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On-line *versus* off-line solid-phase extraction in the determination of organic contaminants in water

Advantages and limitations

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ABSTRACT

Solid-phase extraction (SPE) has become and important and frequently employed method for the preconcentration of organic pollutants from water samples. From the methodological standpoint, two basic approaches can be recognized, on-line and off-line, each of which has its advantages and limitations. The original off-line modification is simple and highly flexible. Therefore, it is often used in analytical research and in quick testing methods. On the other hand, the possibility of automation and the high sample throughput of on-line SPE are the major reasons for its growing use in routine target analyses and in analytical methods for continuous monitoring of water quality. While the growing interest in automation in laboratory practice leads to promising perspectives for on-line techniques, the relatively unlimited flexibility of the classical off-line SPE makes it always a suitable procedure to be used for trace enrichment purposes in diverse analytical applications. This paper gives an overview of the basic principles and possibilities of both off-line and on-line SPE approaches and provides a brief survey of their benefits and limitations.

INTRODUCTION

Low concentrations (ng/l–μg/l) of toxic non-volatile organic compounds in water samples and the complexity of environmental matrices require the application of a suitable sample handling procedure prior to any assessment of water pollution via chromatographic techniques. The role of such a sample-handling step is to enrich all analytes of interest and to purify the original sample matrix by removing as much of the interfering components as possible. The present state of analytical chemistry can be characterized as a situation where highly efficient separation and detection systems are usually coupled with laborious and time-consuming sample handling procedures which limit the sample throughput and, often, also the overall performance of the method [1]. Thus, at present, sample handling is being considered to be the weakest aspect of environmental chromatographic analysis. This situation implies the need for the development of novel approaches to sample handling or, at least, for the thorough improvement of the effectiveness of current methods.

The techniques used most frequently for the preparation of aqueous samples prior to chromatographic analysis are liquid–liquid extraction (LLE) and solid-phase extraction (SPE). The greatest advantages of LLE are its simplicity and numerous practical verifications and performance evaluations. SPE, developed intensively in the last 20 years, has become a powerful alternative technique owing to its simplicity, flexibility and high sample throughput. Comparisons of LLE and SPE can be found in the literature

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[2–4] and experimental comparative tests performed by various workers [5,6] have shown a prevalence of SPE over LLE in many applications, especially when more polar compounds were to be preconcentrated.

The basic principle of SPE is the transfer of analytes from the aqueous phase to the active sites of the adjacent solid phase. This transfer is stimulated by the selection of appropriate operational conditions in the system of three major components, water (liquid phase)–sorbent–analyte. After the replacement of the water by a suitable liquid phase in this system, the analyte can be desorbed from the sorbent and further analysed. Usually, the SPE process is carried out in the column and is often referred to as low-performance liquid chromatography. Owing to the use of two extreme mobile phases, other synonyms such as digital chromatography, on–off or stop–go chromatography are also in use [7].

Many factors influence the efficiency of the SPE process, but the two most important are capacity and retention. An insufficient capacity of the sorbent surface can cause its overloading and, consequently, earlier breakthrough of analytes. However, this situation is considered not to be very likely owing to low concentrations of organic compounds in treated water samples and the relatively high sorption capacities of applied sorbents [8]. The more critical factor is the retention of analytes, which should be maximum in the water–sorbent–analyte system and minimum in the eluent–sorbent–analyte system. The existence of these two contradictory demands on the strength of the sorbent–analyte interactions leads to the necessity to make a compromise during the selection of working conditions for sorption and desorption so as to obtain an optimum preconcentration.

The retention of an analyte in the SPE column during sample application can be expressed via its breakthrough curve, *i.e.*, the dependence of the ratio of the effluent concentration (C) to influent analyte concentration (C_0) on the volume of the aqueous sample percolated through the SPE column (Fig. 1). In principle, the breakthrough curve has the integral shape of a common Gaussian curve. The retention time of an analyte (the maximum of the Gaussian peak) corresponds to the inflection point of the breakthrough curve. The breakthrough volume can be defined as the volume after passage of which a certain level of breakthrough of an analyte occurs. In practical applications, the breakthrough level, defined as the ratio of the outlet to inlet analyte concentration or as the fraction of the total mass of an analyte which has passed

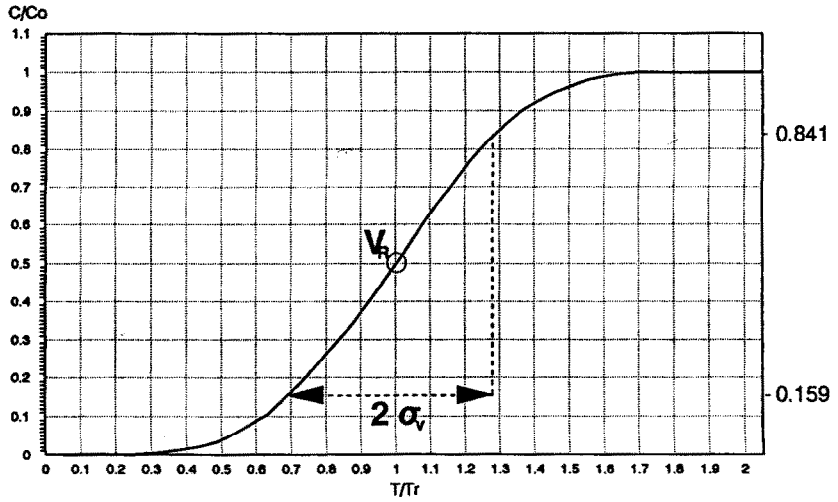

Fig. 1. Breakthrough curve. C is the solute concentration in the effluent, C_0 is the solute concentration in the sample (*i.e.*, in the influent), T is time, T_R is the retention time of the solute, V_R is the retention volume of the solute and σ_V is the elution band broadening.

out of the column, is set to 1–10% (*i.e.*, $C/C_0 = 0.01–0.1$; Fig. 1).

Having information of the shape of the breakthrough curve, the optimum volume of the preconcentrated water sample can be set so that the maximum recovery will be obtained ($V < V_B$) or the maximum amount of an analyte will be preconcentrated ($V > V_R + 3\sigma_V$; see Fig. 1), where V is the volume of the sample passed through the column, V_B is the breakthrough volume of the analyte and σ_V is the dispersion. If more analytes are to be preconcentrated on the same SPE column simultaneously, the selection of the sample volume has to be a compromise between losses of less retained components and low enrichment factors for highly retained compounds. The other solution of this problem is to use more SPE columns connected in series. The breakthrough curve can be measured experimentally either using direct methods based on the on-line (off-line) detection of the analyte in the SPE column effluent or by indirect methods, *i.e.*, evaluation of the effect of the volume of the sample on the recovery of the preconcentration.

The retention of an analyte in the SPE column during the desorption process can be demonstrated by the elution curve, *i.e.*, by the concentration profile of an analyte in the SPE column effluent (Fig. 2). From the shape of the elution curve the optimum volume of the solvent required for the quantitative desorption of the analyte can be obtained. In on-line applications the measurement of the elution curve is of minor importance as adequate information is provided by the character of additional band broadening in the analytical column.

The most often used sorbent materials in SPE applications are chemically bonded silicas, polymers and carbons. They bind analytes primarily due to hydrophobic interactions, but the secondary interactions can play a significant role in some instances, *e.g.*, ionic interactions of residual silanol groups on bonded silicas with positively charged analytes. For trapping of some groups of analytes, ion-exchange or ligand-exchange processes can also be applied using silica- and polymer-based ion exchangers or metal-loaded sorbents.

With respect to the system approach two

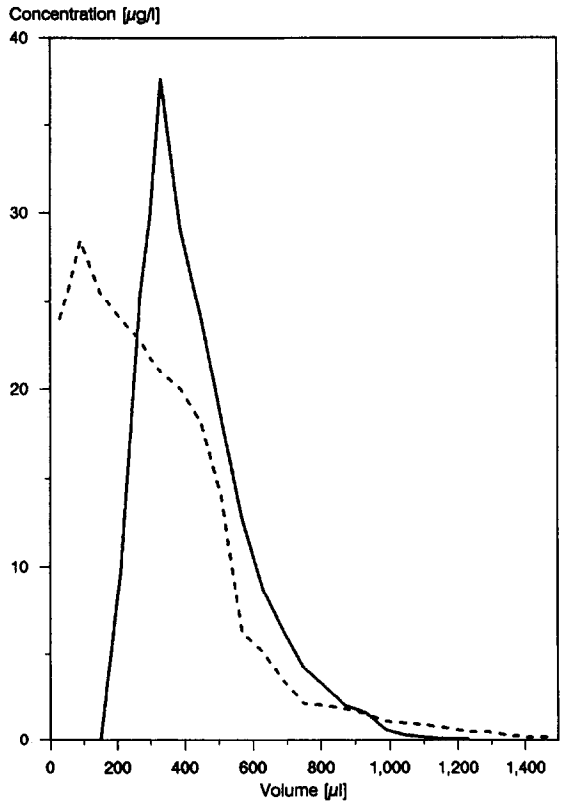

Fig. 2. Elution curves measured for (solid line) cyclohexanone and (dashed line) aniline on a C_{18} SPE column with an internal volume of 1 ml. The eluting solvent was methanol.

modes of SPE can be distinguished: off-line and on-line. In the on-line configuration the SPE column output is connected directly to the analytical column, so that elution and separation of analytes are performed in one step. In the off-line configuration, the elution and separation of analytes are two separate steps. The chemistry and general principles are the same for both of these variants, but the differences in their methodology are sometimes the reason for certain drawbacks or advantages of one of these approaches in a particular application. Nowadays, the opinions of analytical chemists on the usefulness of either of these two approaches are widely polarized.

The objective of this paper is to compare on-line and off-line SPE so as the advantages and drawbacks of these two approaches can be highlighted.

OFF-LINE APPROACH

Principles

In off-line SPE the analyte is accumulated from the water sample by a convenient SPE column and subsequently it is eluted with a suitable solvent. The eluate from the column is collected in a vial. Analytes in the eluate can be further concentrated by partial solvent evaporation, and finally, an aliquot of the eluate is injected into the chromatographic system. The volume of the water sample applied usually ranges from several tens of millilitres to tens of litres. In early SPE applications, the sorbent material was laboratory packed into a column made of stainless steel, glass or polymer with an internal volume of 1–100 ml. The introduction of commercially available disposable cartridges and suitable devices for forcing the water sample through these cartridges reduced the use of laboratory-packed columns substantially.

There is a wide choice of disposable cartridges packed with chemically bonded silicas. Cartridges with graphitized carbon black and ion exchangers are also available from several suppliers. The particle size of the sorbent packed in the SPE column is usually 40–60 μm. This diameter is sufficient to ensure an effective preconcentration and to avoid problems with the back-pressure due to suspended solids in the water sample.

To desorb trapped analytes, various organic solvents (*e.g.*, methanol, acetonitrile, ethyl acetate, diethyl ether) or their mixtures are used. To improve the efficiency of the desorption, the organic solvents can be modified by addition of an acid, base or buffer solution. An aliquot of the eluate is then analysed using one or more separation and detection systems. If needed, the analysis of the eluate can be repeated.

A typical sequence of SPE includes the following steps: activation of the sorbent (wetting with a suitable solvent), conditioning (replacing of the activation solvent by the aqueous phase), percolation of the water sample, clean-up (removal of interfering components), drying of the sorbent bed, elution of accumulated analytes and regeneration of the sorbent (usually not recommended for disposable cartridges because of memory effects [9] and/or hysteresis effects [10]).

As mentioned above, breakthrough and elution characteristics can be employed for optimization of the off-line SPE procedure in order to select suitable volumes of water sample and eluting solvent. Accordingly, the maximum theoretical preconcentration factor (F), given as the ratio of the breakthrough volume (V_B) and the width of the elution curve (W), $F = V_B/W$, was suggested [11]. This factor expresses the maximum possible preconcentration of an analyte in a particular analyte–sorbent–solvent (water/eluent) system while the water sample volume is minimal and the recovery is 100%. The practical significance of this factor is reduced when more analytes are to be preconcentrated simultaneously, but it can be used as a system parameter characterizing the efficiency of a given solid phase to preconcentrate a certain group of analytes.

Empirical approaches to optimization of the SPE procedure can be also found in the literature [12].

Advantages

The major advantages of the off-line approach are its operational flexibility and the simplicity of the equipment required. With certain simplifications, a syringe and an SPE cartridge are sufficient tools for a rapid trace enrichment. In practical applications, to avoid possible sample contamination from the sample-delivery system, a pressure difference (positive or negative, *i.e.*, compressed gas or vacuum) is used as a driving force. There are several types of a simple vacuum manifold available for the percolation of water samples. Such a device usually permits the processing of several samples simultaneously. Another way to force the water through the cartridge is to use a high pressure in the sample reservoir connected to the column inlet. This configuration is preferred in automated SPE systems.

The operational flexibility of the SPE means that there is a wide range available for setting of the operational conditions. The analyst can optimize the amount of sorbent, the type and vol-

ume of eluting solvent, the number of clean-up steps and their working conditions and the scheme of the eluent fractionation. Moreover, one can select a convenient separation and detection technique and the appropriate scale of the SPE procedure (ranging from small micro-columns to large fractionation columns).

One of the large-scale applications of off-line SPE often used in environmental analysis is its incorporation into methods dedicated to the investigation of the occurrence of organic contaminants in waters. For such qualitative broad range analysis, tens to thousands of litres of water sample were forced through a large laboratory-made column and the eluate obtained was further concentrated by evaporation to achieve a high preconcentration factor [13,14]. Such procedures were not sufficiently quantitative, but they provided extensive information on the character of water pollution. From the standpoint of scale, the opposite to large-scale SPE is solid-phase microextraction [15], a rapid, solventless and portable method employing a narrow fibre coated with a film of a suitable phase with thickness ranging from 15 to 150 μm.

To simplify the original complex sample matrix, a single operation to remove undesirable interferences or a suitable combination of several clean-up steps can be performed. Ionized analytes trapped on the solid phase can be separated from other compounds by flushing the column with alkalinized or acidified water. Subsequently, polar compounds can be eluted with appropriate mixture of water and an organic modifier. Finally, remaining non-polar compounds are eluted with pure organic solvent or a mixture of several solvents. Eluates obtained by these procedures are much simpler than the original sample and the subsequent analytical separation can be performed more easily. In principle, the analogous strategy can be used for the fractionation of sorbed analytes. The only substantial difference between clean-up and fractionation is that in clean-up there is usually only one fraction of interest and other fractions are discarded whereas in the fractionation procedure all fractions of the eluate obtained are further analysed. Generally, incorporation of clean-up and/or fractionation steps into the SPE procedure prolongs the analysis time and increases labour requirements, but the resulting information is usually worth this effort. The information gain provided by fractionation can be increased when several separation and/or detection techniques are used simultaneously.

Valls *et al.* [16] combined LLE and SPE for the accumulation of ionic and non-ionic organic contaminants from urban wastewaters and coastal sea waters. After sorption, the organic extracts were further fractionated by column chromatography and fractions were analysed by high-resolution GC–MS using different ionization techniques. This procedure allowed the identification of 290 anthropogenic contaminants in the different aquatic compartments. Asafu-Adjaye *et al.* [17] separated kepone from other pesticides by flushing the loaded SPE column with hexane, which eluted DDT, DDE and HCB. Kepone and its metabolites were subsequently eluted with a mixture of hexane and diethyl ether. Wells *et al.* [7] discussed SPE for the selective fractionation of wastewater effluents.

To optimize the recovery of a particular analyte sorbed on a given sorbent, a wide range of eluting solvents or their mixtures can be employed. Bacaloni *et al.* [18] tested nine eluents to desorb organochlorinate pesticides (OCPs) from graphitized carbon black. They found hexane–diethyl ether (50:50) to be the most efficient desorbing medium for OCPs. The recoveries for other groups of compounds showed that, using this eluent, OCPs could be completely separated from polycyclic aromatic hydrocarbons and, to a certain extent, from polychlorinated biphenyls. Combination of more SPE columns and the use of several eluting solvents can also increase the efficiency of the SPE procedure. DiCorcia *et al.* [19] extracted phenoxyacetic acid herbicides from water using a miniaturized cartridge containing graphitized carbon black (GCB) at the top and a silica-based strong anion exchanger at the bottom. After the percolation of the water sample through this cartridge the anion exchanger was activated by sodium acetate solution. Sorbed analytes were then transferred from the GCB to the anion exchanger using methyl-

ene chloride–methanol basified with sodium hydroxide. After washing, herbicides were desorbed from the anion exchanger with water–methanol containing trifluoroacetic acid and potassium chloride. A similar procedure with a cation exchanger was applied to chloroanilines [20]. The detection limits in both instances were in the ng/l range.

The great amount of work required in off-line SPE is, on the other hand, compensated for by the possibility of using various separation and/or detection techniques simultaneously, with only negligible technical limitations. Aliquots of an eluate can be injected into GC and/or HPLC systems and also transferred to other separation systems (*e.g.*, isotachophoresis, planar chromatography). The range of available GC detectors enables high sensitivities to be achieved (*e.g.*, an electron-capture or nitrogen–phosphorus detector) [21–27] and also considerable qualitative information (using MS) [28,29].

Screening methods for monitoring pesticides and other water pollutants have been at the focus of environmental analytical chemistry for a long time. Many of the screening procedures developed recently prefer the use of SPE for handling water samples. Off-line screening requires simple instrumentation and enables low detection limits to be achieved owing to the possibility of partial evaporation of the eluate from the SPE cartridge. Di Corcia and Marchetti [30] presented an off-line approach to monitoring a large group of pesticides in ground and river waters. The method incorporated fractionation of the analytes into basic, neutral and acidic compounds, based on two different interaction mechanisms on graphitized carbon black. Processing of large volumes of water (0.5–2 l) and evaporation of the eluates led to detection limits lower than 0.1 μg/l for most pesticides (Fig. 3).

The ease of manipulation with disposable SPE media has been further enhanced. Recently, membrane extraction discs have been introduced as an attractive kind of SPE material. These membranes consist of a fibrillated PTFE matrix in which sorbents such as bonded silicas, polymers or ion exchangers are enmeshed. Owing to the internal structure of the discs, high flow-rates of water samples can be achieved and the decrease in recovery due to channelling is avoided. The easy manipulation with discs makes them suitable for rapid testing methods. Hagen *et al.* [31] used discs containing C_{18} and C_8 bonded silica for the enrichment of phthalates and pesticides and Kraut-Vass and Thoma [32] preconcentrated pesticides and other pollutants. Owing to the obvious benefits of their use, discs are becoming preferred media for drinking water test methods as defined by US Environmental Protection Agency [33].

Limitations

The flexibility of the off-line approach is offset by the labour required for the overall SPE procedure. This is usually acceptable in the case of an occasional broad-range analytical survey, but it may become an obstruction when complex continuous monitoring or any similar water quality control programme with a large number of samples and high sampling frequency is to be carried out. Off-line procedures require a lot of manual work that can be automated only by means of robotic systems, at considerable financial cost.

In general, to achieve an exact determination, all measurements of the volume should be as exact as possible. In off-line SPE problems can sometimes arise due to handling of relatively small volumes of several tens of microlitres (*e.g.*, after partial evaporation of the eluate solvent after desorption). During this operation the loss of several microlitres of the eluate solvent can lead to a considerable error in the final result. Moreover, the poor reproducibility of such manual operations (especially between different operators) can also adversely affect the precision of the procedure. Injection of relatively small aliquots of the eluate increases the detection limit of the analytical procedure. This is not critical for non-polar compounds for which the handicap of aliquot injection is compensated for by the large volumes of water sample processed and by evaporation of a substantial part of the eluate solvent, but it becomes a serious problem in the case of polar and/or more volatile compounds which have low breakthrough volumes and high losses during evaporation.

The use of disposable cartridges for handling

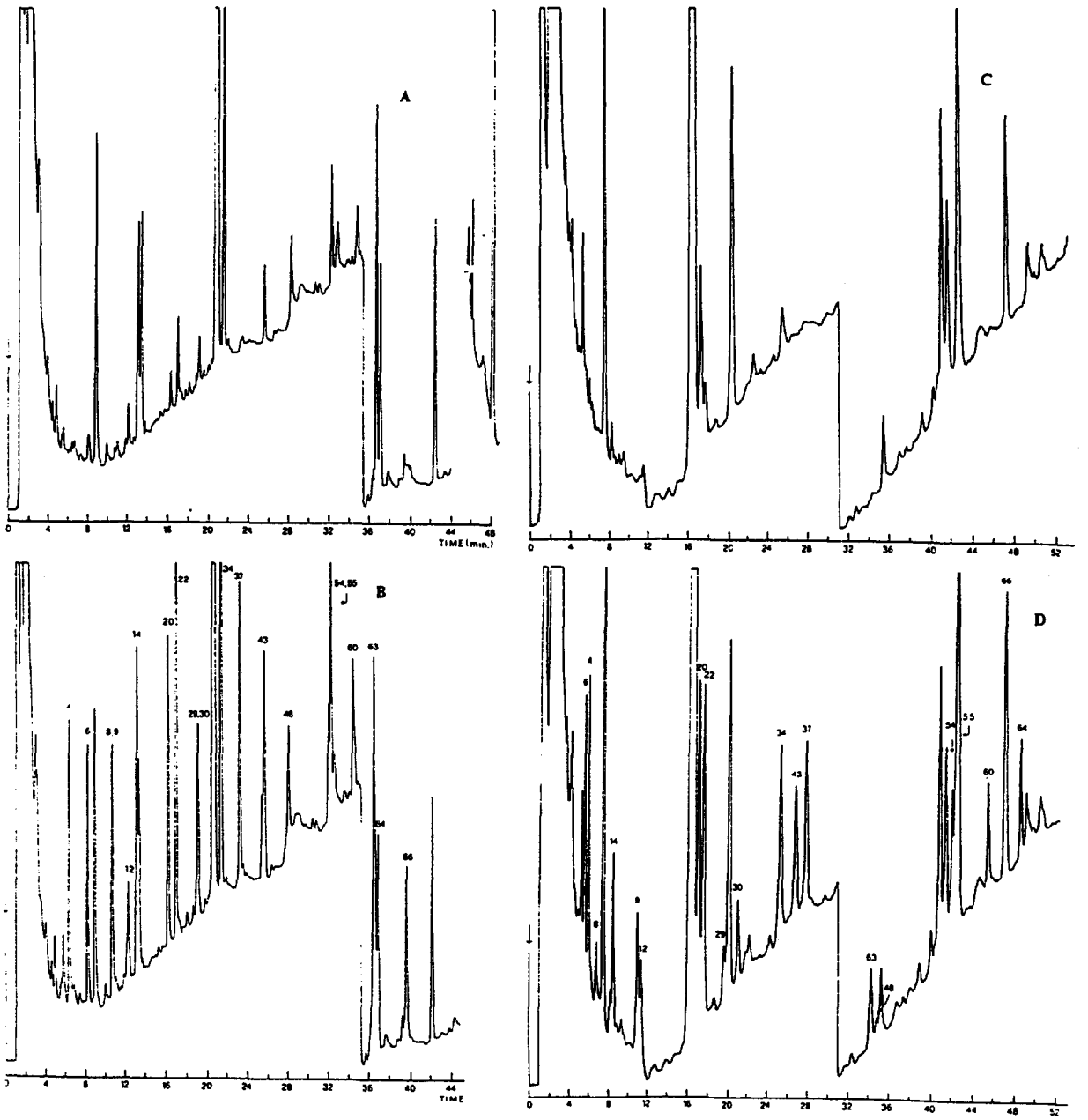

Fig. 3. Chromatograms obtained from 1.5 l of a well-water sample. Extract of unspiked sample chromatographed on the (A) C_{18} DB column and (C) the cyano column. Extract of the spiked sample chromatographed on the (B) C_{18} DB column and (D) the cyano column. The spiking level was 100 ng/l of each pesticide [except for chloridazon (peak 4; 50 ng/l) and carbaryl (peak 22; 25 ng/l)]. The extract volume injected on to the cyano column was 20 μl; UV DAD detection, attenuation 0.005 AUFS. The extract volume injected on to the C_{18} DB column was 40 μl; UV DAD detection, attenuation 0.01 AUFS. Peaks: 4 = chloridazon; 6 = mevinphos II; 8 = aldicarb; 9 = metoxuron; 12 = monuron; 14 = metribuzin; 20 = chlortoluron; 22 = carbaryl; 29 = metobromuron; 30 = paraoxon; 34 = propanil; 37 = linuron; 43 = prpyzmide; 48 = malathion; 54 = fenthion; 55 = parathion-ethyl; 60 = phoxim; 63 = butylate; 64 = metoxychlor; 66 = pendimethalin. From ref. 30 (© American Chemical Society).

water samples reduces the consumption of organic solvents when compared with LLE, but the costs of the sorbent material and solvents necessary for off-line SPE are still higher than those for on-line precolumn techniques.

Nowadays, there a broad range of bonded silica disposable cartridges are commercially available, but there is only limited access to cartridges packed with other kinds of sorbents such as polymers or carbons, which are suitable for trapping more polar compounds. Laboratory packing of the disposable cartridges can be a solution to this problem, but it increases the labour needed for the SPE procedure.

Drying of the wet cartridge prior to elution of sorbed non-polar analytes is usually applied to ensure quantitative collection of the eluate and the exact evaluation of its volume. Such operation is not difficult with this type of compound, but the application of a drying step in the SPE of some more polar compounds can adversely affect their recovery [11]. This problem can be solved by fractionation of the eluent, but it further increases the time and labour needed for the sample-handling procedure.

ON-LINE APPROACH

Principles

The chemical principle of the on-line approach is the same as that for the off-line version. The major difference is the direct transfer of sorbed analytes from the SPE column (precolumn, concentrator column) to the analytical column after changing the position of a switching valve. Hence this approach is also called precolumn switching or precolumn technology. Conventional or microbore HPLC columns usually serve as the analytical column, but GC capillaries have also been applied for this purpose. The principles and technical aspects of the on-line approach, especially for HPLC analyses, have been thoroughly described [8,34–37] and many applications have been published in environmental and biomedical analysis.

A typical simple on-line system is shown in Fig. 4. It consists of two circuits connected together by a switching valve. An aqueous sample is introduced into the precolumn by the

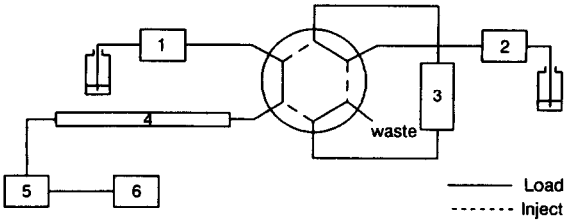

Fig. 4. Basic on-line precolumn set-up. 1 = Mobile phase pump; 2 = sample pump; 3 = precolumn; 4 = analytical column; 5 = detector; 6 = recorder/computer.

pump in the low-pressure (preconcentration) circuit and subsequently the precolumn is switched to the high-pressure circuit where the analytes are eluted by the mobile phase directly into the analytical column. The sample volume applied usually ranges from 10 to 200 ml, depending on the analytes and on the total organic load of the water sample. The internal volume of the precolumn ranges from several tens to several hundreds of microlitres. The optimum volume depends on the volume and the plate number of the analytical column and on the value of the capacity factor (k') of the analyte to be preconcentrated [38,39]. In general, the precolumn volume should be small compared with the volume of the analytical column and have a similar or smaller diameter [8]. These factors are important in minimizing the additional band broadening in the analytical column.

Theoretically, the sorbent material in the precolumn should be identical with the packing material in the analytical column. If two different sorbents are used, the retention of the analyte in the precolumn should be lower than that in the analytical column [8,37]. There is no problem in complying with this rule during the preconcentration of non-polar compounds. However, it becomes more critical in the trace enrichment of polar compounds, when a sorbent having stronger interactions with these analytes than a conventional C_{18} material should be used in the precolumn. If the precolumn containing more hydrophobic material is connected to a C_{18} analytical column, the danger of additional band broadening is obvious. However, investigations of the peak shape deterioration and some on-line applications published recently [40–44] showed that a styrene–divinylbenzene spherical copoly-

mer could retain many polar compounds sufficiently and that its connection to a C_{18} analytical column produced negligible or only low additional band broadening.

The particle size of the precolumn material should be the same as that used in the analytical column, but it is possible to use particles of a larger diameter when processing water samples with a high concentration of suspended solids to prevent problems with high back-pressure.

Advantages

The on-line approach minimizes the operator labour required for the sample preparation and his or her contact with the sample. This eliminates losses which can occur in off-line SPE during handling of the eluate (collection and evaporation, volume measurement) and during drying of the SPE column. The elimination of losses and the introduction of the whole amount of the preconcentrated analyte into the separation–detection unit improve both the sensitivity and the reproducibility of the analysis.

The regulating unit of an on-line system is the switching valve. It can be operated manually, but the whole preconcentration process can be automated using microprocessors for valve(s) control. The automation reduces the labour and time required for the analysis and allows easier control of multi-step SPE procedures. Consequently, this results in a high sample throughput. The basic on-line system (Fig. 4) can be converted into a more sophisticated set-up by incorporation of additional valves, precolumns and/or LC pumps. Such systems usually employ two or more precolumns with different sorbents connected in series (Fig. 5). Subra *et al.* [43] preconcentrated organic pollutants using C_{18} and polymeric precolumns in series connected to a C_{18} analytical column. Non-polar compounds were extracted by the first precolumn whereas the moderately and relatively polar compounds were recovered from both precolumns. The ratio of amounts preconcentrated on C_{18} and polymer (PRP-1) precolumns and variations of this ratio with the sample volume served as useful information for the identification of solutes (Fig. 6). Nielen *et al.* [45] used for the preconcentration and fractionation of organic pollutants in

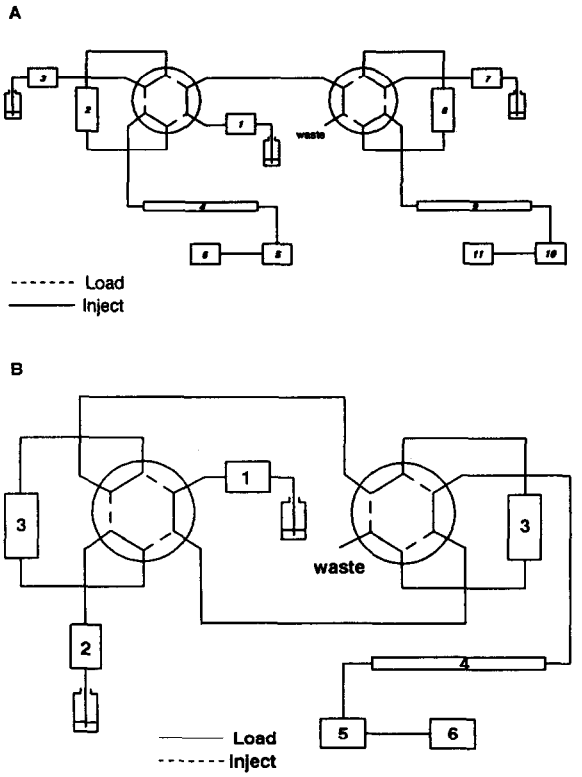

Fig. 5. Two precolumn on-line systems with (A) two or (B) one analytical column. The system components are as follows: (A) 1 = sample pump; 2, 8 = precolumns; 3, 7 = mobile phase pumps; 4, 9 = analytical columns; 5, 10 = detectors; 6, 11 = recorders/computers; (B) 1 = mobile phase pump; 2 = sample pump; 3 = precolumns; 4 = analytical column; 5 = detector; 6 = recorder/computer.

industrial effluents three precolumns in series (C_{18}, PRP-1 and cation exchanger) and eluted all precolumns separately. Brouwer *et al.* [46] connected two polymer (PLRP-S) precolumns in series and the outlet of each precolumn was directed on-line to a separate PLRP-S analytical column. While the first precolumn was operated in the reversed-phase mode, the trace enrichment in the second precolumn was based on an ion-pairing mechanism. This allowed the preconcentration of acidic and basic compounds within one analysis. The same group developed a similar system with one HPLC analytical column [47]. In this system they used as precolumns specially designed holders packed with membrane extraction discs. The advantage of this type of precolumn packing was that its amount

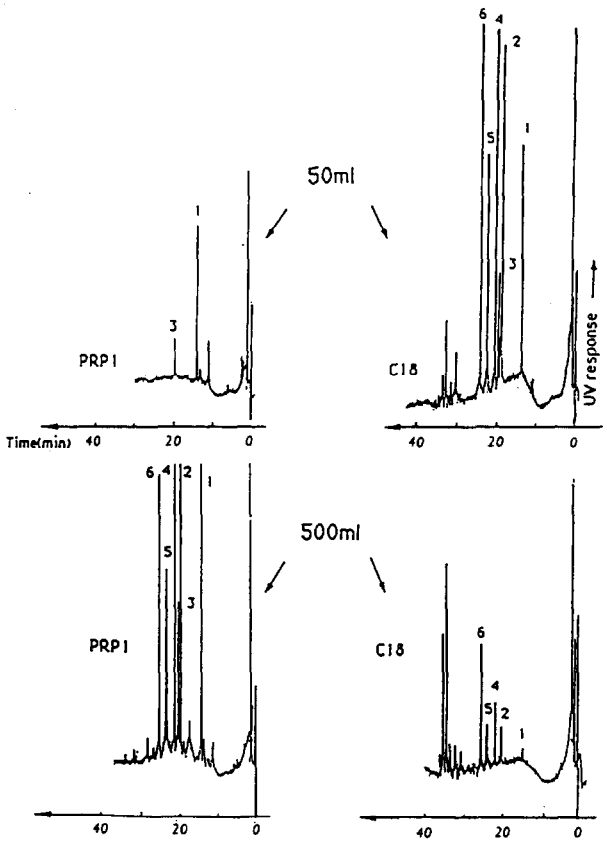

Fig. 6. On-line preconcentration of two samples of different volumes (50 and 500 ml) of a standard solution of phenylureas. The amount of the compounds is the same in the two samples (different concentrations). Solutes: 1 = metoxuron (0.83 μg); 2 = monolinuron (0.51 μg); 3 = buturon (0.83 μg); 4 = chlortoluron (0.89 μg); 5 = diuron (1.09 μg); 6 = linuron (1.18 μg). Preconcentration through two precolumns (10 × 2.1 mm I.D.) in series packed with RP-18 silica (10 μm) at a flow-rate of 3 ml/min; elution to the analytical column (150 × 4.6 mm I.D.) packed with ODS-2 silica (5 μm) at a flow-rate of 1.5 ml/min; mobile phase, acetonitrile gradient with a solution of 0.1 *M* potassium acetate–acetic acid (pH 6) and acetonitrile from 15% acetonitrile from time 0 to 5 min, 20% at 8 min, 50% at 25 min and 80% at 35 min; UV detection at 278 nm, sensitivity 0.01 AUFS. From ref. 43 (© Gordon and Breach).

was easily adjustable in accordance with the needs of a particular application.

The advantage of the high sample throughput can be demonstrated by an automated on-line system such as that developed by Ramsteiner [48]. This system allowed the selection of the sample volume to be preconcentrated and unattended processing of up to 32 water samples. He found this method to be advantageous from the viewpoints of sensitivity, rapid sample handling and costs when large monitoring programmes were to be performed and he demonstrated its superiority over previously used labour-intensive off-line techniques.

The need for continuous monitoring of water quality initiated the development of rapid screening on-line SPE–HPLC methods with UV diode-array detection. The use of on-line systems for this purpose allowed rapid access to information on water quality and a relatively high frequency of sampling. Reupert *et al.* [49] applied C_{18} materials for on-line preconcentration. They compared different C_{18} reversed phases with respect to their retention capacity. Their method was optimized for about 40 individual substances, mostly triazines and phenylureas.

The use of C_{18}, however, does not provide satisfactory results when compounds with higher polarity are to be preconcentrated. In this case the use of polymeric material in the precolumn increases the breakthrough volumes and, despite the fact that the k' values of analytes on the polymer are higher than those on C_{18}, its contribution to additional band broadening on a C_{18} analytical column is still acceptable. Such a combination of sorbents (*i.e.*, polymer in the precolumn and C_{18} in the analytical column) was tested in analyses for more than 50 compounds in aqueous matrices and for most of these compounds the results obtained were satisfactory [41]. The PLRP-S–C_{18} SPE–HPLC system with UV diode-array detection developed in that work was able to detect a wide range of water pollutants at the low- to sub-μg/l concentration level in less than 100 min (Fig. 7). After automation and optimization of all steps, this system was demonstrated to be a powerful tool for the rapid screening of surface water quality and its efficiency had already been recognized by its practical use in a surface water quality monitoring station [50].

The on-line coupling of SPE to GC is more complicated than that to HPLC owing to problems with the liquid phase–gas phase interface. However, interest in on-line LC–GC coupling is continuously increasing, as can be seen from the number of LC–GC applications in the review

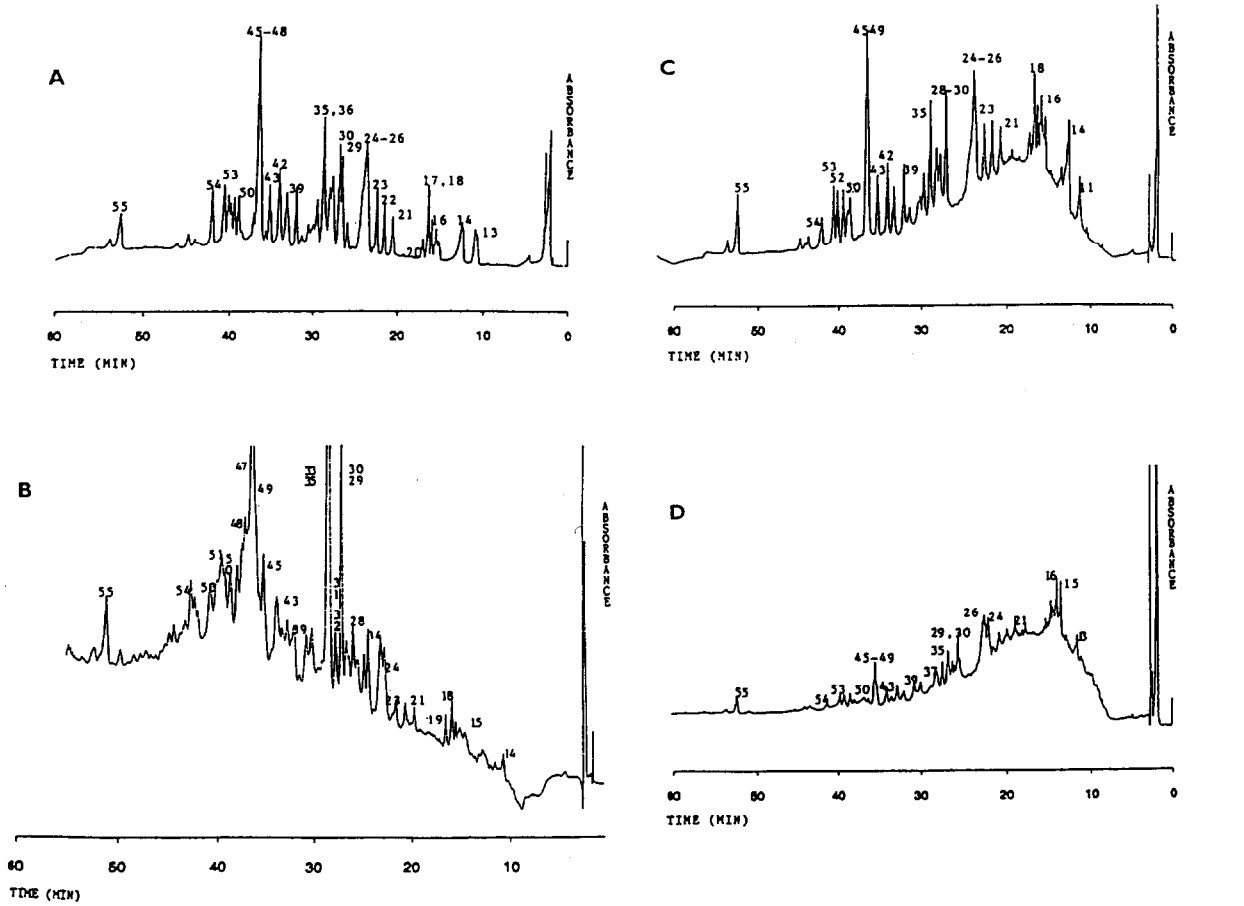

Fig. 7. Chromatograms of (A) HPLC-grade water spiked with the test compounds at concentrations of 2.5–5 μg/l, (B) HPLC-grade water spiked with the same compounds at a 25 times lower concentration, (C) non-buffered river Rhine water spiked with the test compounds at concentrations of 2.5–5 μg/l and (D) non-buffered river Rhine water spiked with the test compounds at a five times lower concentration. Detector scale 0.1 AUFS. Test compounds: 1 = diquat; 2 = paraquat; 3 = maleic hydrazide; 4 = ethylenethiourea; 5 = propylenethiourea; 6 = metham sodium; 7 = aniline; 8 = methyl isothiocyanate; 9 = aldicarb sulphone; 10 = benzenesulphonamide; 11 = asulam; 12 = oxamyl; 13 = fenaminosulf; 14 = carbendazim; 15 = metamitron; 16 = isocarbamide; 17 = 2,6-dimethylaniline; 18 = chloridazon; 19 = dimethoate; 20 = dicamba; 21 = aldicarb; 22 = bromacil; 23 = simazine; 24 = 2-chloroaniline; 25 = 2-nitrophenol; 26 = benzothiazole; 27 = bentazon; 28 = atrazine; 29 = 2,6-dichlorophenol; 30 = bromoxynil; 31 = thiram; 32 = diuron; 33 = triclopyr; 34 = monolinuron; 35 = DNOC; 36 = propachlor; 37 = dichlorprop; 38 = mecoprop; 39 = warfarin; 40 = metazachlor; 41 = linuron; 42 = 3,3-dichlorobenzidine; 43 = sethoxydim; 44 = coumafuryl; 45 = 2,4,5-trichloroaniline; 46 = captan; 47 = alachlor; 48 = metolachlor; 49 = barban; 50 = alloxydim sodium; 51 = dinoterb; 52 = dinoseb; 53 = pentachlorophenol; 54 = phoxim; 55 = permethrin. From ref. 41 (© Gordon and Breach).

paper by Davies *et al.* [51]. SPE–GC, which is also referred to as trace enrichment LC–GC, has been used for the determination of pesticides in water [52]. For that purpose a six-port switching valve was modified to incorporate an internal microcolumn packed with C_8-bonded silica. Pesticides were adsorbed on the precolumn from an aqueous sample, the precolumn was then dried by helium purging and vacuum and the analytes were eluted with hexane directly to a GC column using a retention gap. In the SPE–GC system developed by Van der Hoff *et al.* [53], the SPE precolumn served for the clean-up of the LLE extract prior to GC analysis. This approach of on-line clean-up of LLE extracts was developed by the same group also for HPLC analyses of environmental pollutants [54–57]. They used precolumn switching in combination with off-line

LLE and obtained a considerable selectivity enhancement. The main advantage of this approach, in which the on-line coupled precolumn was used only for clean-up purposes, was the removal of the large matrix peak from the chromatogram (this peak is a frequent interference in on-line SPE–HPLC systems). Even though the off-line extraction required a time-consuming manual operation, owing to the application of column switching a considerable gain in sample throughput was achieved.

The application of a sophisticated clean-up procedure is not the only possibility for achieving high selectivity. The alternative way is to use a highly selective solid phase, *e.g.*, ion exchangers, metal-loaded sorbents or a phase with immobilized enzymes. The procedures utilizing these materials are complicated and require a skilled operator, but their efficiency and suitability for special target analyses has been demonstrated by many workers [8,37,58].

Limitations

Loading of the whole amount of an analyte from the water sample on to the precolumn is usually associated with the delivery of a large number of other matrix components, unless a thorough clean-up is performed. Such a clean-up operation, however, prolongs the analytical procedure and makes it more complicated. It must be also pointed out that the addition of any further operation to the basic preconcentration cycle increases the probability of losses of analytes. Moreover, from the technical and financial standpoints, the addition of further steps to the procedure requires the incorporation of additional pumps, valves and other equipment.

Manipulation with sophisticated on-line systems also requires adequately trained technical personnel. Even though the fully automated systems can be operated, with certain simplifications, by pressing the "start" button only, troubleshooting of any minor malfunctions appearing in such a system will certainly require a skilled operator.

In on-line applications, prior to forcing the water sample through the precolumn, this sample is usually filtered to remove the suspended solid particles in order to prevent clogging of the precolumn. As a consequence, part of the analyte adsorbed on these particles cannot be trapped by the solid phase in the precolumn, and it is therefore lost for further analysis.

Using an on-line sample-handling procedure for polar compounds, it is possible to reach a relatively high preconcentration factor owing to the transfer of the whole mass of an analyte from the processed sample on to the precolumn. On-line preconcentration of polar compounds enables detection limits at low-ppb to high-ppt concentration levels to be achieved much more easily and faster than when the off-line approach is used. However, to reach the low-ng/l level, the volume of water sample to be processed has to be so large that the operation of small on-line precolumns is often problematic owing to interferences, clogging, time needed for sorption, etc. Hence for such applications many analysts prefer the use of larger off-line cartridges, which allow the processing of large volumes and which can sustain higher flow-rates. The increased volume of the eluate from a large SPE column is then reduced by partial evaporation.

Major problems can also occur during the on-line preconcentration of non-polar compounds. The very low detection limits required for these compounds generate similar difficulties with the processing of large sample volumes to those mentioned above. Moreover, the strongly hydrophobic character of such analytes causes their strong adsorption within the whole preconcentration system, which leads to so-called memory effects with the risk of obtaining false-positive results. The elimination of memory effects through extensive flushing is not always efficient. It is clear that off-line procedures that utilize disposable cartridges are more protected against memory effects.

The low flexibility in setting the desorption conditions in on-line configurations is another problem which has to be coped with when one tries to obtain an elution profile in the precolumn that is as narrow as possible and optimum separation in the analytical column. The composition of the mobile phase in an on-line SPE–HPLC system has to be primarily adjusted in accordance with the requirements for a good separation in the analytical column, hence the best solution for improving the shape of the desorption curve (*i.e.*, minimization of additional

band broadening) is focusing via an appropriate gradient profile of the mobile phase in LC.

CONCLUSIONS

Even though the development of environmental analytical chemistry towards rapid and efficient methods has led to an increase in interest in automated on-line sample-handling procedures, the high versatility and simplicity of off-line SPE often makes this approach a method of choice for a particular application. Considering the character of the reviewed applications it can be assumed that the off-line procedures are to be preferred when a complex analytical survey on water quality, requiring complicated fractionations and/or the use of several separation and detection techniques, is to be performed. The off-line approach is also often advantageous when a simple, inexpensive target method is required which can be executed in any common laboratory and also under field conditions.

In the future, the major part of routine off-line target methods will probably be gradually replaced by automated techniques. However, because of their great flexibility and simplicity, the off-line procedures will always be a valuable tool primarily in the area of analytical research and in diverse on-site applications.

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