating triazines and basic drugs, combining hydrogen bonding, cation-exchange, and Van der Waals interactions [211,143]. Empore disks of PS–DVB–RPS have been commercially available with a sulfonated PS–DVB resin embedded. Two new high-capacity carboxylic acid functionalized resin were described and applied to the solid-phase extraction of a broad range of organic compounds [213,214].

A novel anion-exchange polymeric resin containing three amino groups was prepared by reaction of a chloromethylated PS–DVB resin with diethylenetriamine was applied to the extraction of both inorganic and organic anions [215].

Applications to the determination of basic drugs and pharmaceuticals are numerous and can be easily found in application books provided by the companies.

5.6. Normal-phase solid-phase extraction sorbents

Normal phase sorbents include bare silica, alumina, Florisil (synthetic magnesium silicate) and silica chemically modified by polar groups such as amino, cyano or diol groups. The basic principle which governs the SPE process with these polar sorbents is adsorption chromatography, so that only analytes dissolved in samples made of a (usually) non-polar organic solvent can be handled. Few natural matrices are readily soluble in these solvents (oil, lipids, cosmetics). Therefore, these sorbents are used for the clean-up of extracts mainly obtained from solid or liquid matrices. The dry extract is dissolved in a non-polar solvent, typically hexane or isooctane, and percolated through the sorbent after conditioning. Step elution with solvent of increasing polarity allows a separation into fractions on the basis of polarity difference. Florisil, silica gel, alumina and silica modified with polar aminopropyl groups have been successfully used for clean-up of various extracts from complex matrices such as contaminated water, soils, sludges, biological tissues, food [149,216–218]. Clean-up should be seen as a simple SPE process and automation of the sequence should be more often integrated in the methods as it is for preconcentration [219,220]. One example is for the clean-up of water samples extracts for the determination of organochlorine and pyrethroid insecticides using silica cartridges [221]. The extract was obtained from 15 ml of surface water by LLE with hexane and was further evaporated down to 1 ml. Clean-up was made with a 100-mg silica cartridge and the whole sequence – i.e., conditioning with 2 ml of hexane–isopropanol (80:20, v/v) and 6 ml of hexane, application of 500 μl of the extract,

washing with 1 ml of hexane and desorption with 1 ml of hexane–toluene (70:30, v/v) – was automated by the ASPEC from Gilson, which was coupled on-line to capillary GC–electron-capture detection by means of a loop-interface equipped with a solvent vapor exit. The complete analytical procedure was greatly facilitated by automation with a considerable reduction in the sample volume required, thus allowing determination of synthetic pyrethroids at ppt levels in surface water.

5.7. Restricted access matrix sorbents

Mixed-mode sorbents integrate the clean-up of sample in the SPE process, but the sequence is typically an off-line procedure and the deproteination of plasma and serum samples is usually required before extraction. There is an interest in having on-line techniques for the handling of untreated biological samples. In recent years, special sorbent possessing restricted access properties have been developed to allow the direct injection of biological matrices into on-line SPE–LC systems. These sorbents – so-called restricted access materials (RAMs) – combine size exclusion of protein and other high-molecular-mass matrix components with the simultaneous enrichment of low-molecular-mass analytes at the inner pore surface [222–230].

The low-molecular-mass analytes are retained by conventional retention mechanisms such as hydrophobic, ionic or affinity interactions. To prevent the access to proteins, there is either a physical diffusion barrier by an appropriate pore diameter (ChromSper 5 Biomatrix from Chrompack, LiChrospher ADS from Merck) or a chemical barrier by an appropriate polymer at the outer surface of the particles (Hisep from Supelco, Ultrabiosep from Hypersil, Biotrap 500 from Chromtech, Capcell Pak from Shiseido). In addition to the size-exclusion process, special treatment of the outer surface is performed by an hydrophilic polymer or a protein coating in order to avoid irreversible adsorption of proteins and to be able to re-use the precolumns several times.

Internal surface reversed-phase (ISRP) supports are the most popular RAMs [231,232]. The bonded reversed-phase covers the internal pore surface of a glyceryl-modified silica, the ligand being a C_4, C_8 or C_{18} moiety. These sorbents are known as alkyl-diol silica (ADS) and they allow the extraction of a wide range of analytes. Such an alkyl-diol silica precolumn was selected for the analysis of the local anesthetics bupivacaine in human plasma because of its ability to rapidly separate the analytes from the proteins and polar endogenous compounds [233]. It was also shown to provide efficient on-line clean-up and trace-enrichment for the determination of several drugs and their metabolites in various biological fluids (serum, urine, intestinal aspirate, supernatants of cell cultures and supernatants of protein denaturation) [234].

The influence of the mobile phase conditions on the clean-up effect of such RAM precolumns were studied for plasma samples [235]. The necessary washing time, buffer pH, type and content of organic modifier were evaluated and best results were obtained using a phosphate buffer near the physiological pH with small amounts of 2-propanol or acetonitrile when 500 μl of direct plasma was injected. More than 93% of the proteins in a plasma matrix could be recovered within 3 min from a 25×4 mm I.D. alkyl-diol silica C_{18} precolumn. The same authors have recently evaluated the new BioTrap 500 C_{18} precolumn also is based on porous silica [236]. More than 95% of the plasma could be excluded form the matrix within 3 min and one precolumn (20×4 mm I.D.) could tolerate up to 15 ml of plasma without noticeable change in retention and pressure. Clogging was observed after 45 ml of samples.

LC–MS with on-line SPE using a restricted access C_{18} precolumn was described for the analysis of cortisol and prednisolone for plasma analysis and arachidonic acid for urine samples [237]. The only off-line sample pre-treatment step required was centrifugation to remove particulate matter. A precolumn packed with 25 μm C_{18} alkyl diol silica was coupled to LC without transfer loss. An on-line heart-cut technique was employed and only the analyte-containing fraction eluting from the LC column was directed to the MS to protect the LC–MS interface and ion-source from contamination. The analysis time was 5 and 9.5 min for cortisol in serum and arachidonic acid in urine, respectively. Fig. 14 shows the determination of cortisol, cortisone, prednisolone and fludrocortisone in untreated plasma using on-line SPE–LC–MS. Cortisol and

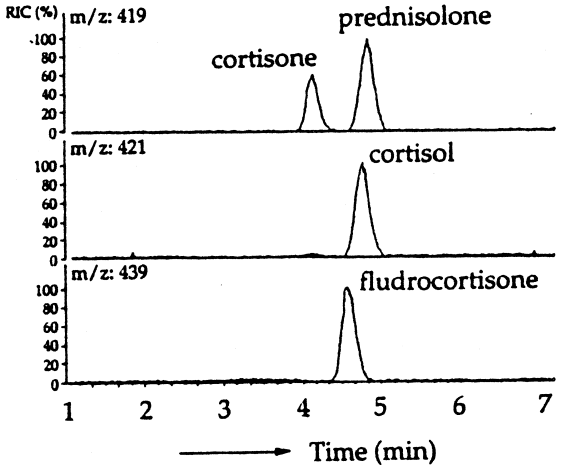

Fig. 14. Determination of cortisol, cortisone, prednisolone and fludrocortisone (100 ng/l each) in untreated plasma using on-line SPE–LC–APCI-MS. Precolumn: 15×4 mm I.D. packed with LiChrospher RP-18 ADS, 25 μm; LC separation on a 125×4 mm I.D. RP-18, 5 μm. From Ref. [237].

related compounds were quantitatively recovered from plasma with a detection limit for prednisolone of 2 ng/ml with a direct injection of 100 μl on the precolumn.

Another selective multicolumn LC method was described for the analysis of six cardiovascular drugs in serum. The method consisted in an on-line sample clean-up using a Pinkerton GFF2 restricted access precolumn, LC of the drugs on a microsphere non-porous silica C_{18} column, and front-cutting to perform a chiral separation of pindolol enantiomers on a second LC system [238]. After filtration spiked and non-spiked serum samples (20 μl) were loaded onto the precolumn and then the washing conditions were optimized in order to have an efficient removal of matrix interferents using a minimum of washing solvent with a retention of all the analytes on the precolumn. This criteria were achieved with a washing of 800 μl of 10 m*M* phosphate buffer at pH 7. Fig. 15 shows the chromatogram corresponding to the on-line analysis of a drug-free serum, the standards and a spiked serum. No interferences are detected on the serum chromatogram.

5.8. Immunoaffinity extraction sorbents

The use of non-selective sorbents can be a major problem when analytes of interest are at trace-level and interferences at higher concentrations. As an example, most of the polar organic compounds cannot be determined at trace-level by LC due to their co-elution with humic and fulvic substances present in high amount in soil and natural waters. Evidence of these compounds are usually seen as an important interfering matrix peak at the beginning of the chromatogram or a large hump in the first part of the chromatogram depending on the gradient shape. Additional clean-up procedures are usually required prior to the final chromatographic analysis [149].

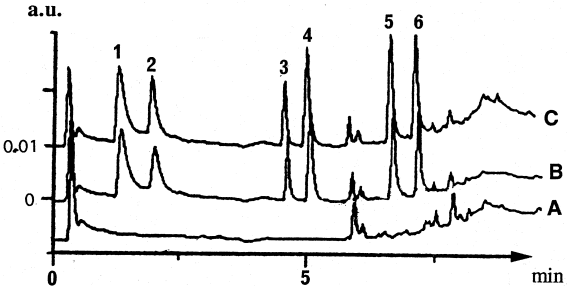

Fig. 15. On-line clean-up and analysis of cardiovascular drugs in serum with HPLC system. (A) Drug-free serum, (B) standards in buffer and (C) spiked serum. Precolumn packed with a Pinkerton GFF2 restricted access sorbent; analytical column Micra RP-18 non-porous silica. UV detection at 220 nm. Peaks: 1=lidocaine, 2=pindolol, 3=metoprolol, 4=oxprenolol, 5=diltiazem, 6=verapamil. From Ref. [238].

There a considerable interest in having highly selective SPE sorbents allowing extraction, concentration and clean-up in a single step. That was achieved by using materials involving antigen–antibody interactions, thus providing selective extraction methods based on molecular recognition. Antibodies are covalently bonded onto an appropriate sorbent to form a so-called immunosorbent (IS), to be packed into a solid-phase extraction cartridge or precolumn. Since antibodies are highly selective towards the analyte used to initiate the immune response with a high affinity, the corresponding immunosorbent may extract and isolate this analyte from complex matrices in a single step, and the problem of the co-extraction of matrix interferences is therefore circumvented.

First ISs have been described in the biological field because of the availability of antibodies which can be very selective for large molecules and easily

obtained. Obtaining selective antibodies for small size molecules is more difficult and the development of immunochemical methods in the solid-phase extraction field targeting low-molecular-mass analytes is recent. The binding of analyte to antibody is the result of a good spatial complementary and is a function of the sum of intermolecular interactions. Therefore, an antibody can also bind one or more analytes with a structure similar to the analyte which has induced the immune response, and this is the so-called cross-reactivity of antibodies. It is usually a negative feature for immunoassay, but it was exploited in extraction, so that ISs have been made for single analyte, single analytes and its metabolites or a class of structurally related analytes. The first commercial ISs have been introduced during the last decade for the clean-up of samples for the analysis of aflatoxins, ochratoxin and veterinary drugs such as clenbuterol.

The first extensive studies demonstrating the advantages of coupling immunoextraction to LC have been made by Farjam and co-workers for the determination of estrogens and nortestoserone (19-α/β) in plasma and urine and aflatoxins in milk and urine [239–244]. Sepharose- or silica-based ISs are now used for preparing immunoextraction sorbents because they do not give raise to non-selective interactions and extraction can occur only by the selective immunoaffinity interactions. Attempts have been made to use hydrophilic polymers for bonding antibodies mainly because oriented bonding is possible [245]. However, although very hydrophilic, some non-specific hydrophobic interactions occurred between the polymer matrix and the analytes mainly due to the occurrence of π-bonds. The selectivity of the IS was therefore reduced. The advantage of silica is its pressure resistance so that it can be used directly in on-line set-up in a precolumn [246,247]. However, several works reported the use of precolumns packed with a Sepharose-based IS, but then desorption occurred at low pressure in a second precolumn – often a C_{18} silica precolumn – which can be coupled on-line to the LC system [239–244,248,249].

5.8.1. Immunosorbents for single analyte, metabolites and a class of analytes

The affinity is usually the highest between antibodies and analyte selected as the hapten which has induced the immune response (so-called analyte–antigen). Rule and co-workers reported for on-line immunoaffinity chromatography with coupled column LC for extraction and detection of two basic drugs in diluted urine using a protein G immunoaffinity column [250]. The same authors used aldehyde activated silica for bonding antibodies anticarbofuran and demonstrated excellent specificity toward this single analyte with direct extraction and detection at low levels (40 ng/l) in spiked water [249]. The selectivity was shown with the analysis of a crude potato extract. Other studies were published targeting pesticides such as atrazine and terbutylazine [251], atrazine and its major metabolites [252], chlortoluron [253], isoproturon [254] or carbendazim [255]. Relevant examples have been described also in the biological field such as trace determination of LSD and its metabolites in urine [256,257].

The cross-reactivity of antibodies was also exploited for developing ISs that could selectively extract a whole class of structurally related compounds. ISs have been tailored by several authors for the extraction of groups of organic compounds including triazine and phenylurea pesticides, BTEXs (benzene, toluene, ethylbenzene and xylene isomers), polyaromatic hydrocarbons (PAHs), benzidine and related azo dyes [245–247,258–262]. In order to recover the whole class, sometimes two antibodies have been mixed in the cartridge bed [263–265]. A recent study reports the on-line coupled immunoextraction combined with LC–MS for the determination of corticosteroids [266].

5.8.2. Features of immunosorbents

A typical SPE sequence using an immunosorbent packed in a disposable cartridge is very similar to that using a conventional C_{18} cartridge. It includes a conditioning step with a few ml of pure water, the sample percolation, a desorption step with a methanol–water mixture and a reconditioning with a PBS (phosphate-buffered saline) solution. The conditions for desorption of analytes are measured with the analyte–antigen because it will be the more difficult to be desorbed. In the literature large molecules are traditionally desorbed using chaotropic ions, high salt concentration and low pH buffers. However,

Farjam et al. were the first to clearly demonstrate that these conditions were not efficient to desorb low-molecular-mass organic molecules from the immunosorbent such as the β-19-nortestosterone hormone [240]. Desorption was better achieved using a high amount of organic solvent or a cross-reacting displacer at high concentration. An amount of 70% methanol was required to desorb aflatoxins, conditions which are unusually strong for antibodies [244]. The silica-based ISs developed for trapping phenylureas or triazines have shown that desorption required 2 or 3 ml of a buffer solution containing up to 70% of methanol [246,258]. Desorption of the 16 priority PAHs from and anti-pyrene IS required a mixture containing 70% of acetonitrile [261]. The anti-phenylureas IS developed by Stevenson et al. [263] required 2 ml of a PBS solution containing 50% of ethanol at pH 2.

The high affinity for the analyte antigen is seen by the high sample volume that can be handled without breakthrough. The group of Stevenson reported that 1 l of water containing 100 ng/l of isoproturon can be handled with a 100% recovery using 1 g of silica modified by 200 μl of crude anti-isoproturon serum [254]. Our results were similar using anti-triazine or anti-phenylureas ISs when water is spiked with the analyte–antigen alone. When water is spiked with the whole group of analytes, the affinity is not the same for all the analytes and this can be seen by a simple measurement of recoveries which are not 100% for all the analytes [245,247,258].

The low capacities together with incomplete recoveries are the major difference with conventional C_{18} silicas. In a solid-phase extraction process, an incomplete recovery is explained by analyte breakthrough which can be the result either of insufficient retention or overloading of the capacity of the sorbent. The capacities of the ISs are sufficient for the analyte–antigen, but this condition is not straightforward for the related analytes, especially those having a low affinity for the antibodies. From a practical point of view, quantitative analysis can be made only if recoveries are constant over the whole linear calibration range and if the calibration curves do not depend on the competition process. In other words, the calibration curves and recoveries should be similar, independent of whether the antigen–analyte or any other related analyte is on its own or in a mixture of related compounds. This is the situation that occurs in an unknown sample, because one does not know the number of compounds present in the sample and their respective concentrations. Typical linear ranges were 0 to 10 μg/l for most of the phenylureas, with a lowest linear range of 0 to 5 μg/l using 220 mg of anti-isoproturon IS with a sample volume of 50 ml [258]. Since the probability of all these compounds being each one at levels of 5 μg/l in an unknown environmental sample is very small, one can consider that there should be no limitation of recoveries by overloading the capacity with such ISs.

The selectivity is the most important feature of these SPE materials and has been demonstrated in complex solid matrices such as soil, sediments, sludges, plant tissue and food. For these samples, there is a real interest in having rapid methods for extracting as much as possible the analytes from solid matrices and then applying immunoextraction to the extract. Supercritical fluid extraction (SFE) coupled to immunoextraction clean-up has been investigated for the trace analysis of organic pollutants including PAHs and pesticides from soil and soots [267]. The analysis of phenylureas and triazines in several food samples (carrots, celery, corn, grapes, onions, potatoes and strawberries) was also shown to be highly simplified [268,269]. Methanolic extracts of the plant tissues were simply concentrated and then diluted with water before passage through the IS. Thanks to the high degree of clean-up, this approach was very rapid compared to actual methods and eliminates the requirements of solvents such as hexane, dichloromethane, acetone and others commonly used for adsorption chromatographic clean-up of sample extracts. PAHs could be determined in waste sludges and sediments using LC–UV DAD. The method was validated using certified reference sludges and sediments and the clean-up provided by an antifluorene IS was shown to be better than that obtained using conventional silica clean-up [270].

The commercialization of immunosorbents will require the availability of reproducible antibodies, which can be better guaranteed by monoclonal antibodies. Further large-scale production does not any longer require animals. The class-specific properties of ISs for phenylureas using monoclonal antibodies against isoproturon, atrazine and simazine

have been compared and the first results indicate that higher capacities are obtained because of the higher concentration of the monoclonal antibodies [264]. The fact that monoclonal antibodies show almost the same cross-reactivities as polyclonal antibodies, can be easily explained by the small size of the molecules. When molecules of interest are very small, the polyclonal antibody mixture cannot contain a large number of specific antibodies for the different parts of the small molecule and the probability is high for having similar properties between monoclonal and polyclonal antibodies.

5.8.3. On-line use of immunosorbents

One main interest for integrating IS in on-line technology is the selectivity of the extraction, because extraction and clean-up are achieved in the same step. So, chromatograms present a clear baseline, allowing quantification at low level and better identification of the analytes by classical UV diode array detectors. Therefore, the sample volume can be reduced.

First examples using on-line techniques with antibodies immobilized on Sepharose were for the determination of the anabolic hormone β-19-nortestosterone and its metabolites α-19-nortestosterone in calf urine by Farjam et al. [240]. Urine was directly loaded on the immunoprecolumn packed with Sepharose-immobilized polyclonal antibodies against β-19-nortestosterone. Because Sepharose was not pressure resistant it was necessary to use a second C_{18} precolumn for the selective desorption and re-concentration of analytes from the immunocolumn. But as far as desorption could not be made without a high proportion of organic solvent, direct desorption and re-concentration became impossible without modification of the basic on-line set-up. This problem was circumvented by using a displacing solution containing 5% acetonitrile and a high concentration of the cross-reacting steroid hormone norgestrel for the desorption and transferred to the second precolumn. Then the classical C_{18} precolumn–C_{18} analytical column system was used. The chromatogram represented in Fig. 16 corresponds to the on-line analysis of 26.5 ml of urine, filtered and diluted in the same volume of water containing 10% of acetonitrile and spiked with 300 ng/l of each α- and β-19-nortestosterone. The selectivity of the immunoextraction is clearly demonstrated by this chromatogram.

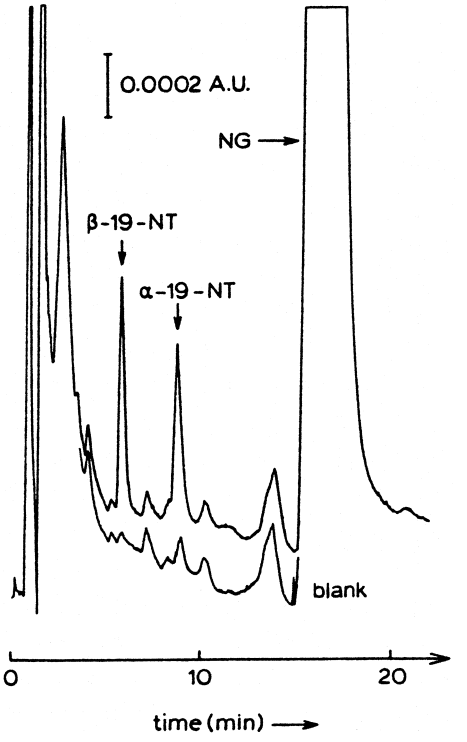

Fig. 16. Chromatogram of a calf urine sample spiked with 300 ng/l of each β-19-nortestosterone and α-19-nortestosterone, and the corresponding blank urine. The samples were filtered and diluted with an equal amount of acetonitrile–water (10:90). An aliquot of 53 ml (containing 26.5 ml urine) was loaded on the precolumn (10×10 mm I.D.) packed with an immunosorbent made of sepharose and immobilized antibodies against β-19-nortestosterone. On-line elution with a displacer according to the text. From Ref. [240].

Another option when using a Sepharose precolumn in an on-line set up was given for the determination of the aflatoxins [243]. After percolation through the immunoprecolumn, the analytes were desorbed with methanol–water (70:30, v/v), diluted on-line with water, subsequently re-concentrated on a second C_{18} precolumn and finally transferred to the LC separation column. The classical on-line set was modified by the addition of a pump for adding water to the desorption solution from the immunoprecolumn. The selectivity of the system was demonstrated with spiked urine and the immunoprecolumn could be re-used. Unfortunately its re-

usability was strongly shortened with milk samples and that was explained by the occurrence of proteolytic enzymes in milk which may be at the origin of the degradation of the immobilized antibodies. A solution was to add a dialysis unit to the system [244]. Fig. 17a shows the chromatogram corresponding to the on-line analysis of a crude milk sample non-spiked and spiked with 50 ng/l of aflatoxin M_1. As a comparison, the chromatogram corresponding to the on-line analysis of the C_{18} precolumn alone is given in Fig. 17b. The selectivity is again clearly illustrated.

The on-line set-up using a silica based immunoprecolumn is very simple and does not differ from that using a single C_{18} or PS–DVB precolumn. The on-line analysis of phenylureas represented in Fig. 18 allowed quantification at the 0.1 μg/l level from a sample volume as low as 50 ml of surface water (River Seine in Paris) and using a simple UV diode array detector. No interferences from humic acids and other analytes are observed. The selectivity can be seen by comparing the two chromatograms corresponding to drinking and river water and also by comparison with the on-line PLRP-S precolumn LC of Fig. 9, where 150 ml of drinking water were analysed. The class-selectivity is also illustrated since 11 phenylureas of the 13 in the mixture are showing up in this chromatogram. Ferrer et al. combined the selectivity of the IS with the high sensitivity achieved by LC–atmospheric pressure chemical ionisation MS and demonstrated that detection and confirmation could be obtained at the low ng/l from preconcentration volumes as low as 20 ml [271,272]. Other example of the practicality of on-line immunoaffinity extraction techniques for sample clean-up and trace enrichment as a sample preparation for LC–MS–MS with application has been described in a recent review [273].

An important feature of the IS is good stability. ISs can easily be regenerated using a PBS solution, even after being submitted to a high proportion of acetonitrile or methanol. It was verified that after 50 runs the loss in capacity was less than 10% [245,246]. The ISs can be submitted to a high proportion of organic solvent, which destroys the antigen–antibody interaction, probably by deformation of conformation of the protein, but in a reversible way, because antibodies have been stabilized by the covalent binding to the silica. However, when possible, it is recommended to switch the valve from

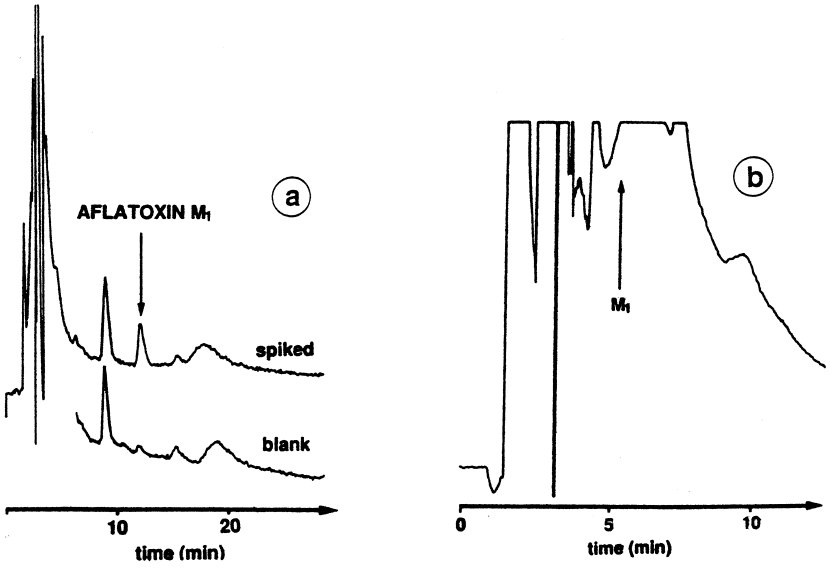

Fig. 17. (a) LC of a crude milk sample spiked with 50 ng/l of aflatoxin M1 and the corresponding blank. Hollow fiber dialysis/immunoaffinity preconcentration was performed with donor and acceptor volumes of 25 ml each. Mobile phase acetonitrile–methanol–water (20:5:75, v/v/v). (b) LC of a non-spiked milk sample analysed according to a flat membrane dialysis/C_{18} preconcentration procedure. Donor and acceptor volumes are 6.5 and 5.3 ml, respectively. Mobile phase acetonitrile–methanol–water (16:24:60, v/v/v). Fluorescence detection. From Ref. [244].

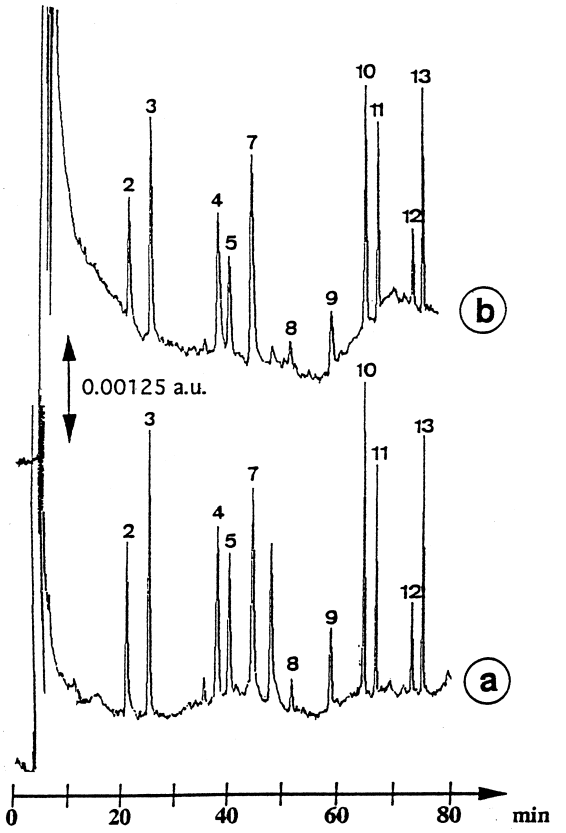

Fig. 18. On-line solid-phase immunoextraction–LC analysis–UV diode array detection of water samples spiked with a mixture of 13 phenylureas using a precolumn (30×4.6 mm I.D.) packed with an anti-chlortoluron I.S. (a) 50 ml of drinking- and (b) 50 ml of River Seine (Paris city) water samples spiked with 0.5 μg/l of each analyte. From Ref. [249]. Reversed-phase LC with water–acetonitrile gradient. Solutes: 1=fenuron, 2=metoxuron, 3=monuron, 4=methabenzthiazuron, 5=chlortoluron, 6=fluometuron, 7=isoproturon, 8=difenoxuron, 9=buturon, 10=linuron, 11=chlorbromuron, 12=difluzbenzuron, 13=neburon. UV detection shown at 244 nm. From Ref. [264].

the inject position to the load position when the mobile phase reaches 50 to 70% of organic solvent, in order to better preserve the lifetime of the IS.

The on-line coupling is particularly appropriate for the trace analysis of volatile analytes because no evaporation of samples occurs. PAHs are ubiquitous pollutants and the 16 priority PAHs list of the US EPA contains some 2–3 ring PAHs which are both volatile and hydrophobic. Due to their hydrophobicity, the addition of an organic solvent in the samples before percolation was necessary in order to avoid the adsorption of the PAHs on container walls or connection tubes [260,261]. Immunoextraction was coupled on-line to LC followed by fluorescence and UV DAD in series. The fluorescence is very sensitive and as shown before, it is possible to quantify analytes with only 10 ml of sample. However, except a recent one, fluorescence detectors do not have the capability of providing spectra for identification as UV diode array detectors have. The advantage over other sorbents is that the sample volume could be increased to 80 ml, so the sensitivity of the fluorescence associated to the selectivity of the IS allows to quantify individual PAHs in contaminated surface water at the ng/l level and below the 20 ng/l level (regulatory level for some PAHs in Europe for surface water taken for drinking water plants) and several of them could be confirmed by spectral identification using UV DAD.

Other applications deal with the determination of polar analytes because of the many interferences early eluted usually observed when surface water or waste water are analyzed. Relevant examples have been presented in recent reviews [264,265].

5.9. Molecularly imprinted polymers

The high selectivity provided by immunoextraction has led to attempt to synthesize antibody mimics. One approach has been the development of molecularly imprinted polymers (MIPs) these recent years. They involve the preparation of polymers with specific recognition sites for certain molecules. The synthesis is made by assembly of monomers around a template molecule and a subsequent polymerization using a cross-linker providing thus a rigid material. Then, the template molecules are removed and the resulting polymers have cavities which are the ''imprints''. These cavities are the recognition sites allowing binding of the template molecule. Like immunosorbents, the recognition is due to shape and a mixture of hydrogen, hydrophobic and electronic interactions. However, they have the advantages to be prepared more rapidly and easily, using well defined methods, and to be stable at high temperature, in a large pH range and in organic solvents. MIPs have found applications in liquid chromatography as normal and chiral stationary phases [274–278] and in areas where they can be substitutes of

natural antibodies, i.e., immunoassays and sensors [279–282] and solid-phase extraction [283–289]. MIPs are today a challenge as seen by several recent reviews [274,290–295].

5.9.1. Features of molecularly imprinted polymers and consequences for their use in solid-phase extraction

Non-covalent imprinting protocols are the most commonly used for preparing MIPs using acrylic or metacrylic monomers – often metacrylic acid – and ethylene glycol dimetacrylate as cross-linker [296,297]. The molecular imprinting is then accomplished through non-covalent interactions and the solvent, so-called porogenic solvent, has been shown to be one important factor for the determination of the effective molecular recognition. Since non-covalent interactions are involved, many of the techniques reported up to now have employed relatively apolar and aprotic solvents and chromatographic characterization also tends to occur in the same solvents. The various techniques of polymerization, the effect of the different parameters and the monomer-template rigidity have been discussed and reviewed in several recent publications [277,296–300].

Therefore, the retention mechanism involved in MIPs for SPE is apparent to normal-phase LC and it was shown that optimum selectivity is obtained using the porogenic solvent. Changing to an aqueous medium, the binding and selectivity decrease. This is important for the SPE sequence, because up to now, aqueous samples cannot be processed directly and require a previous extraction in order to be in an organic compatible solvent. The sample solvent and the conditions for elution are to be carefully selected and this is not straightforward up to now.

Several studies have pointed out two specific problems associated to the use of MIPs in SPE. A first one is the difficulty in removing all the template analyte, even after extensive washing. This potential risk of leakage of the imprints molecules is a real problem when analytes are to be determined at the trace level because large amounts of templates (at the mg levels) are used for the synthesis. A second problem is the difficulty in establishing quantitative and rapid desorption due to the high avidity of the MIP for the analyte.

5.9.2. Selected examples

The potential of MIPs as SPE sorbents was first described by Sellergreen in 1994 for the selective determination of the drug pendamidin in urine samples [284]. An enrichment factor of 54 was obtained at the 30 n*M* concentration level which allowed the drug to be directly detected in the desorption step, thus eliminating the need for a successive chromatographic analysis. A mixture containing 70% of acetonitrile and 30% of phosphate buffer at pH 5 was used for loading the sample (100 ml) and the same mixture with the buffer at pH 3 was used for elution. Martin et al. [286] evaluated the elution conditions for a propranolol-derived MIP. They used radiolabeled propranolol for imprinting and were able to overcome the problem of template leakage. Three structural related aminoalcohols and two dissimilar acids were taken as model molecules. Several results were obtained. First, a ionic modifier was necessary to achieve quantitative recoveries and its selection was of prime importance in the selectivity of the extraction. Solvents containing trifluoroacetic acid provided good recoveries of the analytes but without selectivity whereas the use of triethylamine allowed the selective extraction of propranolol from the solid.

Anderson et al. [285] presented a novel approach to eliminate the problems associated with the leakage of remaining imprint molecules. They prepared an MIP for preconcentration of samaredine by using a close structural analogue as the imprint species.

Muldoon and Stanker used MIPs as specific binding matrices for the solid-phase extraction and clean-up of biological samples [287]. They evaluated an anti-atrazine polymer to clean-up organic extracts of beef liver. They studied the specific binding properties of their MIP by equilibrating imprinted and non-imprinted polymers by atrazine in chloroform, acetonitrile dimethylformamide and water. In organic solvent an increase in specific binding was consistent with a decrease in the electron pair donicity of the solvent. High non-specific binding was obtained in water and atrazine retention was greatest in chloroform with a binding capacity of 19 μmol of atrazine per gram of MIP. Therefore, chloroform was used for extraction of the beef liver and purification was optimized by a washing with 3% of acetonitrile in chloroform followed by elution

with 50% of chloroform in acetonitrile. Purified and non-purified beef liver extracts were analyzed by both LC and enzyme-linked immunosorbent assay (ELISA). The use of MIP-SPE improved the accuracy and precision of the LC method and lowered the detection limits. Atrazine recoveries as determined by LC from spiked extracts were 88.7% following MIP-SPE and 60.9 for unpurified extracts. For the ELISA, the MIP-SPE also improved recoveries and accuracy.

A relevant example apparent to on-line techniques was shown for the SPE of theophylline in serum. An extensively cross-linked MIP was prepared using theophylline as print molecule and was carefully washed by Soxhlet extraction [301]. The polymer was shown to be highly selective and could be used as a stationary phase material for LC separation of theophylline from other structurally related drug compounds. Mobile phase tests confirm that the retention mechanism was governed by the rules of normal-phase LC and that the selectivity of the MIP could be controlled by a combination of the mobile phase and the sample solvent. Chloroform was shown to enhance the specific molecular recognition to such an extent that total retention of theophylline occurred on the column. Conversely, the addition of 1% of methanol in chloroform allows elution of theophylline as shown in Fig. 19a. Under optimal conditions the MIP column functions like a solid-phase sorbent for theophylline extraction with a mobile phase made of chloroform. Then rapid elution was achieved in a pulse format through injection of 20 μl of methanol in order to disrupt the electrostatic and hydrogen bonding between theophylline and binding sites. The resulting chromatogram is shown in Fig. 19b and is a nice illustration of the performance of the method. Using a sample volume of 300 μl, a 40 ng/ml standard solution produced a detectable peak signal. Application to serum demonstrated the selectivity of the method. This last example points out the usefulness of previous LC study in order to optimize the SPE methods.

5.9.3. Potential of molecularly imprinted polymers for solid-phase extraction in aqueous media

The most common and flexible approach for preparing MIPs using only non-covalent interactions between the template and the functional monomer is limited to apolar environment. The bonds formed during prearrangement, hydrogen bonds or electronic bonds are relatively weak. Therefore, in order to obtain the complex formation, non-covalent imprint-

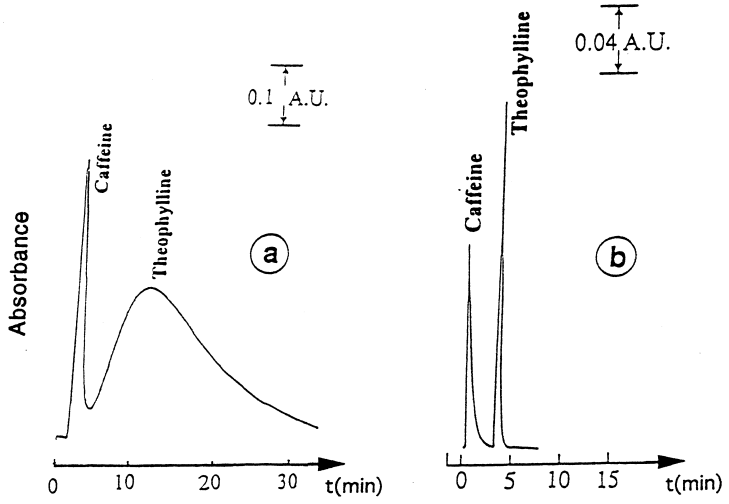

Fig. 19. (a) LC chromatogram of a 1.00 mg/ml theophylline and 0.15 mg/ml caffeine mixture on an anti-theophylline molecularly imprinted polymer column: mobile phase, 1% methanol in chloroform; flow-rate, 0.5 ml/min; sample injected, 20 μl; UV detection at 270 nm. (b) Molecular imprinted polymer-SPE-pulsed elution (MISPE-PE) of a 500 ng/ml theophylline and 50 ng/ml caffeine mixture on an anti-theophylline molecularly imprinted polymer column: mobile phase, 100% chloroform; flow-rate, 1 ml/min; sample injected, 20 μl; pulsed elution methanol, 20 μl; UV detection at 270 nm. Adapted from Ref. [301].

ing has been performed in mostly in apolar organic solvents since in water the presence of complex cannot be stable. A non-covalent molecular imprinting was recently achieved for 2,4-dichlorophenoxyacetic acid in the presence of polar solvents methanol and water [280]. Formation of the prearranged complex relied on hydrophobic and ionic interactions between the template and the functional monomer 4-vinylpyridine. The MIP obtained binds the original template with an appreciable selectivity over structurally related compounds. Although this MIP was not used for SPE applications but for a binding essay, this study has demonstrated that MIP in the presence of polar protic solvent using only non-covalent interactions is possible. Ramström et al [278] prepared a MIP using 2-vinylpyridine and/or metacrylic acid as functional monomer in a self assembly imprinting protocol. The resulting polymers were analyzed as an LC stationary phase in aqueous media, and the effect from the pH of the mobile phase and the degree of added organic solvent were investigated. The results indicated that such MIP prepared in organic phase may perform adequately in aqueous media and pointed out the importance of ionic bonds in conjunction with hydrophobic interactions in the formation of the complex between the analytes and the polymer.

Since MIPs are nowadays extensively studied by several groups one can expect many developments and improvement in a near future.

5.10. Metal-loaded sorbents

Organic compounds which can form complexes with metal ions can be preconcentrated selectively by metal-loaded sorbents. A silica containing the functional group 2-amino-1-cyclopentene-1-dithiocarboxylic acid (ACTA) loaded platinum (IV) – irreversibly retained aniline from water [302]. This sorbent was used to remove interfering anilines in the determination of phenylurea herbicides. The mercury-8 hydroxyquinoline phase allowed the preconcentration of 2-mercaptobenzimiodozole [303] whereas Ag(I) oxine was preferred for the determination of buturon in water [304].

Preconcentration on silicas modified with complexation properties has been reviewed by Veuthey et al. [305]. Some applications of on-line preconcentrations with metal-loaded precolumns have been reported by Nielen et al. [8].

6. Conclusion and future of solid-phase extraction

It is clearly established now that SPE can be a powerful method for sample preparation and that today any laboratory can do without it. The trends are clearly to simplify the labour of sample preparation, increasing its reliability, and eliminating the clean-up step by using more selective extraction procedures. The development of more selective sorbents will remain an active research area, as it is today for molecularly imprinted polymers or immunoextraction sorbents. The development of more easy-to-use sorbents with a simplified procedure and a diminution of the risks of errors shows the interest of the companies in this area.

There will never be a universal SPE method because one has to bear in mind that the sample pretreatment depends strongly on the analytical demand. But, there will always be a demand for more selectivity, sensitivity, reliability and rapidity. Today, in the biological field, there is a tremendous increase in speed of the whole analysis and one can think that miniaturization of the LC system is compulsory because the pressure to decrease solvent usage continues. The demand of LC–MS(–MS) in laboratories has already induced the reduction of the SPE format and the automation for a high throughput using microplates. This area will certainly be expanded in the near future. The key features of automation include miniaturization, throughput, reproducibility and traceability.

Contrarily, in the environmental field the trends are to multiresidue analysis with an increasing number of analytes. Monitoring the environment and preserving the water quality will be a key-issue of the next century. Besides well established on-line laboratory methods allowing extraction, separation, identification and quantification of any analyte, automated, on-site and on-line techniques are the priority. Efficient monitoring tools will be required using field methods such as sensors, biosensors and other fields tests. Most of these methods lack of sensitivity or are strongly perturbated from matrix effects in contami-

nated samples. These methods will gain in interest by coupling simple SPE methods, removing the matrix effect and providing an easy-to-obtain enrichment factor of 10 to 100.

As pointed out in this review, tremendous effort has been made these recent years by companies for targeting the extraction of polar analytes with the introduction on the market of new polymers and carbons with high specific surface area. Identifying new environmental problems and looking at the responsible compounds or metabolites often involves the availability of SPE sorbent able to extract very polar analytes in aqueous samples. Some metabolites are not known just because they are not taken into account by the extraction procedure. There is still an effort to be made in the extraction of some highly neutral and water soluble analytes.

References

[1] D. Barcelo, M.-C. Hennion, in: Determination of Pesticides and Their Degradation Products in Water, Elsevier, Amsterdam, 1997, pp. 249–356.
[2] E.M. Thurman, M.S. Mills, Solid-Phase Extraction – Principles and Practice, Wiley, New York, 1998.
[3] N.J.K. Simpson, Solid Phase Extraction – Principles, Strategies and Applications, Marcel Dekker, New York, 1998.
[4] R.E. Majors, LC·GC Int. September (1998) 8–16.
[5] M.C. Hennion, C. Cau-Dit-Coumes, V. Pichon, J. Chromatogr. A 823 (1998) 147.
[6] S. Chiron, A. Fernandez Alba, D. Barcelo, Environ. Sci. Technol. 27 (1993) 2352.
[7] M.C. Hennion, P. Scribe, in: D. Barcelo (Ed.), Environmental Analysis: Techniques, Applications and Quality Assurance, Elsevier, Amsterdam, 1993, pp. 24–77.
[8] M.W.F. Nielen, R.W. Frei, U.A.Th. Brinkman, in: R.W. Frei, K. Zech (Eds.), Selective Sample Handling and Detection in High Performance Liquid Chromatography, Part A, Journal of Chromatography Library, Vol. 39A, Elsevier, Amsterdam, 1988, p. 5, Ch. 1.
[9] M.-C. Hennion, Trends Anal. Chem. 10 (1991) 317.
[10] M.-C. Hennion, V. Pichon, Environ. Sci. Technol. 28 (1994) 576A.
[11] G. Font, J. Manes, J.C. Molto, Y. Pico, J. Chromatogr. 642 (1993) 135.
[12] A.R. Fernandez-Alba, A. Agüera, M. Contreras, G. Penuela, I. Ferrer, D. Barcelo, J. Chromatogr. A 823 (1998) 35.
[13] J.S. Fritz, P.J. Dumont, L.W. Schmidt, J. Chromatogr. A 691 (1995) 133.
[14] E.R. Brouwer, S. Kofman, U.A.Th. Brinkman, J. Chromatogr. A 703 (1995) 167.
[15] L.A. Berruetta, B. Gallo, F. Vicente, Chromatographia 40 (1995) 474.
[16] D. Barcelo, M.C. Hennion, Anal. Chim. Acta 318 (1995) 1.
[17] D. Barcelo, M.-C. Hennion, in: Determination of Pesticides and their Degradation Products in Water, Elsevier, Amsterdam, 1997, pp. 357–428.
[18] U.A.Th. Brinkman, Environ. Sci. Technol. 29 (1995) 79A.
[19] U.A.Th. Brinkman, J. Chromatogr. A 665 (1994) 217.
[20] S. Dupas, S. Guenu S, V. Pichon, A. Montiel, B. Welte, M.C. Hennion, Int. J. Environ. Anal. Chem. 65 (1996) 53.
[21] I. Urbe, J. Ruana, J. Chromatogr. A 778 (1998) 337.
[22] V. Pichon, M. Charpak, M.-C. Hennion, J. Chromatogr. A 795 (1998) 83.
[23] D.D. Blevins, D.O. Hall, LC·GC Int. September (1998) 17–20.
[24] R. Eisert, K. Levsen, J. Chromatogr. A 733 (1996) 143.
[25] J. Dugay, C. Miège, M.C. Hennion, J. Chromatogr. A 795 (1998) 27.
[26] L.S. DeBruin, P.D. Josephy, J. Pawliszyn, Anal. Chem. 70 (1998) 1986.
[27] S. Magdic, A. Boyd-Boland, K. Jinno, J.B. Pawliszyn, J. Chromatogr. A 736 (1996) 736.
[28] R. Ferrari, T. Nilsson, R. Arena, P. Arletti, G. Bartolucci, G. Basla, F. Cioni, G. Del Carlo, P. Dellavedova, E. Fattore, M. Fungi, C. Grote, M. Guidotti, S. Morgollo, L. Muller, M. Volante, J. Chromatogr. A 795 (1998) 371.
[29] H.L. Lord, J. Pawliszyn, Anal. Chem. 69 (1997) 3899.
[30] T. Gorecki, P. Martos, J. Pawliszyn, Anal. Chem. 70 (1998) 19.
[31] R. Eisert, J. Pawliszyn, J. Chromatogr. A 776 (1997) 293.
[32] A. Saraullo, P.A. Martos, J. Pawliszyn, Anal. Chem. 69 (1997) 1992.
[33] D. McDowall, LC·GC Int. 7 (1994) 638.
[34] D.S. Seibert, C.F. Poole, J. High Resolut. Chromatogr. 21 (1998) 481.
[35] M.H. Abrahams, Chem. Soc. Rev. 22 (1993) 73.
[36] K.G. Miller, C.F. Poole, J. High Resolut. Chromatogr. 176 (1994) 125.
[37] P. Subra, M.-C. Hennion, R. Rosset, R.W. Frei, J. Chromatogr. 456 (1988) 121.
[38] C.E. Werkhoven-Goewie, U.A.Th. Brinkman, R.W. Frei, Anal. Chem. 53 (1981) 2072.
[39] W. Golkiewicz, C.E. Werkhoven-Goewie, U.A.Th. Brinkman, R.W. Frei, H. Colin, G. Guiochon, J. Chromatogr. Sci. 21 (1983) 27.
[40] R. Ferrer, J.L. Beltran, J. Guiteras, Anal. Chim. Acta 346 (1997) 217.
[41] M.L. Larrivee, C.F. Poole, Anal. Chem. 66 (1994) 139.
[42] I. Liska, J. Chromatogr. A 665 (1993) 163.
[43] I. Liska, E.R. Brouwer, A.G. Ostheimer, H. Lingeman, U.A.Th. Brinkman, R.B. Geerdink, W.H. Mulder, Int. J. Environ. Anal. Chem. 47 (1992) 267.
[44] J. Slobodnik, H. Lingeman, U.A.Th. Brinkman, Chromatographia, in press.
[45] C.F. Poole, S.K. Poole, D.S. Seibert, C.M. Chapman, J. Chromatogr. B 689 (1997) 245.
[46] D. Seibert, C.F. Poole, Chromatographia 41 (1995) 51.
[47] M.L. Mayer, C.F. Poole, M.P. Henry, J. Chromatogr. A 695 (1995) 267.

[48] M.L. Mayer, S.K. Poole, C.F. Poole, J. Chromatogr. A 697 (1995) 89.
[49] P. Lovkvist, J.A. Jonsson, Anal. Chem. 59 (1987) 818.
[50] M.-C. Hennion, V. Coquart, J. Chromatogr. 642 (1993) 211.
[51] V. Pichon, C. Cau Dit Coumes, L. Chen, S. Guenu, M.-C. Hennion, J. Chromatogr. A 737 (1996) 25.
[52] V. Pichon, L. Chen, S. Guenu, M.-C. Hennion, J. Chromatogr. A 711 (1995) 257.
[53] S. Guenu, M.-C. Hennion, J. Chromatogr. A 737 (1996) 15.
[54] A. Galencser, G. Kiss, Z. Krivaczy, Z. Varga-Puchony, J. Halvay, J. Chromatogr. A 693 (1995) 217.
[55] A. Galencser, G. Kiss, Z. Krivaczy, Z. Varga-Puchony, J. Halvay, J. Chromatogr. A 693 (1995) 227.
[56] K.K. Chee, M.K. Wong, H.K. Lee, Chromatographia 41 (1995) 191.
[57] P.J. Schoenmakers, H.A.H. Billiet, L. de Galan, J. Chromatogr. 282 (1983) 107.
[58] P. Jandera, J. Kubat, J. Chromatogr. 500 (1990) 281.
[59] T. Braumann, G. Weber, L.H. Grimme, J. Chromatogr. 261 (1983) 329.
[60] T. Braumann, J. Chromatogr. 373 (1986) 191.
[61] C. Hansch, A. Leo, Exploring QSAR, Fundamentals and Applications in Chemistry and in Biology, American Chemical Society, Washington, DC, 1995.
[62] E.M. Thurman, R.L. Malcom, G.R. Aiken, Anal. Chem. 50 (1978) 775.
[63] D.S. Seibert, C.F. Poole, Anal. Commun. 35 (1998) 147.
[64] D.S. Seibert, C.F. Poole, M.H. Abraham, Analyst 121 (1996) 511.
[65] D. Bolliet, C.F. Poole, Chromatographia 46 (1997) 381.
[66] D. Bolliet, C.F. Poole, Analyst 123 (1998) 295.
[67] D. Bolliet, C.F. Poole, M. Roses, Anal. Chim. Acta 368 (1998) 129.
[68] S.K. Poole, C.F. Poole, Anal. Commun. 34 (1997) 247.
[69] V. Coquart, P. Garcia-Carmacho, M.C. Hennion, Int. J. Environ. Anal. Chem. 52 (1993) 99.
[70] V. Pichon, M.-C. Hennion, J. Chromatogr. A 665 (1994) 269.
[71] M.C. Hennion, Analusis 26 (1998) 131.
[72] U.A.Th. Brinkman, J. Slobodnik, J.J. Vreuls, Trends Anal. Chem. 13 (1994) 373.
[73] S. Lacorte, D. Barcelo, Anal. Chim. Acta 296 (1994) 223.
[74] S. Lacorte, D. Barcelo, J. Chromatogr. A 725 (1996) 85.
[75] S. Lacorte, D. Barcelo, Environ. Sci. Technol. 29 (1995) 2834.
[76] I. Ferrer, M.-C. Hennion, D. Barcelo, Anal. Chem. 69 (1997) 4508.
[77] J. Slobodnik, M.G.M. Groenewegen, E.R. Brouwer, H. Lingeman, U.A.Th. Brinkman, J. Chromatogr. 642 (1993) 359.
[78] S. Lacorte, J.J. Vreuls, J.S. Salau, F. Ventura, D. Barcelo, J. Chromatogr. A 795 (1998) 71.
[79] D. Barcelo, S. Chiron, S. Lacorte, E. Martinez, J.S. Salau, M.C. Hennion, Trends Anal. Chem. 31 (1994) 352.
[80] S.A. Senseman, T.L. Lavy, J.D. Mattice, Anal. Chem. 67 (1995) 3064.
[81] E. Martinez, D. Barcelo, Chromatographia 42 (1996) 72.
[82] C. Crescenzi, A. Di Corcia, M.D. Madbouly, R. Samperi, Environ. Sci. Technol. 29 (1995) 2185.
[83] G.A. Penuela, D. Barcelo, J. Chromatogr. A 823 (1998) 81.
[84] G. Carrera, P. Fernandez, R. Vilanova, J.O. Grimalt, J. Chromatogr. A 823 (1998) 189.
[85] G.A. Penuela, D. Barcelo, J. Chromatogr. A 795 (1998) 93.
[86] S. Lacorte, N. Ehresmann, D. Barcelo, Environ. Sci. Technol. 29 (1995) 2834.
[87] I. Ferrer, D. Barcelo, J. Chromatogr. A 778 (1997) 161.
[88] M. Castillo, D. Puig, D. Barcelo, J. Chromatogr. A 778 (1997) 301.
[89] I. Liska, K. Bilikova, J. Chromatogr. A 795 (1998) 61.
[90] B.A. Bidlingmeyer, JCS 35 (1997) 392.
[91] B. Buszewski, M. Jizierska, M. Welniak, D. Berek, J. High Resolut. Chromatogr. 21 (1998) 276.
[92] S. Kobayashi, I. Tanaka, O. Shirota, T. Kanda, Y. Ohtsu, J. Chromatogr. A 828 (1998) 75.
[93] Y. Sudo, J. Chromatogr. A 757 (1997) 21.
[94] J.J. Kirkland, J.W. Henderson, J.J. DeStefano, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 762 (1997) 97.
[95] Y. Sudo, T. Wada, J. Chromatogr. A 813 (1998) 239.
[96] A.B. Scholten, H.A. Claessens, J.W. de Haan, C.A. Cramers, J. Chromatogr. A 759 (1997) 37.
[97] J. Nawrocki, J. Chromatogr. A 779 (1997) 29.
[98] R.J.M. Vervoort, M.W.J. Derksen, A.J.J. Debets, J. Chromatogr. A 765 (1997) 157.
[99] E. Cruz, M.R. Euerby, C.M. Johnson, C.A. Hackett, Chromatographia 44 (1997) 151.
[100] M.J. Wirth, D.J. Swinton, Anal. Chem. 70 (1998) 5264.
[101] H. Engelhardt, M. Nikolov, M. Arangio, M. Scherer, Chromatographia 48 (1998) 183.
[102] D.V. McCalley, R.G. Brereton, J. Chromatogr. A 828 (1998) 407.
[103] Y. Berenznitski, M. Joraniec, J. Chromatogr. A 828 (1998) 51.
[104] J.E. O'Gara, B.A. Alden, T.H. Walter, J.S. Petersen, C.L. Nierderlander, U.D. Neue, Anal. Chem. 67 (1995) 3809.
[105] J.J. Kirkland, J.B. Adams, M.A. van Straten, H.A. Claessens, Anal. Chem. 70 (1998) 4344.
[106] B. Buszewski, R. Gadzala-Kociuchn, R. Kaliszan, M. Markuszewski, M.T. Matyska, J.J. Pesek, Chromatographia 48 (1998) 615.
[107] J.A. Blackwell, P.W. Carr, J. High Resolut. Chromatogr. 21 (1998) 427.
[108] D. Sykora, E. Tesarova, M. Popl, J. Chromatogr. A 758 (1997) 37.
[109] A.I. Gasco-Lopez, A. Santos-Montes, R. Izquierdo-Hornillos, J. Chromatogr. Sci. 35 (1997) 525.
[110] P. Martin, E.D. Morgan, I. Wilson, Anal. Chem. 69 (1997) 2972.
[111] D. Puig, D. Barcelo, Chromatographia 40 (1995) 435.
[112] R.A. McLaughlin, B.S. Johnson, J. Chromatogr. A 790 (1997) 161.
[113] K. Kimata, K. Hosoya, H. Kuroki, N. Tanaka, J.R. Barr, P.C. McClure, D.G. Patterson, E. Jakobsson, A. Bergman, J. Chromatogr. A 786 (1997) 237.
[114] A. Di Corcia, M. Marchetti, Environ. Sci. Technol. 26 (1992) 66.
[115] F. Ortiz Boyer, J.M. Fernandez Romero, M.D. Luque de Castro, J.M. Quesada, Chromatographia 47 (1998) 367.

[116] R.W. Fedeniuk, P.J. Shand, J. Chromatogr. A 812 (1998) 3.
[117] K. Kronkvist, M. Gustavsson, A.K. Wendel, H. Jaegfeldt, J. Chromatogr. A 823 (1998) 401.
[118] J. Hempenius, J. Wieling, J.P.G. Brakenhoff, F.A. Maris, J.H.G. Jonkman, J. Chromatogr. B 714 (1998) 361.
[119] G.P. Mc Nahon, M. Kelly, Anal. Chem. 70 (1998) 409.
[120] R. Rhül, H. Nau, Chromatographia 45 (1997) 269.
[121] H. Torenson, B.M. Eriksson, Chromatographia 45 (1997) 29.
[122] H. Bagheri, E.R. Brouwer, E.A. Struys, J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 647 (1993) 121.
[123] C. Aguilar, I. Ferrer, F. Burrull, R.M. Marcé, D. Barcelo, J. Chromatogr. A 794 (1998) 147.
[124] A.C. Hogenboom, U.K. Malmqvist, K. Nolkrantz, J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 759 (1997) 55.
[125] A.C. Hogenboon, P. Speksnijder, R.J. Vreeken, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 777 (1997) 81.
[126] A.C. Hogenboon, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 794 (1998) 201.
[127] A.C. Hogenboon, R.J. Steen, W.M. Niessen, U.A.Th. Brinkman, Chromatographia 48 (1998) 475.
[128] E.R. Brouwer, A.N.J. Hermans, H. Lingeman, U.A.Th. Brinkman, J. Chromatogr. A 669 (1994) 45.
[129] E.R. Brouwer, E.A. Struys, J.J. Vreuls, U.A.Th. Brinkman, Fresenius J. Anal. Chem. 350 (1994) 487.
[130] D. Barcelo, S. Chiron, S. Lacorte, E. Martinez, J.S. Salau, M.-C. Hennion, Trends Anal. Chem. 13 (1994) 352.
[131] V. Pichon, Analusis 26 (1998) 91.
[132] C. Molina, P. Grasso, E. Benfenati, D. Barcelo, J. Chromatogr. A 737 (1996) 47.
[133] M. Castillo, D. Puig, D. Barcelo, J. Chromatogr. A 778 (1997) 301.
[134] A. Junker-Buchheit, M. Witzenbacher, J. Chromatogr. A 737 (1996) 25.
[135] C. Crespo, R.M. Marcé, F. Burull, J. Chromatogr. A 670 (1994) 135.
[136] F. Hernandez, C. Hidalgo, J.V. Sancho, F.J. Lopez, Anal. Chem. 70 (1998) 3322.
[137] J. Hodgeson, J. Collins, W. Bashe, J. Chromatogr. A 659 (1994) 395.
[138] A.M. Kvistad, E. Lundanes, T. Greibrokk, Chromatographia 48 (1998) 707.
[139] C. Aguilar, F. Borrull, R.M. Marcé, J. Chromatogr. A 771 (1997) 221.
[140] T.A. Albanis, D.G. Hela, T.M. Sakellarides, I.K. Konstantinou, J. Chromatogr. A 823 (1998) 59.
[141] I. Tolosa, J.W. Readman, L.D. Mee, J. Chromatogr. A 725 (1998) 93.
[142] L. Schmidt, J.J. Sun, J.S. Fritz, D.F. Hagen, C.G. Markell, E.E. Wisted, J. Chromatogr. 641 (1993) 57.
[143] P.J. Dumont, J.S. Fritz, J. Chromatogr. A 691 (1995) 123.
[144] T.K. Chanmbers, J.S. Fritz, J. Chromatogr. A 797 (1998) 139.
[145] N. Masqué, M. Galia, R.M. Marcé, F. Borrull, J. Chromatogr. A 803 (1998) 147.
[146] N. Masqué, R.M. Marcé, F. Borrull, J. Chromatogr. A 793 (1998) 257.
[147] N. Masqué, M. Galia, R.M. Marcé, F. Borrull, J. Chromatogr. A 771 (1997) 55.
[148] E.S.P. Bouvier, P.C. Iraneta, U.D. Neue, P.D. McDonald, D.J. Phillips, M. Capparella, Y.F. Cheng, LC·GC Int. September (1998) 35.
[149] V. Pichon, C. Cau Dit Coumes, L. Chen, M.-C. Hennion, Int. J. Environ. Anal. Chem. 65 (1996) 11.
[150] Y.F. Chen, D.J. Phillips, U. Neue, Chromatographia 44 (1997) 187.
[151] J. Slobodnik, A.J.H. Louter, J.J. Vreuls, I. Liska, U.A.Th. Brinkman, J. Chromatogr. A 768 (1998) 239.
[152] G.R. Mills, J. Chromatogr. A 813 (1998) 63.
[153] C. Hidalgo, J.V.C. Sancho, F.J. Lopez, F. Hernandez, J. Chromatogr. A 823 (1998) 121.
[154] R. El Harrak, M. Calull, R.M. Marcé, F. Borrull, J. High Resolut. Chromatogr. 21 (1998) 667.
[155] R.J. Vreeken, P. Speksnijder, I. Bobeldijk-Pastorova, Th.H.M. Noij, J. Chromatogr. A 794 (1998) 187.
[156] S. Guenu, M.C. Hennion, Anal. Methods Instrum. 2 (1995) 247.
[157] J.M. Soriano, B. Jimenez, M.J. Redondo, J.C. Molto, J. Chromatogr. A 822 (1998) 67.
[158] D. Puig, D. Barcelo, J. Chromatogr. A 778 (1998) 313.
[159] N. Masqué, E. Pocurull, R.M. Marcé, F. Borrull, Chromatographia 47 (1998) 177.
[160] E. Pocurull, R.M. Marcé, F. Borrull, Chromatographia 41 (1998) 521.
[161] J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallet, R.S. Plumb, J. Chromatogr. A 828 (1998) 199.
[162] A. Di Corcia, S. Marchese, R. Samperi, J. Chromatogr. 642 (1993) 175.
[163] A. Di Corcia, S. Marchese, R. Samperi, J. Chromatogr. 642 (1993) 163.
[164] H. Sabik, R. Jeannot, J. Chromatogr. A 818 (1998) 197.
[165] A. Lagana, G. Fago, A. Marino, J. Chromatogr. A 796 (1998) 309.
[166] C. Crescenzi, A. Di Corcia, A. Marcomini, R. Samperi, Anal. Chem. 67 (1995) 1797.
[167] A. Di Corcia, M. Marchetti, Anal. Chem. 63 (1991) 580.
[168] G. D'Ascenzo, A. Gentili, S. Marchese, A. Marino, D. Perret, Chromatographia 48 (1998) 497.
[169] C. Crescenzi, A. Di Corcia, E. Guerriero, R. Samperi, Environ. Sci. Technol. 31 (1997) 479.
[170] G. D'Ascenzo, A. Gentili, S. Marchese, A. Marino, D. Perret, Environ. Sci. Technol. 32 (1998) 1340.
[171] B. Altenbach, W. Goger, Anal. Chem. 67 (1995) 2535.
[172] A. Di Corcia, A. Constantino, C. Crescenzi, E. Marinoni, R. Samperi, Environ. Sci. Technol. 32 (1998) 2401.
[173] A. Di Corcia, C. Crescenzi, A. Marcomini, R. Samperi, Environ. Sci. Technol. 32 (1998) 711.
[174] B.A. Tomkins, W.H. Griest, Anal. Chem. 68 (1996) 2533.
[175] J.H. Knox, B. Kaur, G.R. Millward, J. Chromatogr. 352 (1986) 3.
[176] M.-C. Hennion, V. Coquart, S. Guenu, C. Sella, J. Chromatogr. A 712 (1995) 287.
[177] V. Coquart, M.C. Hennion, J. Chromatogr. 600 (1992) 195.
[178] S. Guenu, M.C. Hennion, J. Chromatogr. A 665 (1994) 243.
[179] E.Y. Ting, M. Porter, Anal. Chem. 69 (1997) 675.

[180] K. Koizumi, J. Chromatogr. A 720 (1996) 119.
[181] Q.H. Wan, M.C. Davies, P.N. Shaw, D.Z.A. Barrett, Anal. Chem. 68 (1996) 437.
[182] E. Matisova, S. Skrabakova, J. Chromatogr. A 707 (1995) 145.
[183] C. Elfakir, M. Lafosse, J. Chromatogr. A 782 (1997) 191.
[184] P. Chaimbault, C. Elfakir, M. Lafosse, J. Chromatogr. A 797 (1998) 83.
[185] D.A. Barrett, M. Pawula, R.D. Knaggs, P.N. Shaw, Chromatographia 47 (1998) 667.
[186] A. Di Corcia, C. Crescenzi, E. Guerriero, R. Samperi, Environ. Sci. Technol. 31 (1997) 1658.
[187] I. Rodriguez, M.C. Mejuto, M.H. Bollain, R. Cela, J. Chromatogr. A 786 (1998) 285.
[188] C.M. Torres, Y. Pico, J. Manes, J. Chromatogr. A 778 (1997) 127.
[189] S. Guenu, M.C. Hennion, J. Chromatogr. A 725 (1996) 57.
[190] M. Ibanez, Y. Pico, J. Manes, Chromatographia 45 (1997) 402.
[191] M.W.F. Nielen, U.A.Th. Brinkman, R.W. Frei, Anal. Chem. 57 (1985) 806.
[192] V. Pichon, M.-C. Hennion, Anal. Chim. Acta 284 (1993) 317.
[193] J. Dugay, M.-C. Hennion, Trends Anal. Chem 14 (1995) 407.
[194] E.R. Brouwer, J. Slobodnik, H. Lingeman, U.A.Th. Brinkman, Analusis 20 (1992) 121.
[195] F.T. Lange, M. Wenz, H.J. Brauch, J. High. Resolut. Chromatogr. 18 (1995) 243.
[196] T. Reemtsma, J. Chromatogr. A 733 (1996) 473.
[197] C. Sarzanini, M.C. Bruzzoniti, G. Sacchero, E. Mentasti, J. Chromatogr. A 739 (1996) 63.
[198] C.E. Wujcik, T.M. Cahill, J.N. Seiber, Anal. Chem. 70 (1998) 4074.
[199] J.A. Field, J. Monohan, J. Chromatogr. A 741 (1996) 85.
[200] J.A. Field, J. Monohan, R. Reed, Anal. Chem. 70 (1998) 1956.
[201] M.W.F. Nielen, J. de Jong, R.W. Frei, U.A.Th. Brinkman, Int. J. Environ. Anal. Chem. 25 (1987) 37.
[202] V. Coquart, P. Garcia-Camacho, M.-C. Hennion, Int. J. Environ. Anal. Chem. 52 (1993) 99.
[203] V. Coquart, M.-C. Hennion, Chromatographia 37 (1993) 392.
[204] V. Coquart, M.-C. Hennion, Sci. Total Environ. 132 (1993) 349.
[205] R.B. Geerdink, C.A.A. Van Balkom, H. Brouwer, J. Chromatogr. 481 (1986) 488.
[206] E.R. Brouwer, T.M. Tol, H. Lingeman, U.A.Th. Brinkman, Quim. Anal. 12 (1993) 88.
[207] E. Mallat, D. Barcelo, J. Chromatogr. A 823 (1998) 129.
[208] E.G. Gonzales-Mazo, M. Honing, D. Barcelo, A. Gomez-Parra, Environ. Sci. Technol. 31 (1997) 504.
[209] A. Di Corcia, R. Samperi, Anal. Chem. 62 (1990) 1490.
[210] E.M. Thurman, M.S. Mills, in: Solid-Phase Extraction – Principles and Practice, Wiley, New York, 1998, p. 199.
[211] M.S. Mills, E.M. Thurman, M.J. Pedersen, J. Chromatogr. 629 (1993) 11.
[212] S. Rudaz, W. Haerdi, J.L. Veuthey, Chromatographia 44 (1997) 283.
[213] D.L. Ambrose, J. Fritz, M.R. Buchmeiser, N. Atzl, G.K. Bonn, J. Chromatogr. A 786 (1997) 256.
[214] K. Eder, M.R. Buchmeiser, G.K. Bonn, J. Chromatogr. A 810 (1998) 43.
[215] J. Li, S. Fritz, J. Chromatogr. A 793 (1998) 231.
[216] L.K. Tan, A.J. Liem, Anal. Chem. 70 (1998) 191.
[217] M.J.C. Rozemeijer, K. Olie, P. de Voogt, J. Chromatogr. A 761 (1997) 219.
[218] H. Carlsson, C. Ostman, J. Chromatogr. A 790 (1997) 73.
[219] G.R. van der Hoff, R.A. Baumann, P. van Zoonen, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 20 (1997) 222.
[220] S. Moret, L.S. Conte, J. High Resolut. Chromatogr. 21 (1998) 253.
[221] G.R. van der Hoff, S.M. Gort, R.A. Baumann, P. van Zoonen, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 14 (1991) 465.
[222] I.H. Hagestam, T.C. Pinkerton, Anal. Chem. 57 (1985) 2445.
[223] K.K. Unger, Chromatographia 31 (1991) 507.
[224] S. Vielhauer, A. Rudolphi, K.S. Boos, D. Seidel, J. Chromatogr. B 666 (1995) 315.
[225] A. Rudolphi, K.S. Boos, D. Seidel, Chromatographia 41 (1995) 645.
[226] B.J. Gurley, M. Marx, K. Olsen, J. Chromatogr. B 670 (1995) 358.
[227] Z. Yu, D. Westerlund, J. Chromatogr. A 725 (1996) 137.
[228] Z. Yu, D. Westerlund, J. Chromatogr. A 725 (1996) 149.
[229] Z. Yu, D. Westerlund, K.S. Boos, J. Chromatogr. B 698 (1997) 379.
[230] K.S. Boos, A. Rudolphi, LC·GC Int. 15 (1997) 602.
[231] K.S. Boos, A. Rudolphi, S. Vielhauer, A. Walfort, D. Lubda, F. Eisenbeiss, Fresenius J. Anal. Chem. 352 (1995) 684.
[232] K.S. Boos, C.H. Grimm, Trends Anal. Chem. 18 (1999) 175.
[233] Z. Yu, D. Westerlund, J. Chromatogr. A 725 (1996) 149.
[234] R. Oertel, K. Richter, T. Gramatté, W. Kirch, J. Chromatogr. A 797 (1998) 203.
[235] Z. Yu, D. Westerlund, Chromatographia 44 (1997) 589.
[236] Z. Yu, D. Westerlund, Chromatographia 47 (1998) 299.
[237] R.A. van der Hoeven, A.J. Hofte, M. Frenay, H. Ikrth, U.R. Tjaden, J. van der Greef, A. Rudolphi, K.S. Boos, G. Marko Varga, L.E. Edholm, J. Chromatogr. A 762 (1997) 193.
[238] F. Mangani, G. Luck, C. Fraudeau, E. Verette, J. Chromatogr. A 762 (1997) 235.
[239] A. Farjam, A.E. Brugman, A. Soldaat, P. Timmerman, H. Lingeman, G.J. de Jong, R.W. Frei, U.A.Th. Brinkman, Chromatographia 31 (1991) 469.
[240] A. Farjam, J. de Jong, U.A.Th. Brinkman, W. Haasnoot, A.R.M. Hamers, R. Schilt, F.A. Huf, J. Chromatogr. 452 (1988) 419.
[241] W. Haasnoot, R. Schilt, A.R.M. Hamers, F.A. Huf, A. Farjam, R.W. Frei, U.A.Th. Brinkman, J. Chromatogr. 489 (1989) 157.
[242] A. Farjam, R. de Vries, H. Lingeman, R.W. Frei, U.A.Th. Brinkman, Int. J. Environ. Anal. Chem. 44 (1991) 175.
[243] A. Farjam, C. van der Merbel, H. Lingeman, R.W. Frei, U.A.Th. Brinkman, Int. J. Environ. Anal. Chem. 45 (1991) 73.

[244] A. Frajam, N.C. van der Merbel, A.A. Nieman, H. Lingeman, U.A.Th. Brinkman, J. Chromatogr. 589 (1992) 141.
[245] V. Pichon, L. Chen, M.-C. Hennion, R. Daniel, A. Martel, F. Le Goffic, J. Abian, D. Barcelo, Anal. Chem. 67 (1995) 2451.
[246] V. Pichon, L. Chen, M.-C. Hennion, Anal. Chim. Acta 311 (1995) 429.
[247] V. Pichon, L. Chen, N. Durand, F. le Goffic, M.-C. Hennion, J. Chromatogr. A 725 (1996) 107.
[248] W. Haasnot, M.E. Ploum, R.J.A. Paulussen, R. Schilt, F.A. Huf, J. Chromatogr. 519 (1990) 323.
[249] G.S. Rule, A.V. Mordehal, J. Henion, Anal. Chem. 66 (1994) 230.
[250] G.S. Rule, J. Henion, J. Chromatogr. 582 (1992) 103.
[251] A. Marx, T. Giersch, B. Hock, Anal. Lett. 28 (1995) 267.
[252] D.H. Thomas, M. Beck-Westermeyer, D.S. Hage, Anal. Chem. 66 (1994) 3823.
[253] S.J. Shahtaheri, M.F. Katmeh, P. Kwasowski, D. Stevenson, J. Chromatogr. A 697 (1995) 131.
[254] S.J. Shahtaheri, P.W. Kwasowski, D. Stevenson, Chromatographia 47 (1998) 453.
[255] K.A. Bean, J.D. Henion, J. Chromatogr. A 791 (1997) 119.
[256] J. Cai, J.D. Henion, Anal. Chem. 68 (1996) 72.
[257] J. Cai, J. Henion, J. Chromatogr. B 691 (1997) 357.
[258] V. Pichon, H. Rogniaux, N. Fischer-Durand, S. Ben Rejeb, F. Le Goffic, M.-C. Hennion, Chromatographia 45 (1997) 289.
[259] S. Ouyang, Y. Xu, Y.H. Chen, Anal. Chem. 70 (1998) 931.
[260] M. Bouzige, V. Pichon, M.-C. Hennion, J. Chromatogr. A 823 (1998) 197.
[261] M. Bouzige, V. Pichon, M.-C. Hennion, Environ. Sci. Technol. 33 (1999) 1916.
[262] M. Cichna, D. Knopp, R. Niessner, Anal. Chim. Acta 339 (1997) 241.
[263] A. Martin-Esteban, P. Kwasowski, D. Stevenson, Chromatographia 45 (1997) 364.
[264] V. Pichon, M. Bouzige, M.-C. Hennion, Anal. Chim. Acta 376 (1998) 21.
[265] V. Pichon, M. Bouzige, C. Miège, M.C. Hennion, Trends Anal. Chem. 18 (1999) 219.
[266] C.S. Creaser, S.F. Feely, E. Houghton, M. Seymour, J. Chromatogr. A 794 (1998) 37.
[267] V. Pichon, E. Aulard-Macler, H. Oubihi, P. Sassiat, M.-C. Hennion, M. Caude, Chromatographia 46 (1997) 529.
[268] J.F. Lawrence, C. Menard, M.-C. Hennion, V. Pichon, F. Le Goffic, N. Durand, J. Chromatogr. A 732 (1996) 277.
[269] J.F. Lawrence, C. Menard, M.-C. Hennion, V. Pichon, F. Le Goffic, N. Durand, J. Chromatogr. A 752 (1996) 147.
[270] S. Perez, I. Ferrer, M.-C. Hennion, D. Barcelo, Anal. Chem. 70 (1998) 4996.
[271] I. Ferrer, V. Pichon, M.-C. Hennion, D. Barcelo, J. Chromatogr. A 777 (1997) 91.
[272] I. Ferrer, M.-C. Hennion, D. Barcelo, Anal. Chem. 69 (1997) 4508.
[273] J. Henion, E. Brewer, G. Rule, Anal. Chem. 70 (1998) 650A.
[274] A.G. Mayes, K. Mosbach, Trends Anal. Chem. 16 (1997) 321.
[275] K. Hosoya, Y. Shirasu, K. Kimata, N. Tanaka, Anal. Chem. 70 (1998) 943.
[276] K. Hosoya, K. Yoshizako, H. Sasaki, K. Kimata, N. Tanaka, J. Chromatogr. A 828 (1998) 91.
[277] O. Ramström, L. Ye, M. Krook, K. Mosbach, Chromatographia 47 (1998) 465.
[278] O. Ramström, L. Ye, P.E. Gustavsson, Chromatographia 48 (1998) 197.
[279] L.I. Andersson, Anal. Chem. 68 (1996) 111.
[280] K. Haupt, A. Dzgoev, K. Mosbach, Anal. Chem. 70 (1998) 628.
[281] K. Haupt, A.G. Mayes, K. Mosbach, Anal. Chem. 70 (1998) 3936.
[282] R. Levi, S. McNiven, S.A. Pilletsky, S. H Cheong, K. Yano, I. Karube, Anal. Chem. 69 (1997) 2017.
[283] B.A. Rashid, R.J. Briggs, J.N. Hay, D. Stevenson, Anal. Commun. 34 (1997) 303.
[284] B. Sellergreen, Anal. Chem. 66 (1994) 1578.
[285] L.I. Anderson, A. Paprica, T. Arvidsson, Chromatographia 46 (1997) 57.
[286] P. Martin, I.D. Wilson, D.E. Morgan, G.R. Jones, K. Jones, Anal. Commun. 34 (1997) 45.
[287] M.T. Muldoon, L.H. Stanker, Anal. Chem. 69 (1997) 803.
[288] J. Matsui, M. Okada, M. Tsuruoka, T. Takeuchi, Anal. Commun. 34 (1997) 85.
[289] K. Yano, I. Karube, Trends Anal. Chem. 18 (1999) 199.
[290] J. Olsen, P. Martin, I.D. Wilson, Anal. Commun. 35 (1998) 13H.
[291] B. Sellergren, Trends Anal. Chem. 16 (1997) 310.
[292] D. Stevenson, Trends Anal. Chem. 18 (1999) 154.
[293] B. Sellergren, Trends Anal. Chem. 18 (1999) 164.
[294] I. Ferrer, D. Barcelo, Trends Anal. Chem. 18 (1999) 180.
[295] A. Zander, P. Findlay, T. Renner, B. Sellergren, A. Swietlow, Anal. Chem. 70 (1998) 3304.
[296] S. Rimmer, Chromatographia 46 (1998) 470.
[297] R.A. Ansell, K. Mosbach, J. Chromatogr. A 787 (1997) 55.
[298] K. Yoshizako, K. Hosoya, Y. Iwakoshi, K. Kimata, N. Tanaka, Anal. Chem. 70 (1998) 386.
[299] M.J. Whitecombe, L. Martin, E.N. Vulfson, Chromatographia 47 (1998) 457–464.
[300] J. Haginaka, H. Takehira, K. Hosoya, N. Tanaka, J. Chromatogr. A 816 (1998) 113.
[301] W.M. Mullet, E.P.C. Lai, Anal. Chem. 70 (1998) 3636.
[302] C.E. Goewie, P. Kwakman, R.W. Frei, U.A.Th. Brinkman, W. Maasfield, T. Seshari, A. Kettrup, J. Chromatogr. 284 (1984) 73.
[303] M.W.F. Nielen, R. Bluker, R.W. Frei, U.A.Th. Brinkman, J. Chromatogr. 358 (1986) 393.
[304] M.W.F. Nielen, H.E. Van Inger, A.J. Valk, R.W. Frei, U.A.Th. Brinkman, J. Liq. Chromatogr. 10 (1987) 617.
[305] J.L. Veuthey, M.A. Bagnoud, W. Haerdi, in: R.W. Frei, K. Zeich (Eds.), Selective Sample Handling and Detection in High-Performance Liquid Chromatography, Part B, Journal of Chromatography Library, Vol. 39B, Elsevier, Amsterdam, 1989, pp. 5–29.