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Review

On-line sample treatment for or via column liquid chromatography

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Abstract

It is generally recognized that sample treatment often is the bottleneck in trace-level organic analysis. As far as column liquid chromatography (LC) is concerned, the design, and commercialization, of on-line and fully automated precolumn/analytical column LC systems with diode-array UV or mass spectrometric detection is a distinct step towards solving the existing problems. Small precolumns that can be packed with sorbents of divergent selectivity, and also on-line (electro)dialysis modules, have been shown to perform well in many environmental and biomedical applications. In addition, it is noteworthy that LC or rather, LC-type, trace enrichment and clean-up on such precolumns are increasingly being used as an on-line treatment step for aqueous samples prior to capillary gas chromatographic analysis.

Contents

1. Introduction

In modern trace-level organic analysis, chromatographic techniques play a predominant role. They are used to create an efficient separation of the analytes of interest in complex environmental, biological, food(stuff) or pharmaceutical samples, prior to the actual measurement, *i.e.*, the detection step. Unfortunately, however, even the combined force of an efficient separation and a sophisticated mode of detection does not always create sufficient selectivity and/or sensitivity for the final goal to be reached: quantification and/or identification of sample constituents typically present at low-ppm to low-ppt (10^{-5}–10^{-11} g/g) levels. In such instances, special attention has to be devoted to sample pretreatment (for trace enrichment and clean-up) and pre- or post-separation derivatization or conversion of the analytes (for improved detection selectivity and/or sensitivity). In recent years, column liquid chromatography (LC), and especially reversed-phase LC with aqueous–organic eluents, has achieved widespread accept-

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ance and popularity. No doubt this is at least partly due to the increasing attention being given to polar compounds, irrespective of whether these are drugs, pesticides, industrial chemicals or their breakdown products. Selectivity and sensitivity enhancement in LC by means of (on-line) postcolumn reaction detection has been the subject of many reviews and books [1–3] and will not be considered here. Rather, attention will be devoted to sample treatment for LC, and emphasis will be given to the use of on-line techniques. These are becoming increasingly important in all those situations where (i) large series of samples have to be analysed routinely, making rapid analysis, (semi-)automation and unattended operation aspects of major concern, and (ii) sensitive trace-level determination requires the analysis of total samples or sample extracts rather than aliquots, under conditions in which analyte losses, due to, *e.g.*, evaporation or irreversible sorption to the vessel walls, and contamination, caused by the solvents or reagents used, laboratory air and/or sample manipulation in general, must be rigorously minimized.

In a majority of such cases, on-line sample treatment for LC is carried out by means of a so-called precolumn technique. Most attention will therefore be devoted to this alternative and, especially, to the role played by the nature of the precolumn packing material. In recent years, techniques such as on-line dialysis and electrodialysis have also received attention and they will be discussed accordingly. On-line precolumn derivatization is another topic of current interest, partly because pre- and postcolumn strategies aimed at enhancing detection sensitivity and/or selectivity can be readily compared. Conventional column-switching LC–LC procedures, which, in essence, are fairly straightforward heart-cutting techniques, will not be considered. The reader interested in state-of-the-art applications should consult refs. 4 and 5, which present several elegant studies on the rapid and automated determination of, *e.g.*, bentazone, isoproturon and ethylenethiourea in ground- and rain water (0.2–1 μg/l level). Finally, some attention will be devoted to on-line LC–GC (capillary gas chromatography), a topic of much current interest in which LC is used for either preseparation or trace enrichment, while the highly efficient separation–detection is performed in the GC part of the set-up.

2. On-line precolumn/analytical column LC

In an on-line precolumn/analytical column LC procedure, four main steps can be discerned (see Fig. 1): (i) loading of the sample (typically an aqueous sample or aqueous extract), which results in trace enrichment of the analytes of interest, *i.e.*, in increased sensitivity; (ii) flushing of the precolumn to wash out potentially interfering sample constituents, which ensures improved selectivity; (iii) desorption of the analytes from the precolumn, a step which should be rapid and efficient in order to ensure that the starting zone on the top of the analytical column will be sufficiently small; and (iv) reconditioning of the precolumn, which is preferred with expensive precolumns and/or when reconditioning is rapid, or exchange of the (disposable) precolumn cartridge which is recommended for all other situations.

Currently, there is an increasing tendency to use relatively small precolumns which typically have dimensions 2–10 mm × 4.6–2 mm I.D. Packed precolumns are commercially available from several manufacturers, but manual slurry or dry packing of a precolumn does not present any

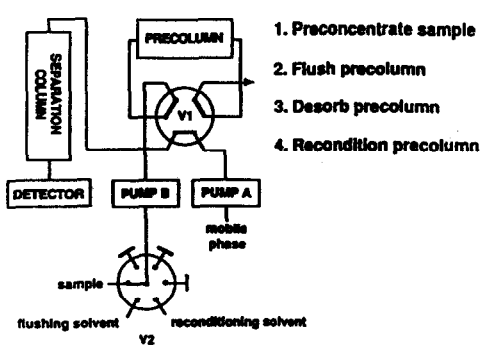

Fig. 1. Set-up of a precolumn sample pretreatment system for LC. V1 = High-pressure switching valve; V2 = low-pressure solvent-selection valve.

real problems and, in addition to rapid exchange, allows the easy screening of new packing materials. Recently, as an alternative to precolumns, so-called Empore membrane extraction discs have been introduced [6,7] that contain a suitable stationary phase (C_{18}-bonded silica, ion exchanger or copolymer; typically about 90%, w/w) enmeshed in a polytetrafluoroethylene network (10%, w/w). The successful use of small 4.6 mm diameter discs for trace enrichment coupled on-line with LC has been reported [7].

In most published procedures for on-line precolumn/analytical column LC, C_{18}- or C_8-bonded silicas are used as the precolumn packing material. A good illustration of the general usefulness of these materials is presented in Table 1 for a number of chlorophenols [8]. Obviously, even compounds as hydrophilic as dichlorophenols will have breakthrough volumes of *ca.* 10 ml on a small precolumn, whereas for a really hydrophobic compound such as pentachlorophenol the breakthrough volume is over 300 ml. Although the latter figure is, of course, more impressive, one should realize that even a 10-ml sample loading represents a 100-fold improvement in analyte detectability over a conventional 100-μl loop injection!

Alkyl-bonded silicas are very effective for trace enrichment, as is well illustrated by the data summarized in Table 2, which deal with a prolonged study on the determination of urapidil and two of its metabolites in serum and urine [9]. However, their general drawback is that together with the analytes of interest, many potentially interfering sample constituents will also be retained. Hence the selectivity will be increased to only a minor extent, if at all. In such instances, enhanced selectivity will have to be provided in the detection step. An example is given in Fig. 2, which shows the trace-level determination of several highly chlorinated phenols in tap water at

Table 1
Breakthrough volumes of selected chlorophenols on precolumns packed with alkyl-bonded silica or polymer materials

Packing material	Breakthrough volume of chlorophenol (ml)				
	Mono-	Di-	Tri-	Tetra-	Penta-
LiChrosorb RP-18	0	12	58	180	320
Hypersil C-18	0	10	35	150	340
Polymer PRP_1	30	200			

2 mm × 4.6 mm I.D. precolumn; sample, water of pH 3.

Table 2
On-line precolumn/analytical column LC of urapidil and its metabolites M_1 and M_2

Parameter	Result[a]
Recovery	95–110%
Accuracy/precision	10% or better
Detection limit:	
ECD	5 ng/ml in serum
UV	50 ng/ml in urine
Precolumn:	
Lifetime	15–20-ml of sample
New column	Every 10 samples

[a] Based on *ca.* 15 000 analyses carried out over a 2-year period; one metabolite stabilized as a result of sorption on precolumn packing material.

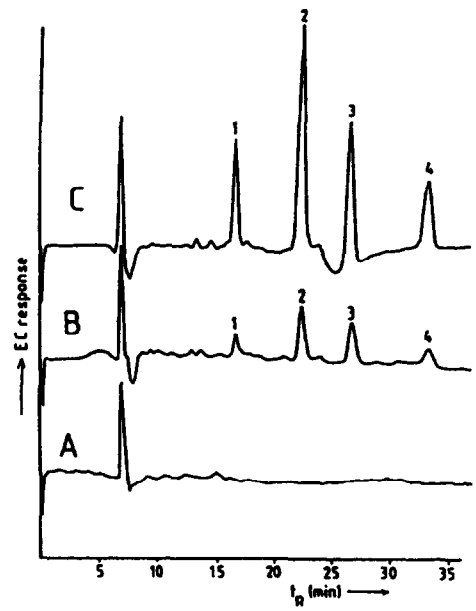

Fig. 2. LC with electron-capture detection of 40 ml of tap water after trace enrichment. (A) Non-spiked, (B) spiked with 14–45 ng/l and (C) spiked with 60–180 ng/l of (1) 2,3,6-tri-, (2) penta-, (3) 2,3,5-tri- and (4) 2,3,4-trichlorophenol. Normal-phase LC on silica with hexane–toluene–glacial acetic acid (79:20:1) as eluent.

the 10–100 ng/l level using trace enrichment combined with normal-phase LC and electron-capture (!) detection [10]. An alternative means of improving selectivity is to use postcolumn reaction detection. In one such study [11,12], N-methylcarbamates were preconcentrated on C_{18}-bonded silica and separated by conventional reversed-phase LC, the LC effluent then being led through a solid-phase reactor containing an anion-exchange resin which was kept at 100°C. The carbamates decomposed and methylamine was formed, which reacted with *o*-phthalaldehyde in a second open-capillary-type reactor. The highly fluorescent reaction product was then monitored directly, because *o*-phthalaldehyde itself does not fluoresce. As a continuation of this work, the determination of twenty parent N-methylcarbamates and twelve major degradation products in surface water was carried out, using 50 ml of water and low-carbon C_{18}-bonded (C_{18}/OH) silica for trace enrichment [13]. Although the published procedure is not fully on-line, the experimental results are highly rewarding, with detection limits of, typically 20–30 ng/l (see Fig. 3).

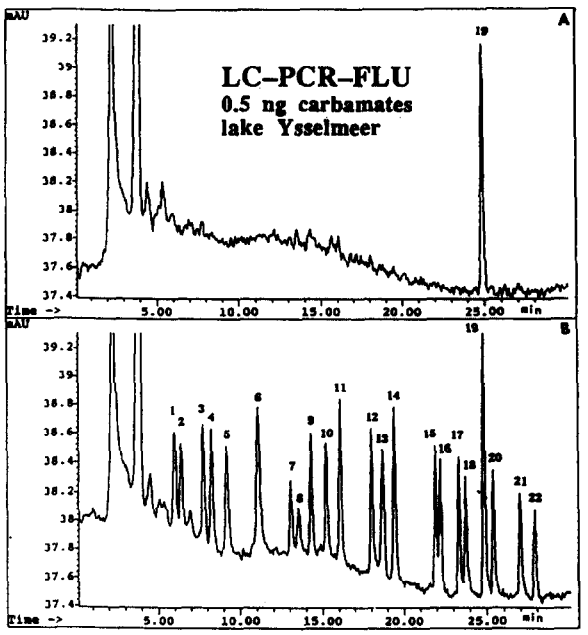

Fig. 3. LC with post-column reaction detection and fluorescence monitoring of lake IJsselmeer surface water samples after trace enrichment of 50 ml of (A) a blank water sample and (B) a water sample fortified with eleven N-methylcarbamates and ten degradation products at the 0.1 μg/l level on 500-mg C_{18}/OH cartridges. Landrin (peak 19; 1.0 ng injected) was used as an internal standard. In the case of 100% recovery, the injected amount (in 100 μl) of all other carbamates is 0.5 ng.

Obviously, if precolumns have to be used in order to also enhance selectivity, one alternative is to use a series of precolumns containing different packing materials or a selective, *e.g.*, a metal-loaded or an antibody-loaded, precolumn. Two well known examples of the former approach deal with monitoring the effluent from a wastewater treatment plant (C_{18}-bonded silica/polymer/ion exchanger precolumns) [14] and with the determination of carbohydrates in fermentation broths and spent sulphite liquor [15]. The set-up of the latter system involves two off-line and two on-line precolumns and, after the LC separation, either UV absorbance monitoring or postcolumn reaction detection using an immobilized enzyme reactor and electrochemical detection. In some instances, clean-up was sufficiently dramatic to allow UV detection of sugars at 195 nm (!).

Metal-loaded packing materials can easily be prepared by flushing an (inexpensive) thiol- or 8-hydroxyquinoline-containing poly(methyl methacrylate) polymer with an excess of an aqueous solution of a suitable metal salt, *e.g.*, silver nitrate. Ag(I)-loaded precolumns have been used to preconcentrate pyrimidine nucleobases such as uracil and thymine, structurally related compounds such as the drugs 5-fluorouracil and AZT and the pesticide bromacil [16]. The precolumn set-up used for the trace-level determination of AZT in plasma is shown in Fig. 4. Initially, the AZT-containing sample was loaded directly on the metal-loaded precolumn (sample pH = 5). Desorption with a very small (60 μl) volume of 0.1 *M* perchloric acid effected the rapid and quantitative desorption of the analyte from the precolumn and its transfer to a conventional reversed-phase LC system. Even after prolonged use, the repeated injection of the plug of strong acid did not cause a noticeable deterioration in the performance of the analytical column. However, the precolumn pro-

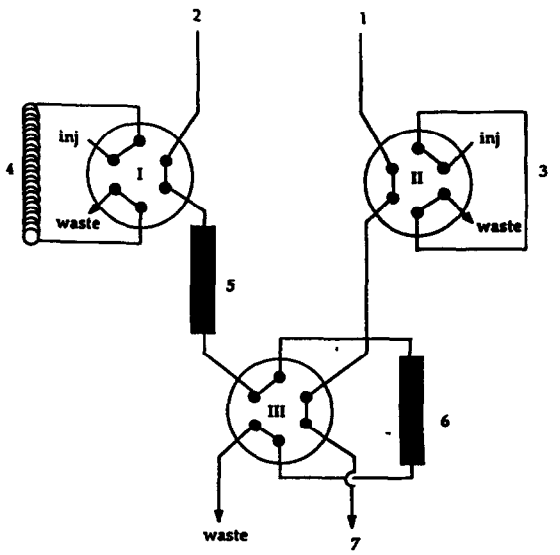

Fig. 4. Scheme of the analytical system used for the determination of AZT in biological samples. 1 = LC pump; 2 = preconcentration pump; 3 = injection loop (perchloric acid); 4 = sample injection loop; 5 = polymer-based clean-up precolumn; 6 = Ag(I)–thiol column; 7 = to LC column and UV detector.

cedure did not remove all endogenous compounds having the –NH(=CO) structure responsible for retention. For that reason, a PLRP-S copolymer precolumn was inserted (Fig. 4; No. 5) to effect additional clean-up. Under these conditions, AZT could be determined in plasma down to a concentration of 10^{-8} *M* using ordinary UV detection at 269 nm.

Interesting studies on metal-loaded precolumns are also being performed by Boos *et al.* [17]. They have synthesized a tailor-made Cu(II)–phthalocyanine-modified porous glass precolumn packing material and have set up a fully automated precolumn/analytical column LC procedure for the low-level detection of free and conjugated 1-hydroxypyrene in urine (detection limit 0.01 pmol).

The use of immobilized antibodies for selective on-line sample treatment in LC has been reported for, *e.g.*, the anabolic steroid β-19-nortestosterone and its main metabolite, α-19-nortestosterone, in calf urine and meat [18]. Other applications include the determination of β-trenbolone, chloramphenicol, clenbuterol and aflatoxin M_1 [19]. After sorption of the analyte(s) of interest from, typically, a biological fluid or milk on to a properly pretreated immunoaffinity precolumn, on-line desorption is carried out either selectively, *i.e.*, by using an essentially aqueous solution containing a so-called displacer (norgestrel in the case of nortestosterone), or non-selectively with a methanol–water or acetonitrile–water mixture containing a high proportion of modifier. In such instances, additional water has to be pumped in between the immunoaffinity precolumn outlet and the inlet of a second precolumn packed with an alkyl-bonded silica to allow refocusing of the analyte-containing zone on the latter precolumn (see Fig. 5). In most studies published so far, detection limits are well below the 1 μg/l level. Some of the immunoaffinity precolumns have been used for over 100 runs. Two relevant applications [19,20] are shown in Figs. 6 and 7.

Under certain conditions, the above line of reasoning regarding selectivity does not provide the proper answer. Within the framework of the Rhine Basin Program, an international partnership of several Dutch, German and Swiss universities, governmental institutes and instrument companies [21], a fully automated precolumn–LC–diode-array UV system has been designed for monitoring polar pesticides in river Rhine water and also water from other Euro-

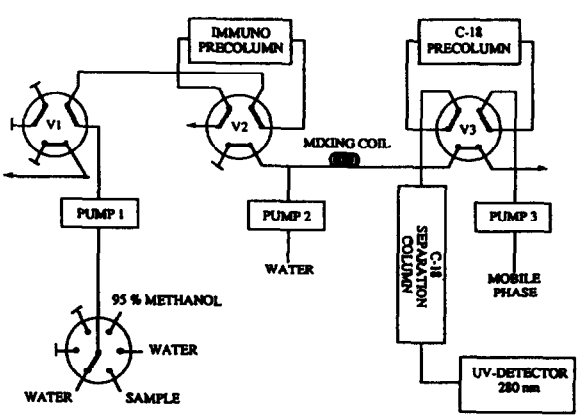

Fig. 5. Typical set-up of an on-line immuno precolumn/analytical column LC system used for non-selective desorption.

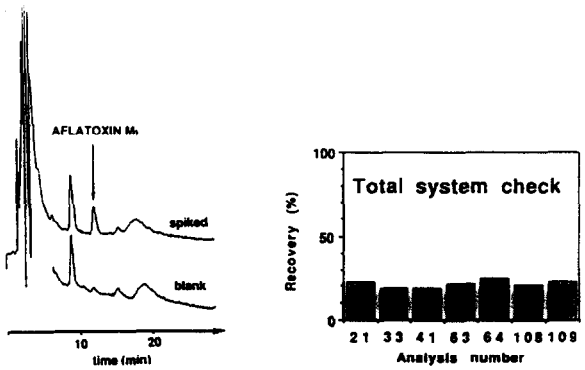

Fig. 6. LC with fluorescence detection of a crude milk sample spiked with 50 ng/l of aflatoxin M_1, and of the corresponding blank. Hollow-fibre dialysis–immunoaffinity preconcentration was performed with donor and acceptor volumes of 25 ml each. Total system check is also shown.

pean rivers (monitoring level 0.5–3 μg/l; at present, 50–80 pesticides are included). In such a case, the presence of even a single pollutant at or near the alert (1 μg/l) or alarm (3 μg/l) level is, and should be, the exception rather than the rule. In other words, instead of trying to improve selectivity by combining several types of precolumns or by using selective stationary phases, one now must try to trap whatever (unknown) pollutant is present in the river water sample on a single phase, obviously the most hydrophobic one available, *i.e.*, on a polymer packing material [22,23]. As an example, Fig. 8 shows the identification of an extremely low level of atrazine in river Rhine water. The completely automated SAMOS (System for Automated Monitoring of Organic compounds in Surface water) LC monitoring system, which combines a Prospekt (Spark Holland, Emmen, Netherlands) sample treatment module and an HP 1090 (Hewlett-Packard, Waldbronn, Germany) LC separation–diode-array detection unit, is commercially available through the latter company.

The same SAMOS approach has also been found very useful in work using on-line precolumn/analytical column LC combined with thermospray mass spectrometric (TSP-MS) detection [24]. Using, for example, 50-ml river Rhine water samples, all fifteen phenylurea herbicides tested could be detected at the 0.1

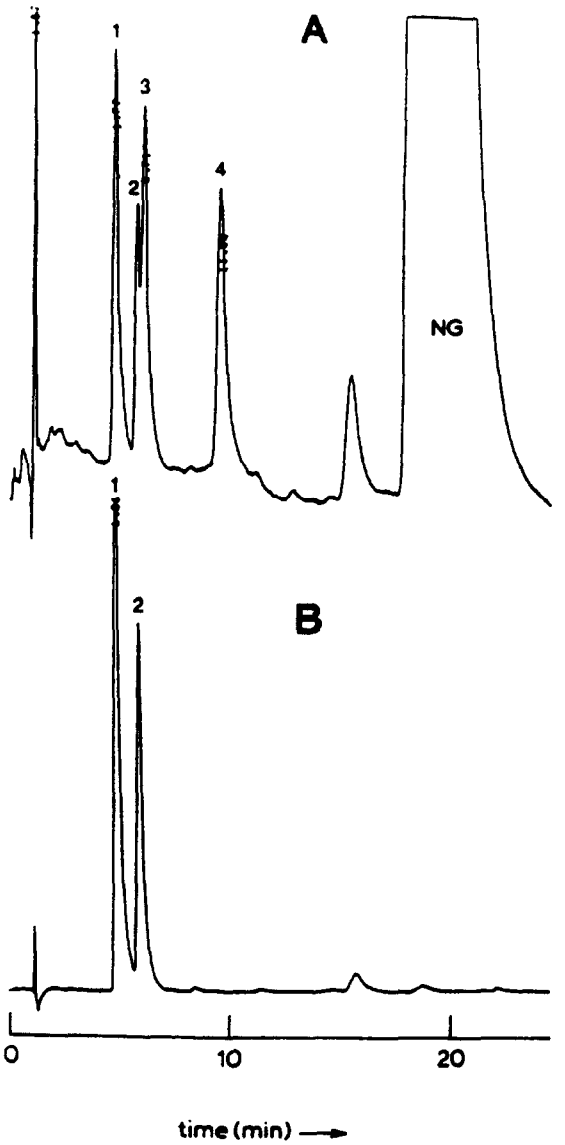

Fig. 7. LC of a mixture of (1) 17-β-trenbolone (1 μg/l), (2) 17-α-trenbolone (2 μg/l), (3) β-19-nortestosterone (0.2 μg/l) and (4) α-19-nortestosterone (0.3 μg/l); 53 ml of this mixture were loaded on to the immuno precolumn. (A) UV detection at 247 nm (0.002 AUFS); (B) UV detection at 340 nm (0.008 AUFS).

μg/l level (selected-ion monitoring). A relevant example of real analysis is given in Fig. 9.

Finally, one should mention the so-called restricted access materials such as internal-surface reversed phases, shielded hydrophobic

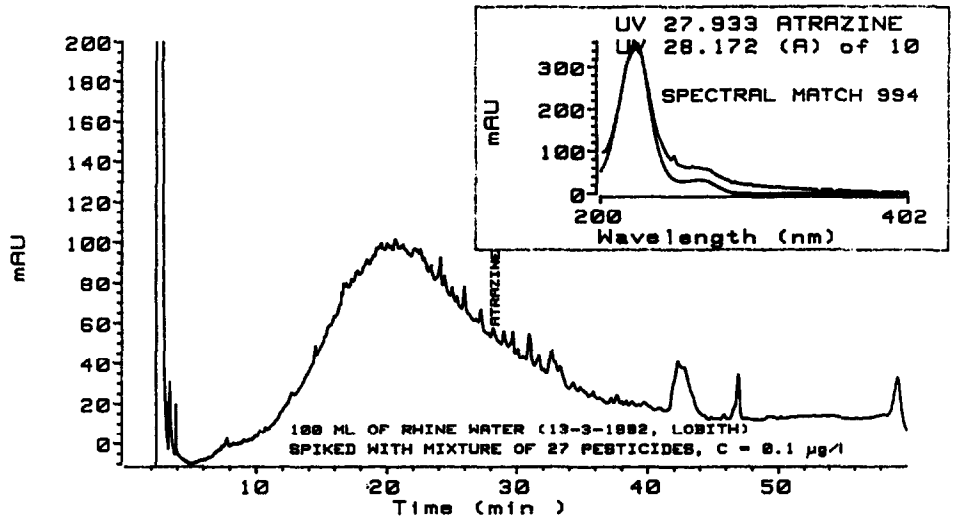

Fig. 8. Identification of a 0.1 μg/l spike of atrazine in 100 ml of river Rhine water using trace enrichment on a polymer precolumn coupled on-line with reversed-phase LC–diode-array UV detection (SAMOS LC system).

phases, semi-permeable surfaces and dual-zone materials [25,26]. The unique feature of these packings is that they prevent the access of matrix components such as proteins (which are allowed to interact only with hydrophilic, non-adsorptive layers on the outer packing surfaces), whilst selectively retaining small molecules such as drugs and their metabolites (which can penetrate

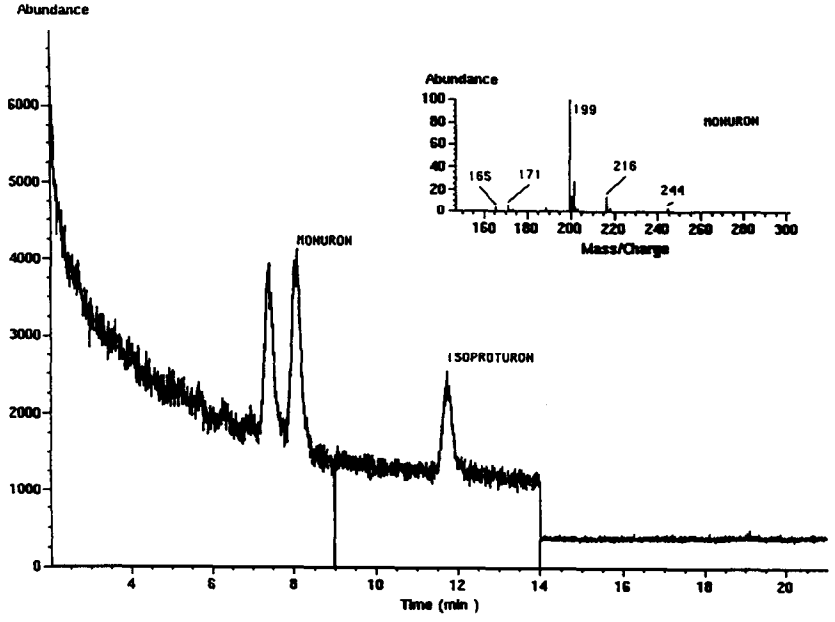

Fig. 9. On-line precolumn/analytical column LC–thermospray MS of 50 ml of river Rhine water using C_{18} membrane extraction discs and a linear methanol–0.1 *M* ammonium acetate gradient. MS monitoring: 0–10 min, *m/z* 199 and 201; 10–14 min, *m/z* 207; 14–21 min, *m/z* 275 (for detection of neburon).

the packings and gain full access to partitioning phases). Although the restricted access materials have not yet gained widespread acceptance in practice, a number of interesting single- and coupled-column mode applications have been reported, and there is no doubt that the possibility to carry out, *e.g.*, drug determinations by direct serum injection will stimulate further activity in this area.

3. Alternative on-line sample treatment techniques

In recent years, the use of dialysis as an on-line sample treatment technique for the removal of macromolecules prior to LC has received much interest; an extensive review is available [27]. A dialysis module consists of two Perspex blocks with the dialysis membrane (molecular mass cut-off typically 10 000–15 000) in between to separate the donor (sample) phase from the acceptor phase. Fig. 10 shows the general set-up of a system. It is important to realize that depending on the aim and the boundary conditions of the analysis (high recovery, rapid analysis, large or limited sample volume), a different donor–acceptor mode of operation should be selected from among the four combinations generally recommended, *viz.*, (i) stagnant–stagnant, (ii) stagnant–flowing, (iii) pulsed–flowing and (iv) flowing–flowing.

Because dialysis is based on molecular diffusion occurring as a result of the concentration gradient across the cell of the analyte of interest, it is obvious that the in itself simple stagnant–stagnant mode will be time consuming and will never effect more than a 50% recovery. In practice, therefore, a flowing acceptor stream is almost invariably selected. Dialysis now proceeds more rapidly and the recovery will be higher. Unfortunately, however, dilution is an unavoidable consequence. It is therefore necessary to insert a precolumn in the system (*cf.*, Fig. 10) to reconcentrate the analytes, while avoiding the use of too large volumes of acceptor solvent which may cause breakthrough. If relatively large sample volumes are available as with, for example, milk, the process can be further accelerated by adding the sample as a number of pulses (thus restoring the initial, high concentration gradient) or by using a continuously flowing donor (sample) stream. Relevant examples include the determination of sulphonamides [28] and nitrofurans [29] in (aqueous extracts of) eggs, meat and milk and of oxytetracycline [30] (Fig. 11) in salmon plasma and whole blood. Hollow-fibre dialysis has been shown to be

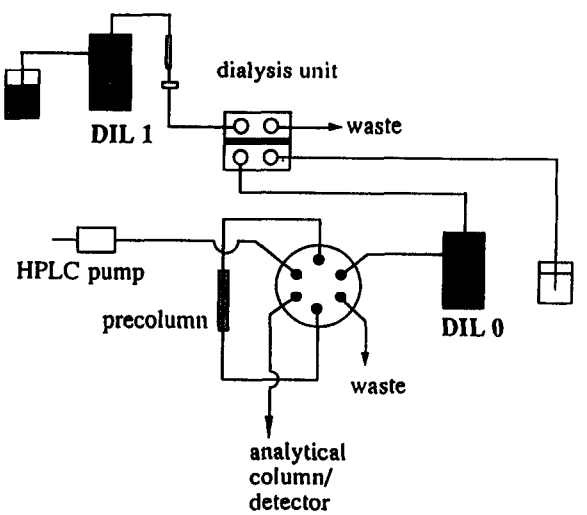

Fig. 10. General set-up for dialysis combined on-line with LC.

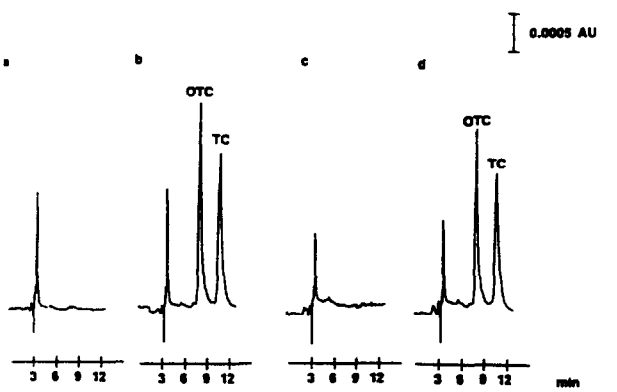

Fig. 11. Reversed-phase LC–UV detection after dialysis of (a) drug-free salmon plasma, (b) salmon plasma spiked with 1 μg/ml of oxytetracycline (OTC), (c) drug-free salmon whole blood and (d) salmon whole blood spiked with 1 μg/ml of OTC. Internal standard: tetracycline (TC).

highly rewarding in the determination of trace amounts of aflatoxins in milk by immunoaffinity precolumn/analytical column LC [20].

Recently, first results have been published [31] concerning electrodialytic sample treatment, where 5–10 V are applied across the dialysis cell (now containing electrode compartments and ion-exchange membranes next to the ordinary cut-off membrane). This promotes the transfer of charged analytes from the donor to the acceptor phase by means of electromigration. In such a case, a slowly flowing donor stream and a stagnant acceptor stream are used, and the dilution effect on the acceptor side, which invariably occurs with conventional dialysis, is absent. Rather, selective enrichment (up to 10–20-fold) of the charged analytes is achieved, as has been shown for, *e.g.*, the determination of several aromatic sulphonic acids in river Rhine water and of paraquat and diquat in groundwater (Fig. 12) [32].

A quick survey of the literature shows that, whereas postcolumn reaction detection is invariably carried out on-line, precolumn labelling or conversion of analytes is usually done off-line. In recent years, however, on-line precolumn derivatization has attracted some attention. Most studies have dealt with the low-level determination of primary and/or secondary amino acids in a variety of samples, using reagents such as 9-fluorenyl(m)ethyl chloroformate or *o*-phthalaldehyde [33–35]. Modern autosamplers or autosampler-related devices are used to carry out the required reactions under such (geometrical) conditions that on-line coupling with the LC part of the system is possible.

When utilizing *o*-phthalaldehyde as reagent, the addition of a mercapto reductant containing a chiral centre allows the separation of D- and L-amino acids, as their diastereomers, on a conventional alkyl-bonded silica column [36]. A similar result can be obtained by using pure enantiomeric (+)-9-fluorenylethyl chloroformate as the reagent (see Fig. 13) [35]. A different type of application [37] deals with the separation of nitrated polyaromatics, *e.g.*, nitrated pyrenes, as their amino analogues. Reduction is carried out on a precolumn filled with a mixture of small zinc particles and glass beads. The (chemiluminescence) detection limits are between 0.1 and

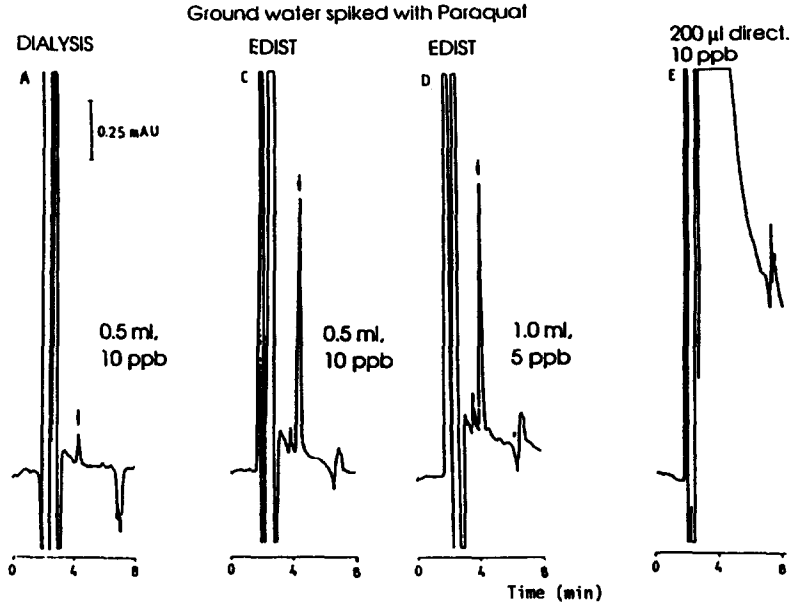

Fig. 12. LC–UV detection of ground water samples spiked with 5–10 µg/l of paraquat (see arrow). Pretreatment by means of dialysis and electrodialysis (EDIST) is compared with direct injection.

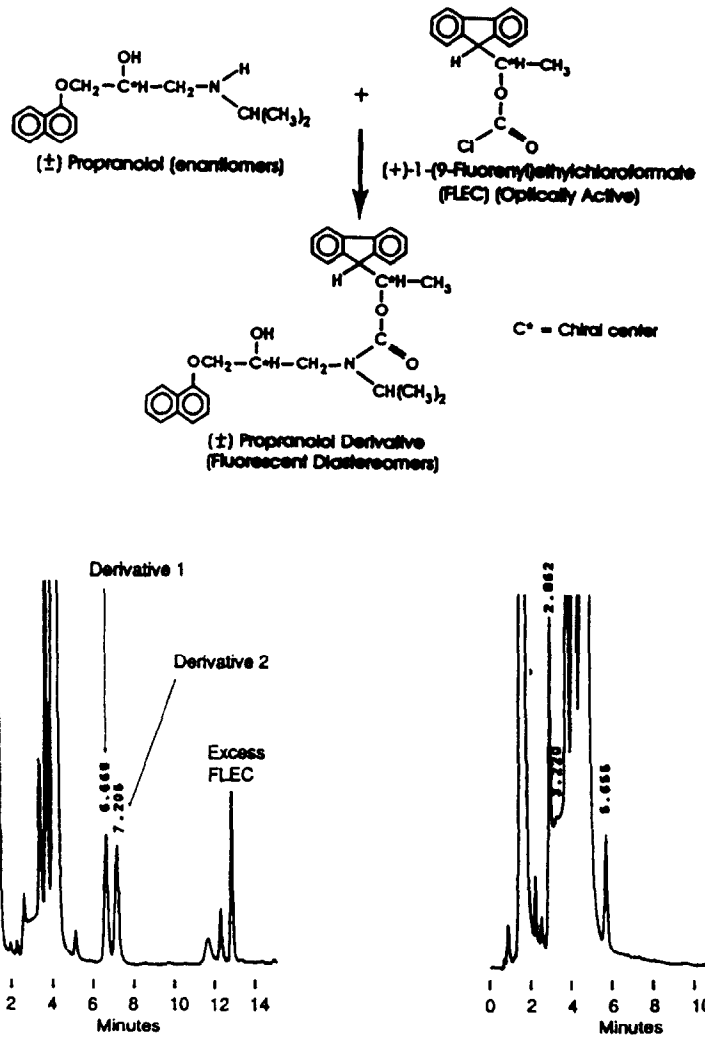

Fig. 13. Determination of (±)-propranolol using on-line precolumn derivatization using an optically active reagent and LC with fluorescence detection. Left chromatogram; FLEC-(±) propranolol, 200 pmol. Right chromatogram: FLEC-buffer (blank). Fluorescence detection: excitation wavelength 265 nm, emission wavelength 345 nm. Mobile phase: acetate–acetonitrile (30:70).

10 pg. It is interesting to add that the reduction can also be carried out successfully in the post-column (Fig. 14). The differences in retention times observed for the precolumn compared with the postcolumn procedure are, of course, due to the fact that the compounds that are actually separated are different, *i.e.*, amino and nitro analogues, respectively. Phase-transfer catalysis has also been used for on-line precolumn analyte derivatization. In one study, solutes such as ethinylestradiol and 4-monochlorophenol were determined in urine samples [38]. Dansylation in an aqueous–organic two-phase system was followed by on-line LC separation and fluorescence detection. In another study [39], a micellar system was used for the rapid derivatization of the drug valproic acid and free fatty acids in plasma using substituted coumarins and acridines as reagents. Under suitable conditions [Arkopal N130 as non-ionic surfactant, tetrakis(decyl)ammonium bromide as transfer catalyst and 9-bromomethylacridine as reagent; 50°C], the reac-

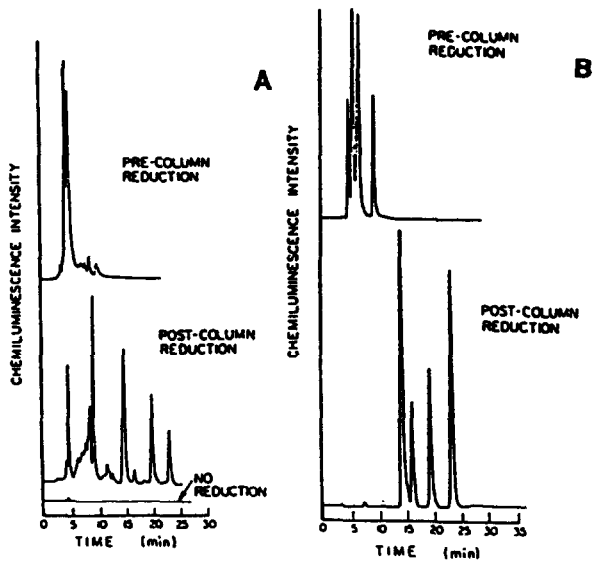

Fig. 14. LC with chemiluminescence detection of (A) a carbon black extract and (B) a nitrated pyrene sample. The nitrated polynuclear aromatics are detected as their amino analogues after on-line pre- or postcolumn reduction with powdered zinc contained in a small reactor.

tion is completed within about 10 min, and on-line coupling to an LC system presents no serious problems. In recent years, the use of off-line and (automated) on-line solid-phase derivatization has repeatedly been reported by Krull and co-workers [40–42], and achiral and chiral derivatizations of both nucleophilic analytes and amino acids have been studied. An interesting recent example is the direct determination of 1-adamantanamine in plasma and urine using a solid-phase reagent containing a covalently bound activated ester of 9-fluoreneacetate. Surprisingly, the technique has not yet gained much acceptance.

4. On-line LC–GC

Another approach to sample treatment that has been shown to be rewarding is the on-line coupling of LC and capillary GC. Admittedly, in an LC–GC system the role of LC is reduced to that of providing sample treatment (either heart-cutting or trace enrichment plus clean-up; see below) while GC provides the real separation. However, the on-line coupling of LC with, in principle, the full range of universal and selective GC detectors makes LC–GC too good a combination to be missed in an overview on pretreatment of (often aqueous) samples. A discussion of the various LC–GC interfaces currently available is inappropriate in the present context. For this aspect, the reader should consult, *e.g.*, refs. 43 and 44. Here, some interesting recent applications will be briefly discussed.

In one (heart-cutting) study [45], olive oil (diluted with hexane) was injected into a normal-phase LC system; pentane containing 2% of methyl *tert.*-butyl ether was used as eluent. The (300 μl) fraction containing the polycyclic aromatic hydrocarbons was transferred on-line via a 3-m retention gap to a GC–MS system and analysed. A relevant result is shown in Fig. 15. Another example [46] deals with the determination of the herbicide fenpropimorph in cereals.

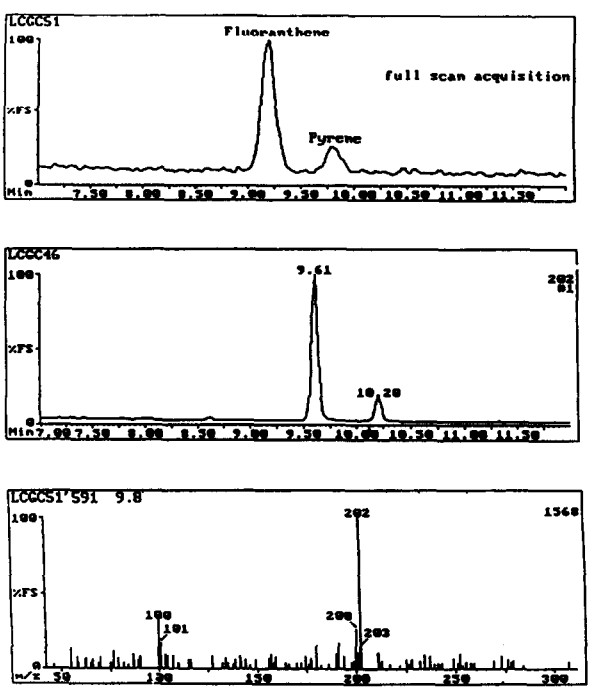

Fig. 15. On-line LC–capillary GC with MS detection of polycyclic aromatics in an olive oil sample (low μg/l level). Part of the final gas chromatogram is shown in the full-scan acquisition and the selected-ion monitoring (SIM) (m/z 202) mode; the mass spectrum of pyrene is also shown.

After reversed-phase LC, the appropriate heart-cut was mixed on-line with an organic solvent. A segmented stream resulted, the analyte of interest being extracted into the organic phase, which was separated from the aqueous phase in a non-membrane-type phase separator, collected in a loop and analysed by GC with thermionic detection (NPD).

In the literature, by far the most attention has been devoted to the heart-cut type of application. We have, however, mainly studied the trace-enrichment alternative, with emphasis on application to aqueous samples. In that case, the main concern is to remove most, if not all, of the water before the analyte-containing zone or plug enters the GC part of the system. A set-up that can be used to that end is shown in Fig. 16. An aqueous sample is loaded on the same type of small precolumn as discussed above for LC operation. Next, ethyl acetate with a flow-rate of, typically, *ca.* 50 μl/min is led through the precolumn. The organic solvent serves the double purpose of pushing any water remaining in the precolumn or the capillaries out to waste and desorbing the trapped analytes of interest, transferring them to the retention gap. Traces of water that dissolve in the ethyl acetate (*ca.* 3%, w/w) will be completely removed during partially concurrent solvent evaporation in the retention gap when ethyl acetate, which forms an azeotrope with water containing *ca.* 8% (w/w) of the latter solvent, is evaporated prior to the actual GC run. Good results have been obtained with polar analytes in surface water [47]. In another instance, three norsteroids were determined at the 0.5–5 ng/ml level in 5 ml of urine [48].

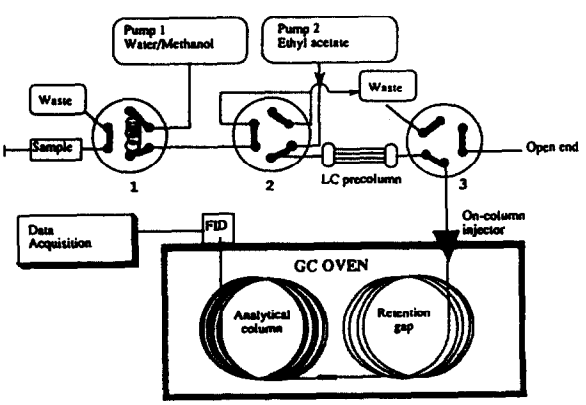

Fig. 16. Scheme of an LC–GC system involving LC-type (aqueous samples) trace enrichment and sample clean-up and on-line GC analysis (SAMOS GC system). The desorption step is shown.

Situations have also been encountered, however, in which the presence of traces of water in the retention gap caused severe peak distortion and even loss of peaks in GC analysis. As an alternative, we therefore introduced the use of Empore membrane extraction discs for trace enrichment in LC–GC. As regards loading of these discs with analytes from aqueous samples, the situation is of course identical with that in LC discussed above. The main advantage of using extraction discs in LC–GC is that on-line drying with a stream of nitrogen takes only 10–15 min at room temperature. Work on a series of organophosphorus pesticides showed that, under these conditions, analyte loss was negligible even at the sub-nanogram level. As an example, on-line membrane extraction–GC–nitrogen–phos-

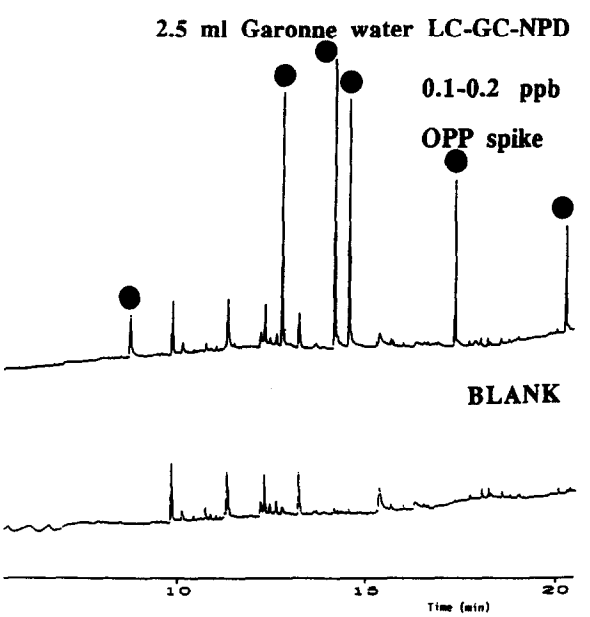

Fig. 17. LC–GC–NPD of (bottom) 2.5 ml of Garonne water and (top) 2.5 ml of Garonne water spiked with six organophosphorus pesticides. The 0.1–0.2 ng/ml spikes (see black spots) are (with increasing retention time) mevinfos, diazinon, fenitrothion, fenthion, triazofos and coumafos.

phorus detection (NPD) analyses of blank and spiked surface water are shown in Fig. 17. The six pesticides were added as 0.1–0.2 ng/ml spikes and trace enrichment was carried out using 2.5 ml of water. The cartridge holder held three 0.5-mm thick, 4.6-mm diameter polymer-loaded extraction discs, which could be re-used at least ten times [49]. Obviously, this meanwhile automated SAMOS GC approach is a promising one to meet the current directives for the determination of trace levels of pesticides in surface and drinking waters. The small volumes of sample required are an additional benefit. Very recently, similar studies have been carried out for LC–GC–MS using both full-scan acquisition and single-ion recording [50]. An interesting result obtained for a 10-ml surface water sample is shown in Fig. 18.

5. Conclusions

Sample treatment is often the bottleneck in modern organic trace-level analysis. Most procedures are still off-line in nature and they tend to be laborious, time consuming and prone to error. As there is an increasing demand for the routine monitoring of ever larger numbers of samples, the development of on-line sample treatment procedures is critically important: sen-

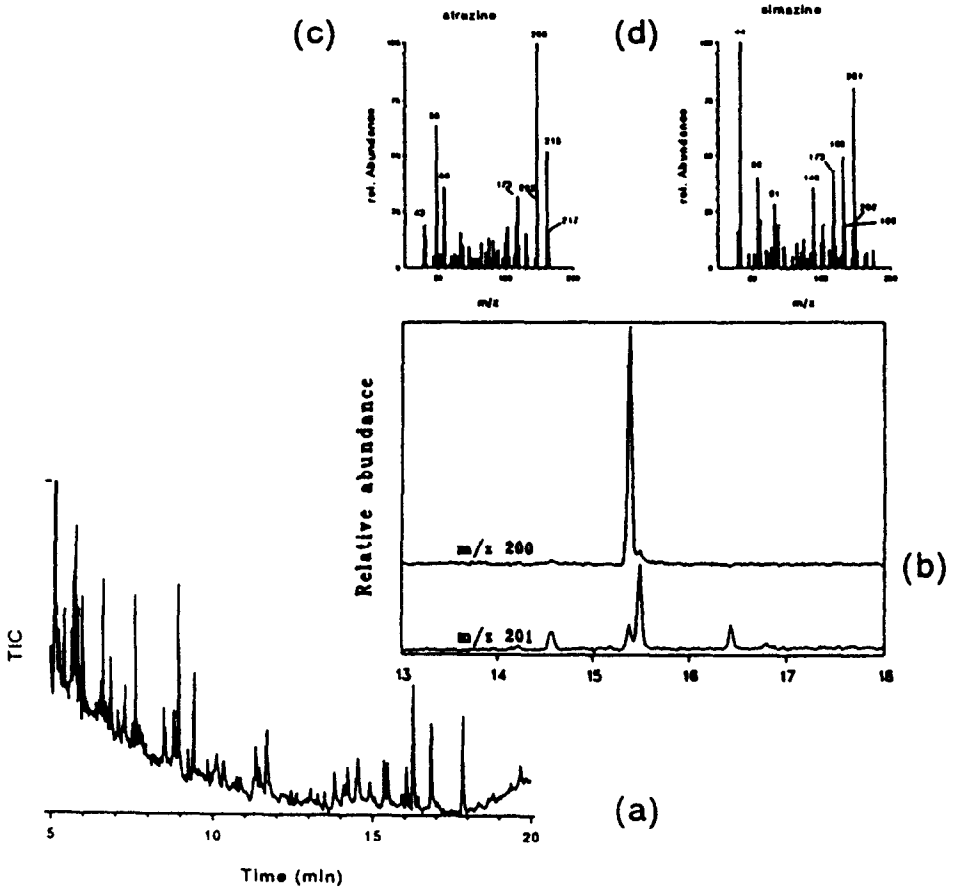

Fig. 18. On-line LC–GC–MS of 10 ml of river Meuse water with full-scan acquisition over the m/z 35–385 range. (a) Total ion current. (b) Reconstructed ion traces for atrazine (m/z 200) and simazine (m/z 201). Mass spectra recorded for atrazine (c) and simazine (d) peaks are also shown.

sitivity increases, losses are prevented and automation becomes readily accessible, as is demonstrated by the development of the SAMOS LC and SAMOS GC systems, and of automated systems for on-line precolumn derivatization.

Today, both disposable and re-usable precolumns filled with a variety of polymers, alkyl-bonded silicas or selective stationary phases are routinely used for on-line precolumn/analytical column LC. Many biomedical and environmental applications have been published and the robustness of such systems is highly satisfactory. Several alternative solutions are increasingly attracting attention. Amongst these, dialysis, with its efficient removal of high-molecular-mass material, already takes a prominent place. The on-line coupling of (reversed-phase) LC or, more correctly, solid-phase extraction of aqueous samples and capillary GC still poses a number of problems. However, the separation and detection power of such a system is sufficiently rewarding to justify the research activities required to solve these problems.

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