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Journal of Chromatography A, 768 (1997) 239–258

JOURNAL OF CHROMATOGRAPHY A

Monitoring of organic micropollutants in surface water by automated on-line trace-enrichment liquid and gas chromatographic systems with ultraviolet diode-array and mass spectrometric detection[1]

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Received 22 August 1996; revised 25 November 1996; accepted 28 November 1996

Abstract

The pollution of the Nitra river (Slovakia), a left-bank tributary of the river Danube, by organic microcontaminants was monitored in 1993 and 1994. Water samples were taken at six sites every two months, transported in a portable refrigerator and processed in the Netherlands. From among five systems tested for their suitability to analyse the samples, solid-phase extraction (SPE)–LC–diode-array detection (DAD UV), SPE–LC–particle beam (PB)-MS and SPE–GC–MS, were selected for regular monitoring. At a later stage SPE–LC–DAD UV and SPE–LC–PB-MS were integrated in one system. The three systems used similar SPE procedures for trace enrichment coupled on-line to LC and GC set-ups. Each method was fully automated by means of an automated cartridge exchange, solvent selection and valve-switching unit, and SPE/WIN software. On-line analysis of 10–200-ml samples allowed the determination of low- to sub-$\mu g\,l^{-1}$ levels of numerous pollutants. Relative standard deviations (R.S.D.) of the retention times were 0.1–0.9% for each system. R.S.D. values of peak areas were 1–15% for SPE–LC–DAD UV, 10–16% for SPE–GC–MS and 17–31% for SPE–LC–PB-MS. The three techniques were found to be complementary. No significant maintenance problems occurred during the project. More than 500 compounds frequently appeared in the sample chromatograms; about 30% could be identified by at least one technique. The majority were industrial pollutants, hydrocarbons, aliphatic alcohols, substituted phenols, sulphur-, nitrogen-, oxygen-, phosphorus- and chlorine-containing compounds, pesticides and their degradation products.

Keywords: Water analysis; Environmental analysis; Detection, LC; Detection, GC; Extraction methods; Pesticides

1. Introduction

The monitoring of organic micropollutants is nowadays one of the major challenges of environmental analytical chemistry. Over 10 million compounds have been registered and the human and/or animal population can come into contact with at least 76 000 of these. Many of these compounds can enter the environment, one route being via surface and ground water [1,2]. To prevent undue pollution, analytical chemists should develop systems which

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[1] Presented at the 10th International Symposium on Advances and Applications of Chromatography in Industry, Bratislava, 30 June–4 July 1996.

PII S0021-9673(96)01050-3

can give fast and reliable information on the identity and amount of suspected pollutants. As regards surface water, the detection of many organic compounds is typically required at levels of 1–3 $\mu g\,l^{-1}$ [3]. Separation of the targeted analytes from each other and the matrix is usually achieved by liquid (LC) or gas (GC) chromatography. Detection can be carried out by a variety of LC [diode-array (DAD) UV, fluorescence, electrochemical] or GC (flame ionization, thermionic, electron-capture) detectors. For obvious reasons the most valued detector is the mass spectrometer (MS) which can be coupled to both LC and GC. Unfortunately, most of the above detectors do not provide sufficient sensitivity and, therefore, trace enrichment of the analytes is required. Here, solid-phase extraction (SPE) is often preferred, especially in the on-line mode because of its rapidity and ease of automation [4]. Following this strategy, several automated systems using SPE coupled on-line to LC–DAD UV, LC–MS and GC–MS were developed in our laboratory [5–10] under the acronym SAMOS (System for Automated Measurement of Organic micropollutants in Surface water).

In this study, three SAMOS systems were employed for a two-year monitoring programme of the Nitra river in Slovakia. One main aim was to test their robustness and practical usefulness in real-world situations and also to study the information content provided by the individual analytical techniques. The Nitra river basin was selected because of the known concentration of large industries and agricultural activities in this area. The second main goal of the study was to get a provisional overview of the type and classes of pollutants present in the basin and to construct pollution profiles.

2. Experimental

2.1. Sampling

Samples were collected at 6–8-week intervals at the end of 1993 and during 1994 at six sampling sites (Fig. 1) selected on the basis of preliminary experiments. Selection criteria were the presence of large industries and/or major agricultural activities in the area upstream of the sampling site and a similar distance between the sampling sites. Samples were taken at a distance of approx. 1 m from the shore and 10–20 cm under the water surface. They were delivered within 24–30 h to the Netherlands. In order to prevent unwanted degradation of polar compounds, the samples were stored in a portable refrigerator at approx. 10°C in the dark during transport. The samples were not acidified because of the best performance of the SPE–LC–DAD UV method for samples with neutral pH values (7.5–8.5) [5]. Immediately after transport, the samples were filtered through a 0.45 μm acetyl-cellulose filter (Schleicher&Schuell, Dassel, Germany) and stored at 4°C. No indication of sample degradation was noticed during the feasibility studies. Samples were analysed using three analytical systems: SPE–LC–DAD UV, SPE–LC–PB-MS and SPE–GC–MS (Fig. 2, for more details, see Ref. [8]). Prior to each analysis, 1 $\mu g\,l^{-1}$ of metoxuron and propazine were added to the sample; these internal standards were selected in order to demonstrate the complementarity of LC (both analytes) and GC (propazine) techniques.

2.2. SPE–LC–DAD UV

2.2.1. Instrumentation

Analyses were performed on a HP 1090 liquid chromatograph (Hewlett–Packard, Waldbronn, Germany) equipped with a built-in HP 1040 DAD UV detector. Later, a stand-alone HP 1040 detector was installed (Fig. 2) and used. A 250 mm×4.6 mm I.D. LC-18-DB analytical column packed with 5 μm, 100 Å C_{18}-bonded silica (Supelco, Bornem, Belgium) was used.

Data were handled by HPLC3D CHEMSTATION software (G1307A, DOS Series) installed on a HP Vectra 486/33 computer. User-made libraries of the DAD UV spectra, PEST.1 and SUPPEST.1 (approx. 150 entries of most common pesticides and related pollutants), were used for automated library searches. On-line sample pretreatment prior to LC analysis was accomplished with a Prospekt (Spark Holland, Emmen, Netherlands). SPE was carried out on 10 mm×2.0 mm I.D. PTFE cartridges packed with 15–25 μm, 100 Å PLRP-S (styrene–divinylbenzene copolymer, Polymer Laboratories, Church Stretton, UK). Communication between the Prospekt and the

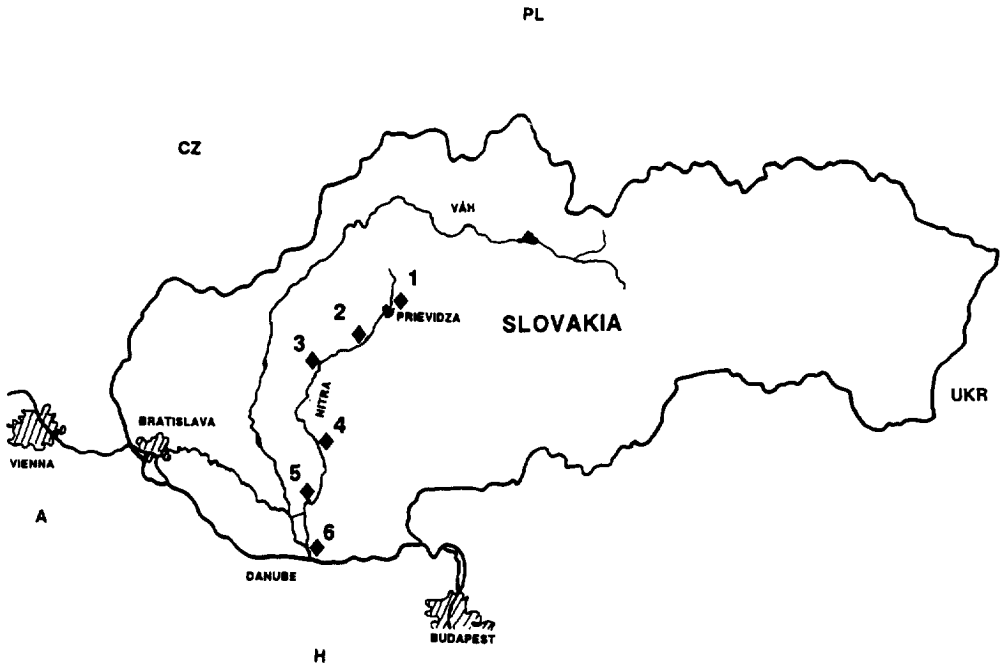

Fig. 1. Sampling sites in the Nitra river basin selected for the 1993–1994 monitoring programme. 1, Nedozery; 2, Chalmova; 3, Praznovce pri Topolcanoch; 4, Cechynce; 5, Komoca; 6, Komarno.

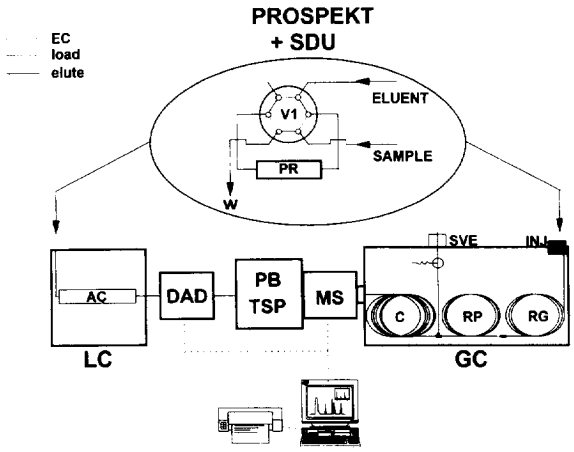

Fig. 2. Scheme of the experimental set-up. ELUENT, LC eluent gradient or ethyl acetate for GC; EC, electronic connections; V1, six-port switching valve; load/elute, positions of V1; PR, precolumn or cartridge; W, waste; AC, analytical column; LC, liquid chromatograph; DAD UV, diode-array detector; PB, particle beam interface; TSP, thermospray interface; MS, mass spectrometer; GC, gas chromatograph; C, GC analytical column; RP, retaining precolumn; RG, retention gap; SVE, solvent vapour exit; INJ, on-column injector.

HP Vectra 486 computer was established by means of the SPE/WIN software (Hewlett–Packard).

2.2.2. Procedures

The SPE cartridge was first conditioned with 4 ml methanol and then with 2 ml HPLC-grade water at 2 and 1 ml min^{-1}, respectively; next, a 100-ml surface water sample was enriched. The cartridge was on-line eluted by the LC gradient: acetonitrile (ACN)–0.01 *M* phosphate buffer (pH 3) using a linear gradient from 5% ACN at 0 min to 90% ACN at 55 min with a return to 5% ACN in 5 min; the flow-rate was 1 ml min^{-1}. The DAD UV detector was operated at 210 nm with 10 nm bandwidth. Retention times and UV spectra of detected peaks were compared with those of standard analytes stored in the spectrum library. In case of good agreement between retention times (5% window) and spectra (match value above 950) the name of the identified compound was assigned to the peak, its concentration calculated from an appropriate calibration table (if satisfactory calibration was established) and the report printed. All above actions were performed

unattendedly, with sequences of 10–15 analyses typically being run overnight. For more details on quantification aspects see Refs. [5,6].

2.3. SPE–LC–PB-MS

2.3.1. Instrumentation

Liquid chromatography was performed on a HP 1090 liquid chromatograph equipped with a UV photometric detector and an automatic six-port switching valve (Rheodyne, Cotati, CA, USA). The analytical column was the same as for SPE–LC–DAD UV. A 59889B (PB) interface was utilised for transfer of the LC eluent to the MS. The temperature of the desolvation chamber was held at 70°C and the pressure of nebulising helium was 35 p.s.i (approx. 2.5 l min^{-1}) (1 p.s.i.=6894.76 Pa). The mass spectrometer, a HP 5989A MS engine was operated in the full-scan EI mode (mass range 65–400 u, scanned at 0.39 scan s^{-1}) with positive ion detection. The temperatures of the ion source and quadrupole were 250°C and 100°C, respectively. The standard HP thermospray interface (TSP) was operated in the positive ion mode with ionization being assisted by the discharge electrode (mass range 85–500 u, scanned at 0.36 scan s^{-1}). The stem temperature was optimised to 96°C, and the temperatures of the ion source and quadrupole were kept at 200°C and 100°C, respectively. Ammonium acetate was added to the LC gradient (1 ml min^{-1}).

Data were acquired by a HP Vectra 486/66 or HP UX 9000/345 computer and processed by MS CHEMSTATION (G1034C, DOS Series) or UNIX CHEMSTATION software (version B 6.01), respectively. On-line sample pretreatment prior to LC analysis was carried out on a stainless-steel 10 mm×2.0 mm I.D. precolumn, slurry-packed with PLRP-S. The precolumn was placed in the automatic six-port switching valve of the HP 1090.

In the last two sampling campaigns (August and October 1994) the SPE–LC–DAD UV and SPE–LC–PB-MS systems were integrated into one system and automated by means of the Prospekt [9]. All parts of the system were controlled from a single (HP Vectra 486/66) computer by combined actions of the MS CHEMSTATION, HPLC3D CHEMSTATION and WIN/SPE software packages. Because of limitations imposed by the LC–MS technique, the flow-rate of the LC eluent was reduced to 0.4 ml min^{-1}; no buffer was used and the enrichment volumes were 200 ml. Changes to the LC gradient are discussed below.

2.3.2. Procedures

The precolumn was conditioned as with the SPE–LC–DAD UV procedure; next, a 200-ml sample was enriched. Compounds trapped on the precolumn were then on-line eluted by the LC eluent (ACN–0.1 *M* ammonium acetate buffer, linear gradient from 10% ACN at 0 min to 90% ACN in 40 min and back to 10% ACN in 5 min; flow-rate 0.4 ml min^{-1}) and analysed. Mass spectra of relevant peaks were compared with those stored in an EI mass spectrum library [16]. In experiments utilising DAD UV no buffer was added to the LC eluent. Semiquantitative evaluations were carried out according to procedures described in Ref. [9] provided standard chemicals of detected compounds were available. For more details, see also Ref. [11].

2.4. SPE–GC–MS

2.4.1. Instrumentation

Analyses were performed on a HP 5890 Series II gas chromatograph equipped with a pressure-programmable on-column injector. The split-splitless injector was replaced by a laboratory-made solvent vapour exit (SVE). On-line sample pretreatment prior to GC analysis was accomplished using the Prospekt. SPE was carried out on the same cartridges as used with LC (see above). Separations were performed on a SPB-1 dimethyl silica (15 m×0.2 mm, 0.2 μm, Supelco) or a HP-5 MS phenyl-methyl cross-linked silica (30 m×0.25 mm, 0.25 μm) columns. The injector was connected to a 5 m×0.32 mm retention gap deactivated with diphenyltetramethyldisilazane (BGB Analytik, Zürich, Switzerland). The 2 m×0.2 mm retaining precolumn contained the same stationary phase as the analytical column. Ethyl acetate was delivered by means of a syringe pump (Gilson, Villiers-le-Bel, France). An HP 5970 quadrupole mass spectrometer was used for detection. Data were handled by MS CHEMSTATION (G1034C, DOS Series) software on a HP Vectra 486/33 computer or on the HP Vectra 486/66 of the Multianalysis system [9]. The automation hardware

and software facilities were the same as for the above systems.

2.4.2. Procedures

A 10-ml sample was subjected to enrichment on the conditioned precolumn at a flow-rate of 2 ml min^{-1}. Salts from the sample were removed by flushing with 1 ml of HPLC-grade water. The precolumn was then dried with a flow of nitrogen (40 l min^{-1}, 30 min) and the analytes trapped on the precolumn were eluted into the retention gap and retaining precolumn with 80 μl of ethyl acetate delivered by the syringe pump at a flow-rate of 100 μl min^{-1}. Excess eluent was removed through the SVE using partially concurrent solvent evaporation [13]. The GC temperature program started at 85°C (hold, 7 min) with an increase to 280°C at 10°C min^{-1}. The helium (carrier gas) pressure was programmed to increase from 6 to 12.4 p.s.i. during the run to approach constant-flow conditions. After closure of the SVE, the analytes were transferred to the analytical column for separation and MS detection. The MS detector was kept at 250°C and data were acquired in the full-scan EI mode (scanned from 35 to 350 u at 1.6 scan s^{-1}). The spectra of relevant peaks were searched in the same library that was used for LC–MS data evaluation. Concentrations of detected analytes were estimated from single-point calibration curves provided standard chemicals were available. For more details and quantification aspects, one may consult [7,9].

3. Results and discussion

3.1. General considerations

At the start of the project three sampling campaigns were carried out in the second half of 1993. The main aim was to test the suitability of five automated chromatographic systems available in our laboratory to analyse surface water. As a result, two systems were not incorporated in further studies.

On-line SPE–GC–atomic emission detection (AED) did provide a good overview of, e.g., the sulphur, phosphorus, nitrogen, chlorine and carbon (sensitivity similar to that of flame ionization detection) profile of the river water. As will be discussed below, quite a number of, e.g., S-containing compounds were indeed identified in the present study by GC–MS. However, in a situation like the present one, where no information regarding the pollution profile is available beforehand, GC–AED does not provide much information that is not also obtained via GC–MS. In addition, the experimental conditions used for conventional GC–MS and GC–AED (see [12]) are somewhat different which complicates peak correlation. Attempts are now being made to solve the peak correlation problem by the use of a dual-hyphenated approach; preliminary results are quite encouraging [14].

On-line SPE–LC–thermospray (TSP) MS showed excellent sensitivity for a large number of compounds (typically 20–40 detected in a single sample), but the mass spectra did not provide much structural information, a complaint often heard about this method of detection [15,16]. Fig. 3 illustrates some advantages and disadvantages of the method. It is no problem at all to selectively record the LC profile of a (unknown) pollutant. In addition, the method is well suited for quantification with the R.S.D. values of the retention times and peak areas of the internal standards, determined for six different Nitra river samples, being less than 0.3% and 7% ($n=12$), respectively. However, the identification of unknowns is rather difficult, as is illustrated by the full-scan TSP-MS spectrum: only molecular mass information is obtained (m/z 233, $[M+H]^+$, m/z 250, $[M+NH4]^+$, i.e., M_r 232). The corresponding peak in the SPE–LC–DAD UV chromatogram of the same sample was rather small and also had to be labelled 'unknown'. Actually, the information provided by TSP-MS was found to be comparable to that of DAD UV; the latter method was therefore used in all further experiments.

The three selected methods, SPE–LC–DAD UV, SPE–LC–PB-MS and SPE–GC–MS, were already applied individually and, on one occasion, combined for the analysis of surface water from several European rivers [9]. In this project, they were combined for the first time for real monitoring purposes rather than for the analysis of a few selected samples. The general strategy was: a 100-ml sample was first analysed by SPE–LC–DAD UV in order to obtain information on the overall pattern of pollution; next, the sample was analysed by both

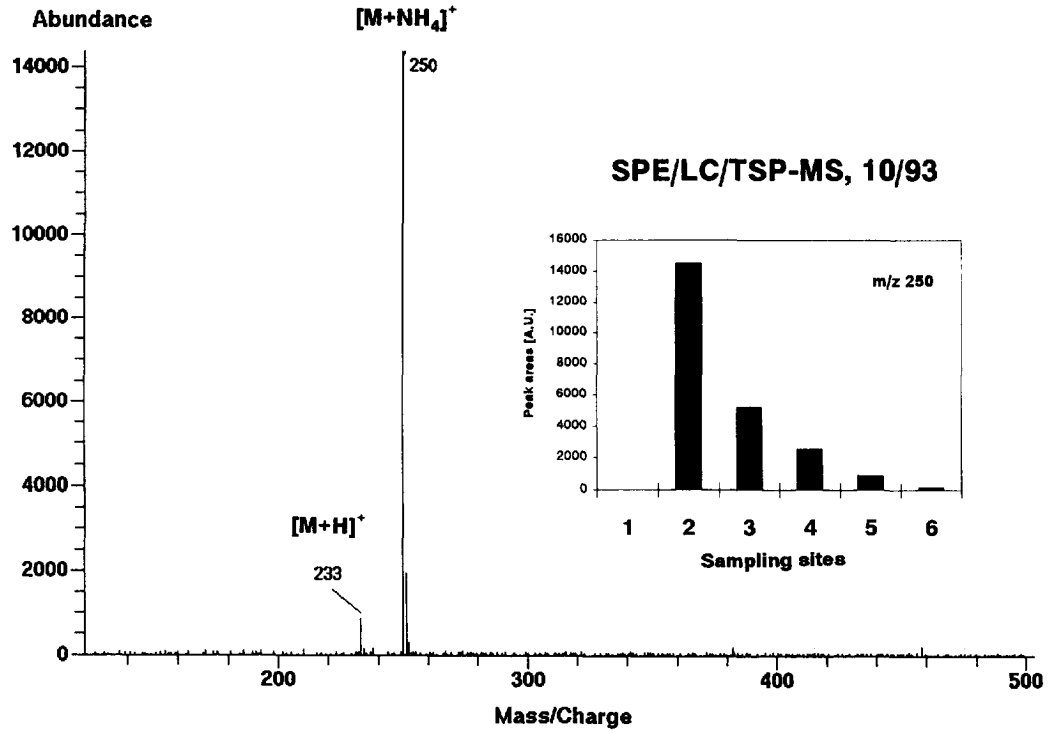

Fig. 3. Spectrum of unknown compound (t_R 32.2 min; M_r 232) obtained by SPE–LC–TSP-MS of 100 ml Nitra river sample (Chalmova, October 1993). Insert, pollution profile constructed from ion chromatograms (m/z 250); for numbering of sampling sites, cf., Fig. 1; TSP-MS operated in discharge-assisted positive ion mode. For other conditions, see text.

SPE–LC–PB-MS (200 ml) and SPE–GC–MS (10 ml) to confirm the presence of specific analytes and to identify unknowns. Once a pollutant was identified, its spectrum and retention time were stored in the UV spectrum library. Subsequently, SPE–LC–DAD UV was used for routine monitoring and quantification purposes. A typical result is shown in Fig. 4. SPE–LC–DAD UV (Fig. 4A, note different numbering in parts B and C) provides good separation of up to 20–30 compounds present in the sample and many of these could be detected at 0.05–0.5 $\mu g\,l^{-1}$. Examples of the provisional identification of atrazine (0.05 $\mu g\,l^{-1}$) and isoproturon (0.1 $\mu g\,l^{-1}$) are shown in the upper part of Fig. 5. The high separation power and good analyte detectability of SPE–GC–MS (Fig. 4C) allowed acquisition of mass spectra of up to 100 compounds from a single sample. An example of positive identification, of 1-chloro-2-(chloromethyl)ethoxypropane, is given in Fig. 5 (detected by this technique only); another example, for 9,10-dihydro-N-methyl-10-acridone, is included in Fig. 10 below.

SPE–LC–PB-MS allowed the detection of, typically, 10–20 pollutants in a single run (Fig. 4B). However, co-elution due to the lower separation efficiency of LC compared with GC and higher chemical noise in the MS ion source created significant problems when trying to obtain good-quality spectra by means of background subtraction. Consequently, library matches generally were lower than in GC–MS. Of course, this does not apply for abundant peaks, such as, e.g., that of tetrabutylammonium cation in Fig. 5. In other words, the use of appropriate trace-enrichment procedures is obligatory to compensate as much as possible for the above disadvantages and to obtain a spectrum quality similar to that of GC–MS. It should be emphasized that the particle beam at present is the only commer-

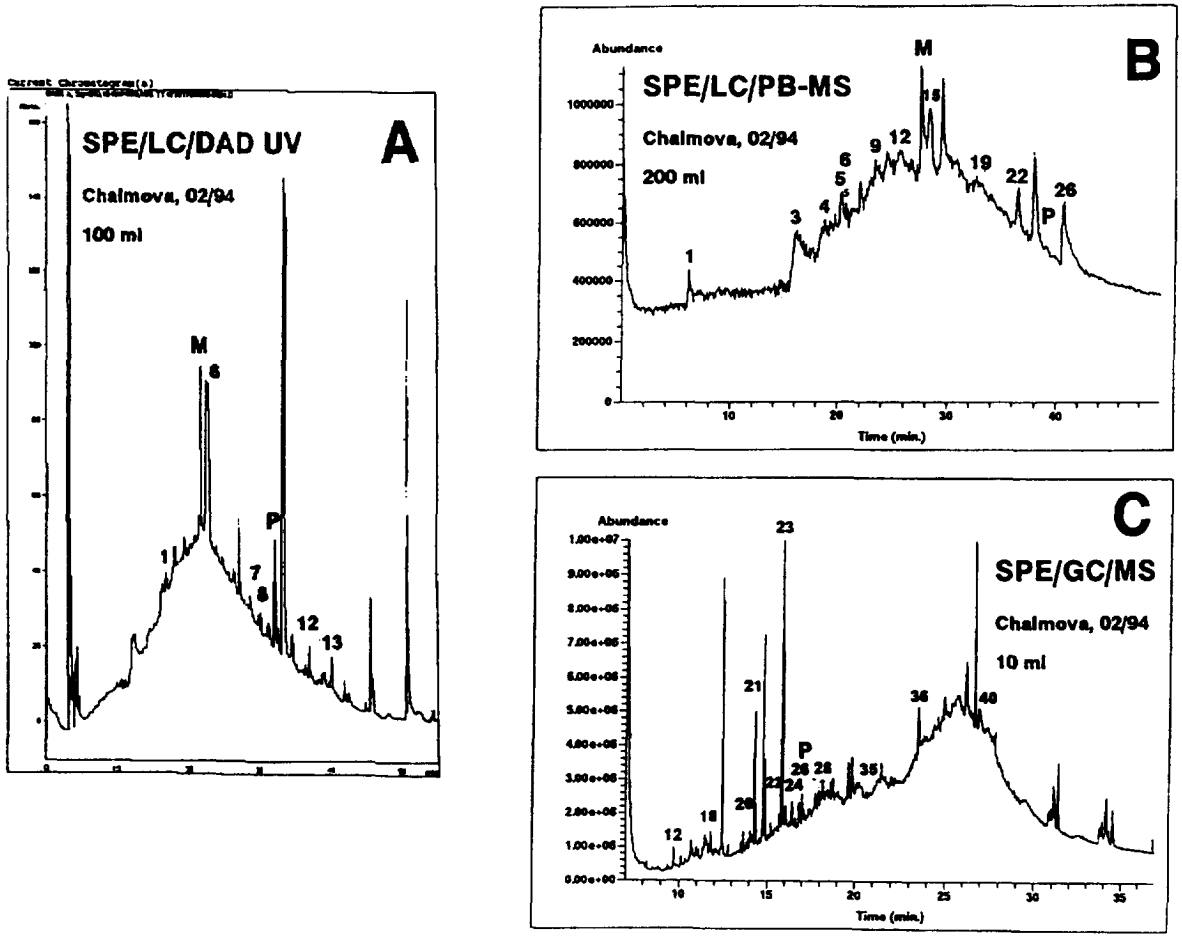

Fig. 4. (A) SPE–LC–DAD UV, (B) SPE–LC–PB-MS and (C) SPE–GC–MS chromatograms of a Nitra river sample (Chalmova, February 1994); for numbering of peaks, cf. Tables 1–3, respectively (another 24 tentatively identified compounds in SPE–GC–MS not included); M and P, internal standards metoxuron and propazine, respectively; concentration 1 $\mu g\ l^{-1}$. For more details, see text.

cially available LC–MS interface providing library-searchable electron impact (EI) and solvent-independent chemical ionization (CI) spectra.

3.1.1. Transport of samples on PLRP-S cartridges

Long-distance transport and storage of water samples in bottles usually require an expensive large-size vehicle equipped with cooling facilities. Spiking with agents which prevent microbial degradation of analytes is also required. The operation can be considerably simplified when the analytes are isolated from the matrix in the field or in a nearby peripheral laboratory. Encouraging results were reported for off-line devices such as 250–500 mg sorbent SPE cartridges or membrane extraction disks [17,18], but there are no such reports for small, on-line cartridges as used in the present study.

In two campaigns 100-ml samples were preconcentrated on PLRP-S cartridges in Bratislava and sent to Amsterdam by mail without any further precautions. Prior to analysis, the cartridges were wet with 1 ml HPLC-grade water and then eluted under the same conditions as applied to the water samples transported to Amsterdam by car, and in a refrigerator. Comparison of Fig. 6A and B shows an almost total disappearance of the characteristic dis-

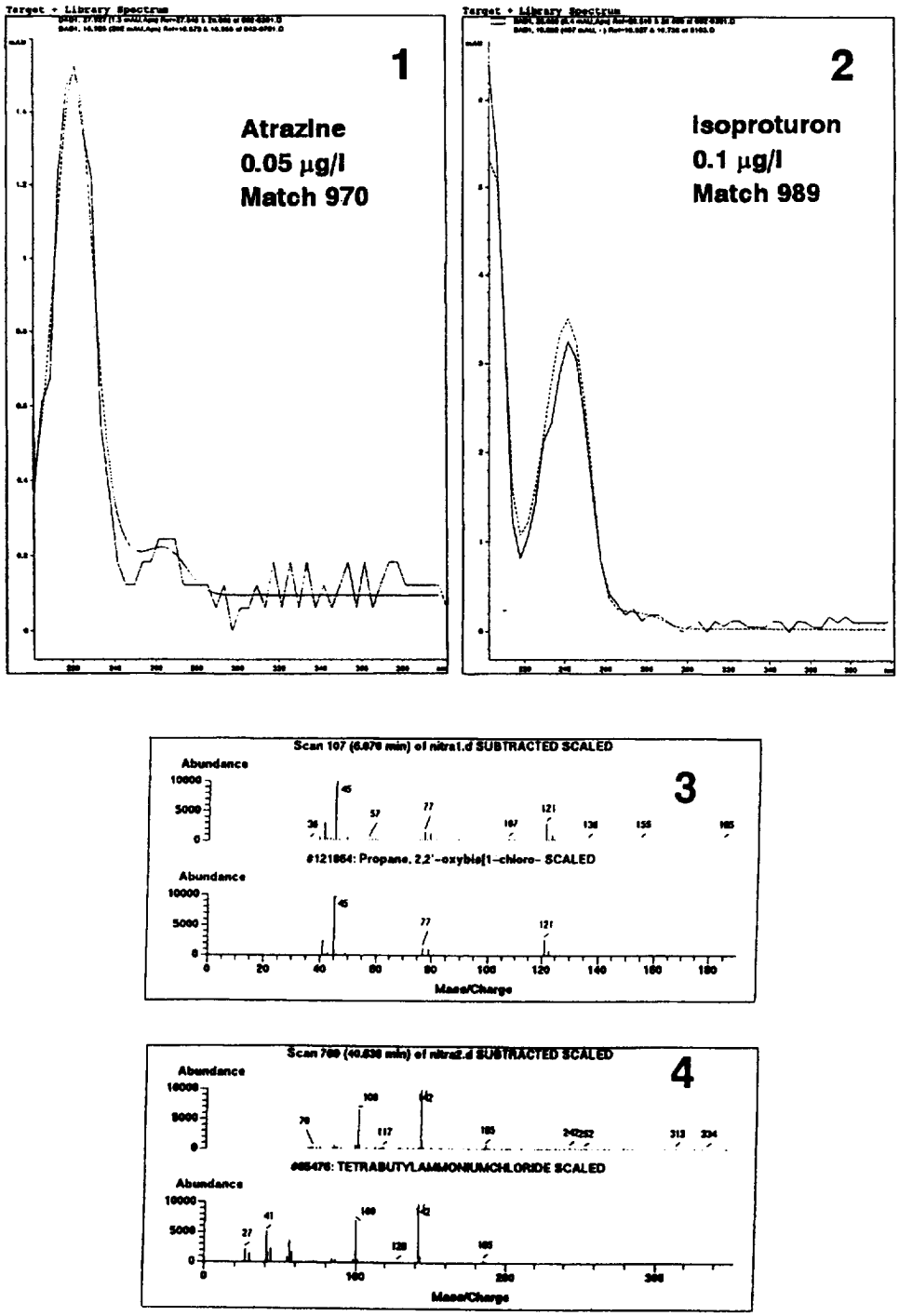

Fig. 5. Results of (1, 2) DAD UV, (3) GC–MS and (4) PB-MS spectrum library search of several pollutants detected in samples from the Nitra river taken at Chalmova. Identified compounds, (1) atrazine and (2) isoproturon (February 1994), (3) 1-chloro-2-(chloromethyl)ethoxypropane (October 1994) and (4) tetrabutylammonium cation (February 1994). For more details, see text.

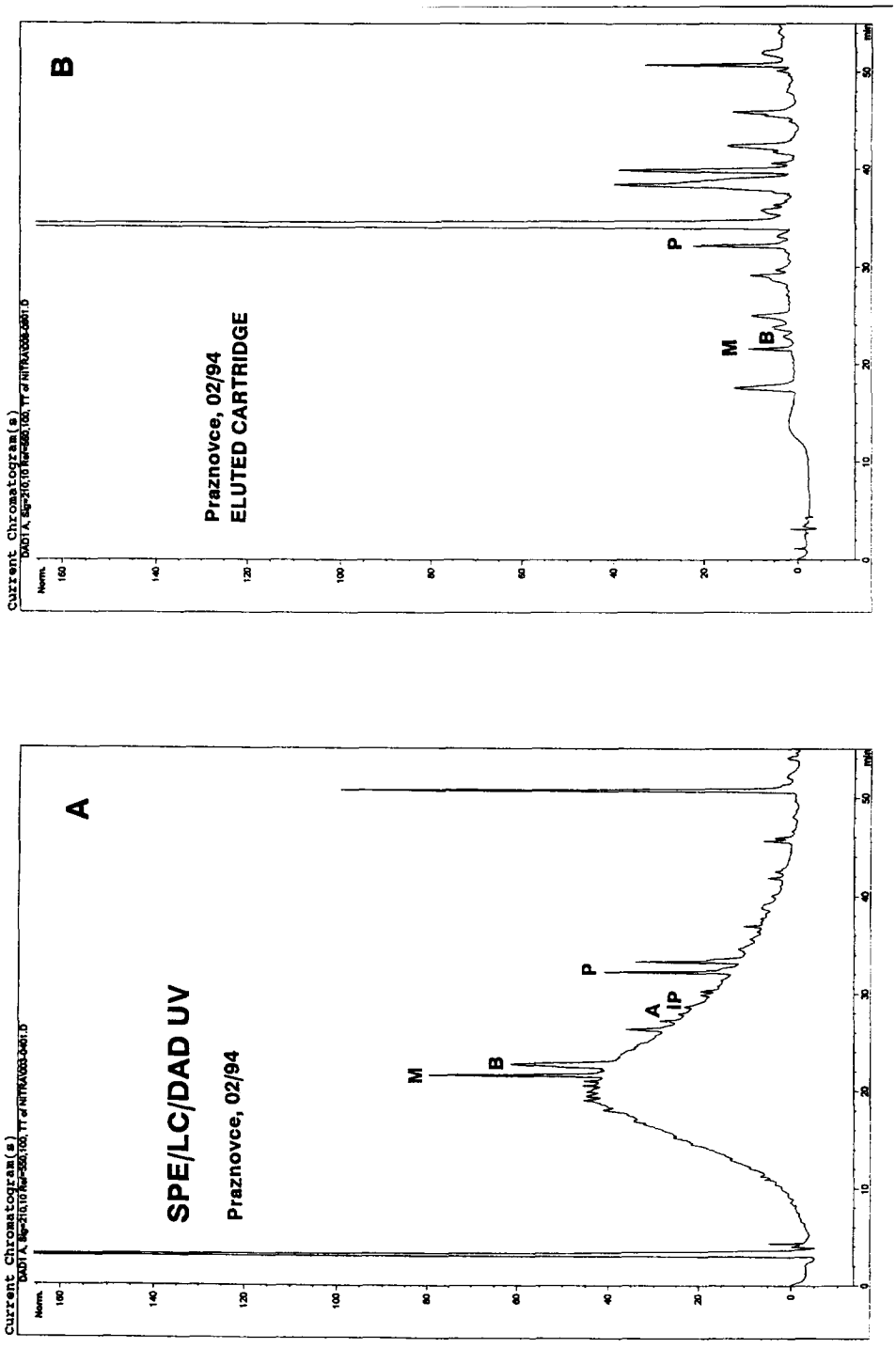

Fig. 6. SPE–LC–DAD UV chromatograms of 100-ml Nitra river samples (Praznovce, February 1994). Samples: (A) transported by car at 10°C; (B) enriched on PLRP-S cartridge in Bratislava and sent to Amsterdam by mail. Internal standards, metoxuron (M) and propazine (P) at 1 $\mu g\,l^{-1}$ level. Benzothiazole (B), atrazine (A) and isoproturon (IP) identified by library search. For analytical conditions, see text.

solved organic carbon (DOC) hump, the presence of a new, abundant peak at 34.5 min (also in all other chromatograms) and a 30–80% reduction of the peaks due to the internal standards, propazine and metoxuron. The signal of benzothiazole, which was present in the sample, was reduced by some 90% while atrazine and isoproturon could not be detected at all. Such behaviour was not observed in an earlier study on C_{18}-bonded phases [19]. Therefore, the problem may well be related to processes occurring inside the pores of a polymeric sorbent [20]. In contrast, in an additional study performed at the Water Research Institute in which loaded cartridges were kept either in sealed plastic bags for several weeks in a refrigerator (4°C), in a freezer (−18°C) or at ambient temperature, and analysed by SPE–LC–DAD UV at regular intervals, none of the chromatograms displayed any of the earlier dramatic changes. This suggests that extreme temperature and/or humidity conditions during transport may well be the principal cause of our problems, and thus have to be avoided. To be on the safe side, in the present project all samples were transported at approx. 10°C and delivered within less than 30 h.

3.2. SPE–LC–DAD UV

An interesting illustration of the usefulness of SPE–LC–DAD UV results is given in Fig. 7. The four chromatograms were obtained from samples collected on 4 February 1994 within a 5-h period. Next to the tentatively identified compounds (for peak assignment, see Table 1) one can see an increase of the DOC hump and a sudden appearance of numerous additional and, as yet, unknown peaks at the sampling site Chalmova. This should be attributed to industrial and/or agricultural activities in this area rather than to the presence of naturally occurring compounds, because the distance between sampling sites 1 and 2 is only about 30 km. The pollution then gradually decreases and a relatively clean chromatogram is obtained in Cechynce and Komoca and also further down the river in Komarno (not shown).

The SPE–LC–DAD UV system and its spectrum library were originally developed for the target analysis of pesticides. Therefore, mainly pesticides from classes such as the triazines, phenylureas, carbamates, organophosphorus herbicides and anilides were frequently detected in our samples (Table 1). Atrazine was found to be present in the surface water throughout the whole 1993–1994 period at concentrations from 0.05 $\mu g\,l^{-1}$ (February 1994) to 0.8 $\mu g\,l^{-1}$ (June 1994). Its presence was confirmed by the other two methods (see below). More recently (August 1995) atrazine was determined in a concentration of 0.25 $\mu g\,l^{-1}$ in another sample of Nitra river by SPE–LC–atmospheric pressure chemical ionization-MS–MS [10]. The other compounds of Table 1 were typically found to be present at the 0.1–3 $\mu g\,l^{-1}$ level. Next to the analytes of Table 1, which were detected at Chalmova, several other pesticides were found at similar concentration levels at the other sites, e.g., the triazines terbutylazine, cyanazine and atraton, the phenylurea fluometuron and 3,4-dichloroaniline (probably a degradation product of phenylurea herbicides [11]). The presence of the above analytes is not unexpected; triazines, anilides and phenylureas are known to be applied seasonally on fields in the Nitra basin [21]. The majority of the quoted microcontaminants are also detected regularly in other European and North American rivers [1,22,23]. Benzothiazole, which is frequently found in surface water [23,24] and its derivatives are used as vulcanisation accelerators [25]. Both point sources, e.g., the rubber industry in the vicinity of the sampling site Chalmova, and non-point sources, e.g., run-offs of tire residues in dumps, should be considered. The highest concentration of benzothiazole was 2.8 $\mu g\,l^{-1}$ (Chalmova, February 1994).

SPE–LC–DAD UV proved to be a suitable approach also for the analysis of samples containing a large amount of particulate matter, such as those collected during a heavy rainfall in June 1994. The only notable difference in the chromatograms was the about two times higher DOC hump which did not seriously affect analyte detectability. As regards provisional identification, one should of course be careful with compounds having no distinct absorption maxima in the recorded UV range such as, e.g., alachlor. To maintain the reliability of the procedure, these compounds were considered identified when the spectrum match was at least 990 (out of 1000), whereas a match of at least 950 was taken as sufficient for other analytes.

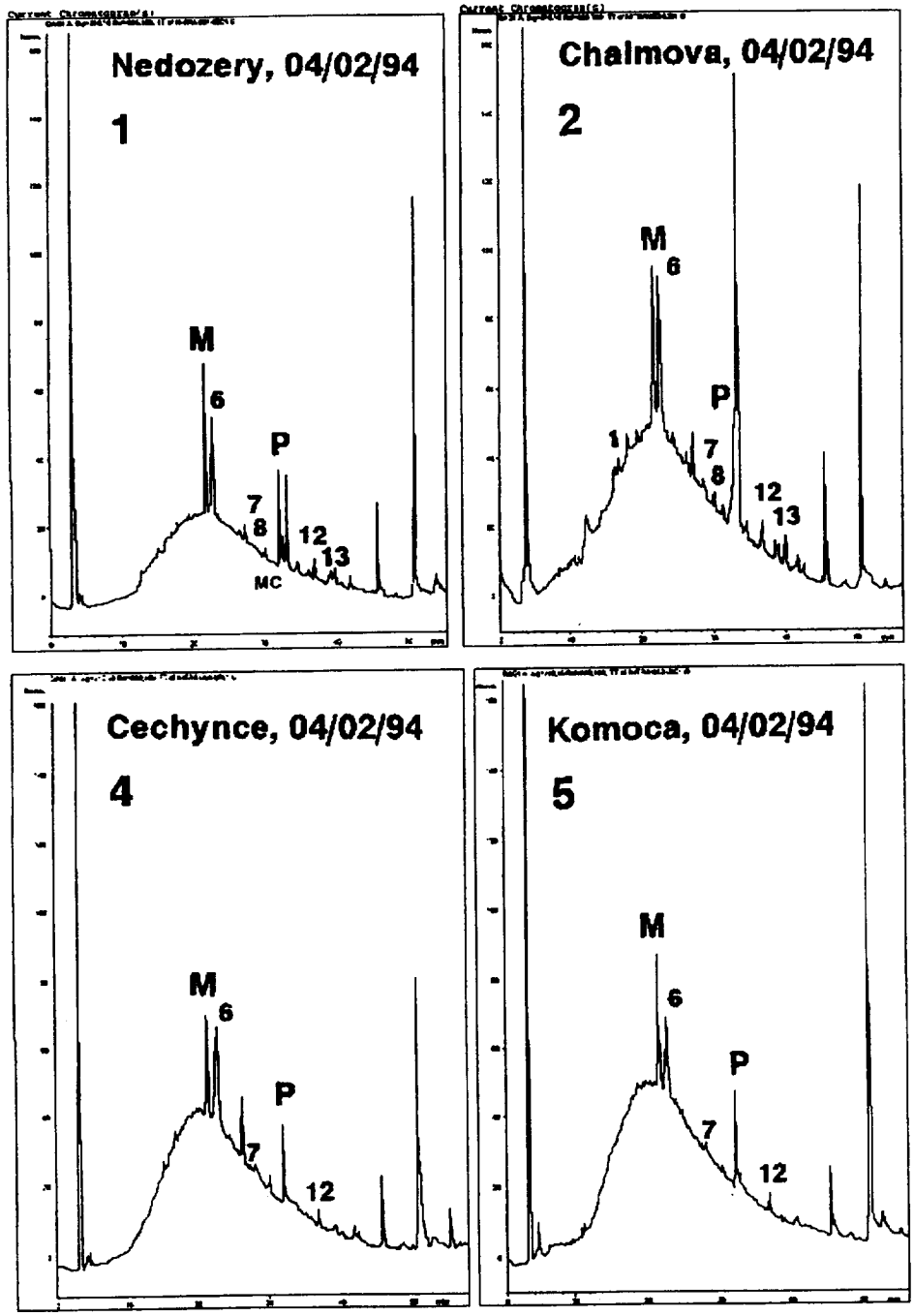

Fig. 7. SPE–LC–DAD UV chromatograms of 100 ml Nitra river samples (February 1994) collected at all sampling sites (cf., Fig. 1). Assigned peaks identified by automated library search. Analytical conditions: eluent, acetonitrile–0.01 *M* phosphate buffer (pH 3) at 1 ml min^{-1}; from 5:95 linearly to 90:10 in 55 min. Internal standards, metoxuron (M) and propazine (P) at 1 $\mu g\, l^{-1}$ level; MC, metazachlor; for numbering of other compounds, cf., Table 1. For other conditions, see text.

Table 1
Compounds identified in Nitra river water (Chalmova) by SPE–LC–DAD UV

No.	Compound	Date of sampling			
		4 February 1994	6 June 1994	19 August 1994[a]	16 October 1994[a]
1	Desmethyl metoxuron	D			
2	Caffeine				D
3	Aldicarb				D
4	Mevinphos				D
5	Malathion				D
6	Benzothiazole	D	D	D	
7	Atrazine	D	D	D	
8	Isoproturon	D			
9	Disulfoton			D	D
10	Terbutryn		D		D
11	Metolachlor			D	D
12	Dichlorprop	D			
13	Alachlor	D	D		D
14	1,2,4-Trichlorobenzene				D

Compounds identified by retention times and DAD UV spectra library search.
D, compound detected at 0.05–3 $\mu g\,l^{-1}$ level with a spectrum match of at least 950 or 990 (cf., text).
[a] LC–DAD UV was combined on-line with PB-MS (cf. text), retention time was not used as a search criterion.

3.3. SPE–LC–PB-MS

As with SPE–LC–DAD UV, and also with SPE–GC–MS (cf., below), the highest pollution of the Nitra river, in terms of number and intensity of detected peaks, was invariably recorded at Chalmova. The structures of numerous pollutants, e.g., *ortho*-tolylbiguanide, trimethoprim, atrazine (cf., above), tetrabutylammonium cation (cf., Fig. 5), 3-phenyl,4-methylisoxazol-5-one, 1-(propyloctyl)-benzene, several substituted phenols and benzenes, aliphatic alcohols, hydrocarbons, S-, O- and N-containing compounds were derived from their EI spectra after a library search (Table 2).

Aliphatic alcohols, with major peaks at m/z 117 and 103 (cf., compound No. 14), were detected also by GC–MS (cf., Fig. 11 below). However, as is to be expected for this procedure aimed at LC—rather than GC—amenable analytes, the majority of the detected compounds did not have corresponding spectra in the MS library and more laborious procedures are required for their identification. One approach is to obtain additional information from a CI run as recently demonstrated for (2-methylthio)benzothiazole, presumably a transformation product of benzothiazole [9]. Because of the time-consuming nature of such an approach, this subject was not pursued any further in the present study.

Total ion SPE–LC–PB-MS chromatograms such as that shown in Fig. 4B, were used for a first indication of peak positions. Next, ion chromatograms were constructed for the base peak in the spectrum of an analyte. A typical result of this procedure is shown in Fig. 8A. The spectrum of peak No. 30 gave a rather low match of 40–50 (out of 100) in the library search (Fig. 8C). However, further processing (data not shown) enabled an improved distinction between matrix interferences and the structurally important ions m/z 90, 118, 121, 134, 161, 178 and 290 of the analyte, which was then identified as the 2-ethylhexyl ester of 3-(4-methoxyphenyl)-2-propenoic acid (M_r 290). The comparison of the LC–PB-MS and GC–MS spectra of a frequently detected unknown compound (No. 15) demonstrates the typical, by 50–100-fold higher sensitivity (see y axes) and the distinctly lower noise level in the low-mass region of GC–MS as compared to LC–PB-MS (Fig. 8B).

The complementarity of the LC–PB-MS and GC–MS procedures is illustrated by the fact that the major ions of 19 out of the 33 compounds of Table 2 were not present in the spectra of approx. 500

Table 2
List of selected pollutants detected by SPE–LC–PB-MS with identification proposed after library search

No.	t_R (min)	B.p.	m/z	m/z	Proposed identification	October 1993	February 1994	August 1994	October 1994
1	6.1	89	133	155	1,4,7,10,13,16-Hexaoxacyclooctadecane		D		
2	13.0	147	106	185	5-Chloro-1,1,3,3-tetracyano-1,3,3A,6 A-TE	D			
3	15.8	107	191	149	*ortho*-Tolylbiguanide		D		
4	18.4	106	132	192	N-[2-(2-Oxopropyl)phenyl]acetamide		D		
5	20.3	201	216	159	Tetraisopropylidenecyclobutane		D		
6	20.4	290	259	275	Trimethoprim		D		
7	22.0	186	201	159	1,1'-(2,2-Indolizinediyl)bisethanone		D		
8	22.9	127	254	98	N-2-Methyloctadecanoyl pyrrolidine				D
9	23.4	215	200	159	2-(Isopropylamino)-1,4-naphthaquinone		D		
10	24.3	129	103	73	3-Hydroxydimethylpentanedionic acid				D
11	24.4	98	84	292	2-(3-Thienyl)ethyl-1-(2-dibenzothienyl)		D		
12	25.6	117	103	175	2,5-Didesoxy-tri-*o*-(trimethylsilyl)pentitol		D		
13	26.0	213	119	228	4,4'-(1-Methylidene)bisphenol	D			D
14	28.1	117	103	175	1-(2-Methoxy-1-methylethoxy)-2-propanol		D		
15	29.5	254	138	97	Unknown	D	D	D	D
16	30.8	117	175		6-Deoxy-2,3,4,5-tetrakis-D-galactose		D		
17	32.3	107	79	92	4-Ethylpyridine			D	
18	33.2	200	215	69	Atrazine			D	
19	33.3	117	175	131	3-Phenyl-4-methylisoxazol-5-one	D	D		
20	36.2	71	83	111	2-(Tetradecyloxy)ethanol	D			
21	36.4	69	83	196	Tetradecene	D			
22	36.5	306	291	247	O,O-Dimethyl ether of nepenthone-A		D		
23	38.8	69	83	210	Pentadecene	D			
24	39.4	149	167	70	Bis(2-ethyl)-1,2-benzenedicarboxylic acid				D
25	40.5	83	97	69	11-Tricosene	D			
26	40.7	142	100	185	Tetrabutylammonium		D		
27	41.8	69	83	224	Hexadecene	D			
28	42.1	91	133	189	(1-Propyloctyl)benzene	D			
29	42.7	193	69	71	2-Anthracenamine	D			
30	43.8	178	161	290	2-Propenoic acid, 3-(4-methoxyphenyl), 2-ethylhexyl ester				D
31	47.9	75	117	313	Octadecanoic acid, 3-methoxy-, methyl ester	D			
32	49.5	149	119	130	Monopentyl-1,1-benzenedicarboxylic acid			D	
33	60.6	237	295	265	Tetraethyl plumbane	D			

Sampling site, Chalmova.
B.p. and *m/z*, three most abundant ions in spectrum. Presence of each ion in spectrum checked manually; matches from library search are typically 50–90.

analytes detected by SPE–GC–MS. A rather special example is the compound eluting at 40.7 min in SPE–LC–PB-MS (Fig. 4B). From its PB-MS spectrum, shown in Fig. 5, the analyte was tentatively identified as tetrabutylammonium cation (M_r 242; concentration approx. 1 $\mu g\,l^{-1}$). A compound with an almost identical spectrum was detected in SPE–GC–MS, albeit at a much lower concentration (close to its limit of detection), and identified as N,N-dibutyl-1-butanamine (M_r 185). Probably, the latter assignment is not correct and was due to the fact that the structurally important ion *m/z* 242 was already below the spectral noise level or that an energetically favourable thermal decomposition of the parent tetrabutylammonium cation took place. The pollution profile revealed that tetrabutylammonium cation is a typical 'point-source chemical' which is presumably released as a result of industrial activity.

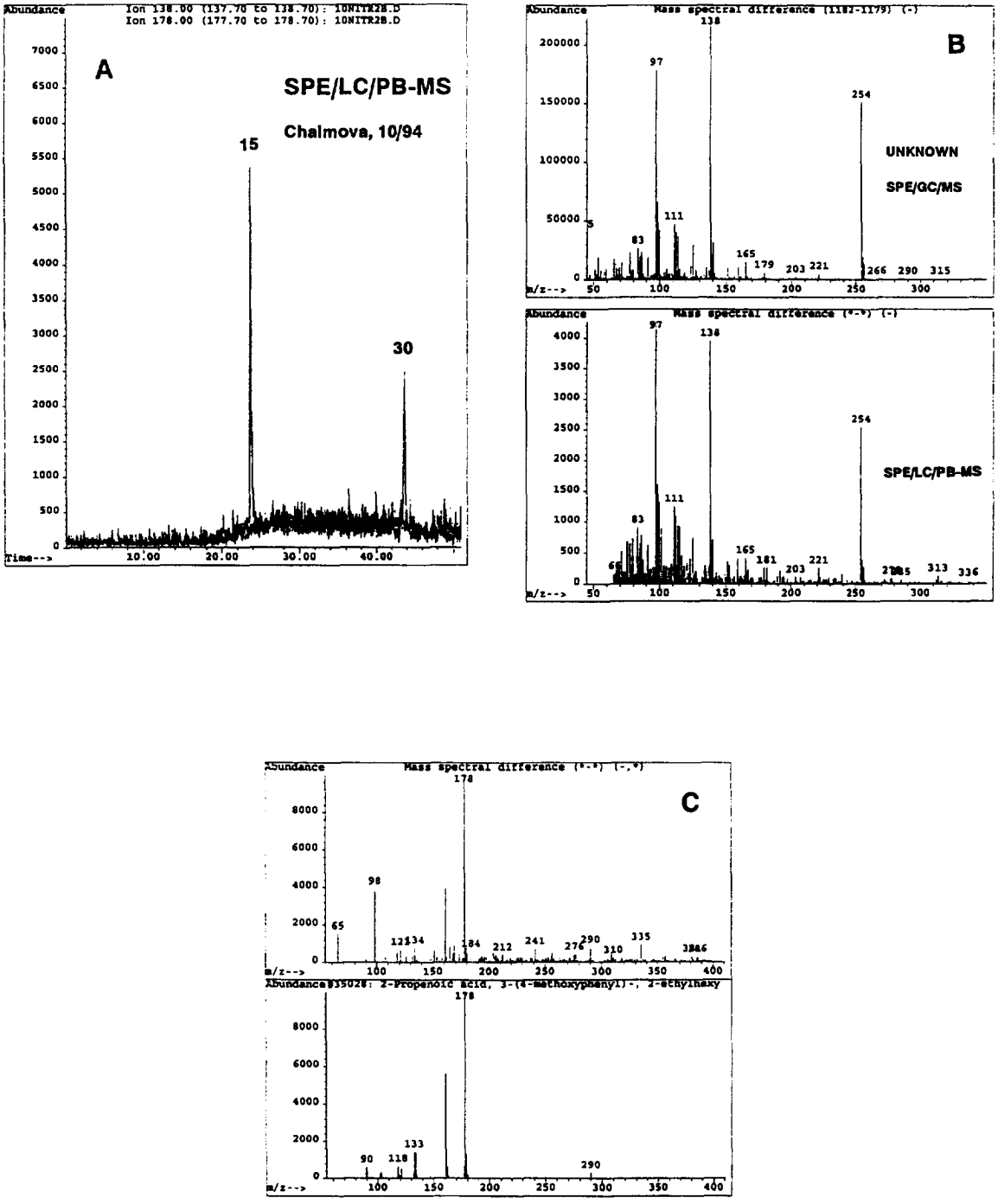

Fig. 8. (A) SPE–LC–PB-MS ion (m/z 128 and 178) chromatograms of 200 ml Nitra river samples (Chalmova, October 1994). For numbering of detected compounds, cf. Table 2. (B) SPE–GC–MS and SPE–LC–PB-MS spectra of frequently detected unknown pollutant (No. 15); (C) result of spectrum library search for compound No. 30 (match 50), ions m/z 65, 98, 241, 310 and 335 are due to matrix interferences. For more details, see text.

3.4. SPE–GC–MS

The excellent sensitivity and separation power of GC–MS, in the EI mode, combined with 10-ml sample preconcentration typically allows detection in the range of 1–10 ng l^{-1} [7,9]. Therefore it is not surprising that analyses of each Nitra river sample revealed the presence of 50–100 compounds (Fig. 4C). More than 30% of these could be identified by a library search. The samples contained a wide variety of industrial and agricultural pollutants such as halogenated hydrocarbons, aliphatic alcohols, chloro- and alkyl-substituted phenols, rubber chemicals, plastic additives, flame retardants and pesticides (Table 3). The table shows only a selection of the more than 500 pollutants detected; the analytes were selected on the basis of frequent occurrence in the samples, high signal intensities and/or environmental relevance. Many are representatives of a class of compounds: Hexadecanoic acid (t_R 19.07 min) represents the nonanoic–octadecanoic acids, two substituted aliphatic alcohols (t_R 8.79 and 11.61 min) represent a whole group of similar compounds (cf., below) and pentachlorophenol (t_R 16.84 min) was selected from amongst several halogenated phenols present.

As an illustration of our findings, data for six compounds are plotted in Fig. 9. They include two compounds which were also found by means of SPE–LC–DAD UV, atrazine and caffeine. The con-

Table 3
List of selected pollutants tentatively identified (D) by SPE–GC–MS

No.	t_R (min)	Compound[a]	Nedozery	Chalmova	Praznovce	Cechynce	Komoca	Komarno
2	5.38	Phenol	D	D	D	D	D	D
3	6.32	2-Ethyl-1-hexanol	D	D	D	D	D	D
5	6.78	2,2'-Oxybis(1-chloro-)propane		D	D	D	D	D
7	7.14	Hexamethylcyclotrisiloxane	D	D	D	D	D	
8	7.72	Undecane	D	D	D	D	D	
10	8.79	1-[1-Methyl-2-(2-propenyloxy)]-2-propanol		D	D			
11	9.27	1 α-Terpineol		D		D	D	D
13	10.00	2-(1-Methylpropenyl)-1,3-dithiolane		D	D	D	D	
14	10.04	Benzothiazole	D	D	D	D	D	D
17	11.61	1,1'-(1-Methyl-1,2-ethanediol)-2-propanol		D	D		D	D
19	12.34	3-(1-Methyl-2-pyrrolidinyl)pyridine	D		D		D	D
20	14.09	2,6-Bis(1,1-dimethylethyl)- 2,5-cyclohexadiene-1,4-dione	D		D	D	D	D
22	15.28	2-(Methylthio)benzothiazole		D	D	D		D
23	15.81	Propanoic acid 2-methyl-,1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	D	D			D	D
24	16.37	Triphenylphosphine oxide	D	D		D		D
25	17.03	Atrazine	D	D	D	D	D	D
26	16.84	Pentachlorophenol		D				
27	17.27	N-Butylbenzenesulfonamide			D	D		D
28	17.46	1st peak in Fyrol PCF		D	D	D		D
29	17.47	Tris(2-chloroethanol)phosphate	D	D	D			
30	18.08	Caffeine	D	D	D	D	D	D
31	18.88	1,1'-Sulfonylbis(benzene)	D	D	D	D	D	D
32	19.07	Hexadecanoic acid		D	D	D	D	D
33	19.88	Trichloroheptafluoro butane	D	D			D	D
35	21.75	Unknown		D	D	D	D	D
37	23.40	10-Methyl-9(10 H)-acridone				D	D	D
38	25.08	1,2-Benzenedicarboxylic acid dinonyl ester	D	D			D	D
39	26.40	1,4,7,10,13,16-Hexaoxacyclooctadecane		D		D	D	D
41	29.96	Cholest-5-en-3-ol	D		D	D	D	

[a] Non-IUPAC names were taken directly from the spectrum library.

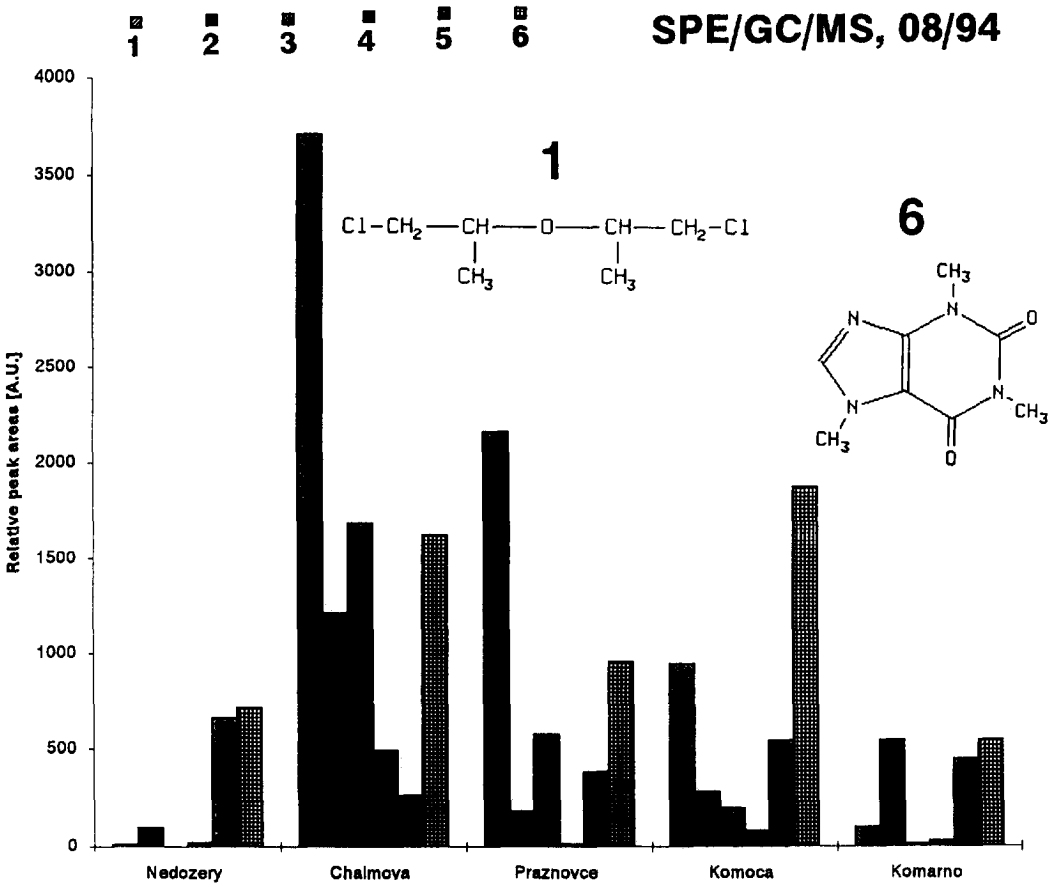

Fig. 9. Pollution profiles of the Nitra river (August 1994) for (1) 1-chloro-2-(chloromethyl)ethoxypropane, (2) 1,1′-sulfonylbisbenzene, (3) 2-(chloromethyl)ethoxy-1,1-dichloropropane, (4) triphenylphosphine oxide, (5) atrazine and (6) caffeine. Data obtained by SPE–GC–MS; all peak areas, except for 1-chloro-2-(chloromethyl)ethoxypropane, enlarged by factor 10–50. For more details, see text.

centration of the latter compound, the presence of which is no doubt due to human activity, was nearly constant at each sampling site throughout the whole campaign. Actually, it is used as an internal marker in river Tyne (UK) studies [26]. Our estimates indicate the concentration of caffeine to be 0.5–2 $\mu g\,l^{-1}$ which is 5–10-fold higher than in other European rivers [23]. This may well be due to the fact that dilution in a small river such as the Nitra is much less than in larger rivers. Non-point source pollution was also observed for the widely used pesticide atrazine. In contrast, 1-chloro-2-(chloromethyl)ethoxypropane (cf., Fig. 5; maximum concentration, 10–100 $\mu g\,l^{-1}$ at Chalmova) and many other halogenated compounds obviously enter the water from a point source. Triphenylphosphine oxide, which is a widely used industrial chemical [11] was something of an exception. Its concentration increased dramatically at Chalmova and, next, became significantly lower at Praznovce and Cechynce (not shown) to increase again at Komoca. Generally speaking, the pollution pattern agreed with that found using the other two techniques.

An interesting example is shown in Fig. 10. In April 1994, 9,10-dihydro-N-methyl-10-acridone suddenly appeared in all chromatograms from Cechynce and the sites downstream. The compound probably is a degradation product of acridine, which is widely

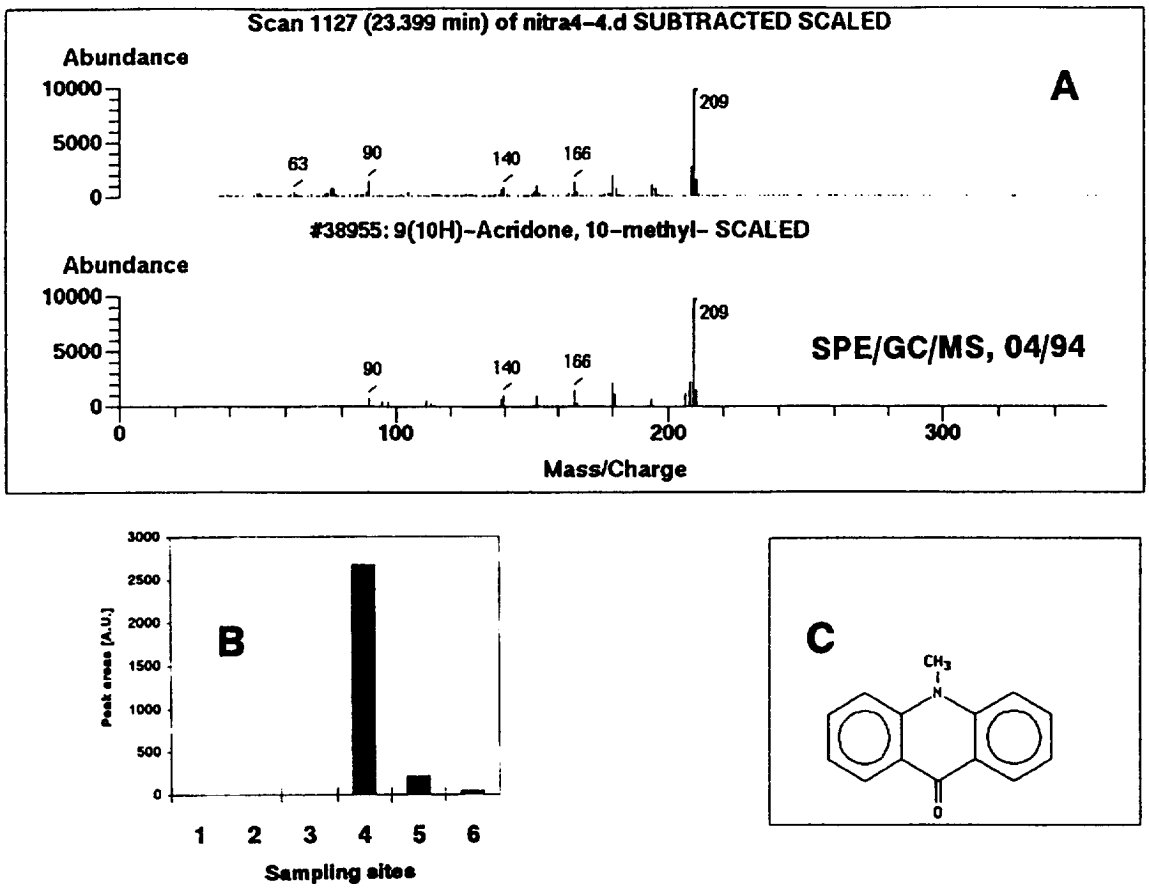

Fig. 10. (A) Result of spectrum library search for compound detected in the Nitra river (Cechynce, April 1994) by SPE–GC–MS; (B) pollution profile; for numbering of sampling sites, cf., Fig. 1; (C) structure of identified 9,10-dihydro-N-methyl-10-acridone. For more details, see text.

used in plastic production. Its presence may therefore well be related to a large plastic producer located a few kilometres upstream from the sampling site.

The selectivity of SPE–GC–MS is nicely demonstrated by the group of substituted aliphatic alcohols in the April 1994 and October 1994 samples. Ion chromatograms of *m/z* 59 ($C_3H_7O^+$ of the alcohols, but also $C_2H_3O_2^+$ of the methyl esters) showed a vast pollution of the river water at Chalmova throughout the year (Fig. 11). The concentrations of the alcohols were about ten times higher in October 1994 (approx. 75 $\mu g\,l^{-1}$ for the most intense peak at 11.5 min) than in April 1994 (approx. 5 $\mu g\,l^{-1}$ for the same peak). Qualitatively, the results agreed well with those of SPE–LC–PB-MS (data not shown) which showed similar peak profiles in ion chromatograms of *m/z* 117, assigned as $C_6H_{13}O_2^+$, usually the second most abundant ion in the EI spectra of the substituted aliphatic alcohols (*m/z* 59 is outside the scan range of the LC–PB-MS).

3.5. Performance of the SAMOS systems

The internal standards were also used to test the long-term stability of the systems with regard to their analytical characteristics. The primary goal was to evaluate the reproducibility within individual sampling campaigns because absolute values of retention times and peak areas could easily be related to those of the internal standards even after slight changes in

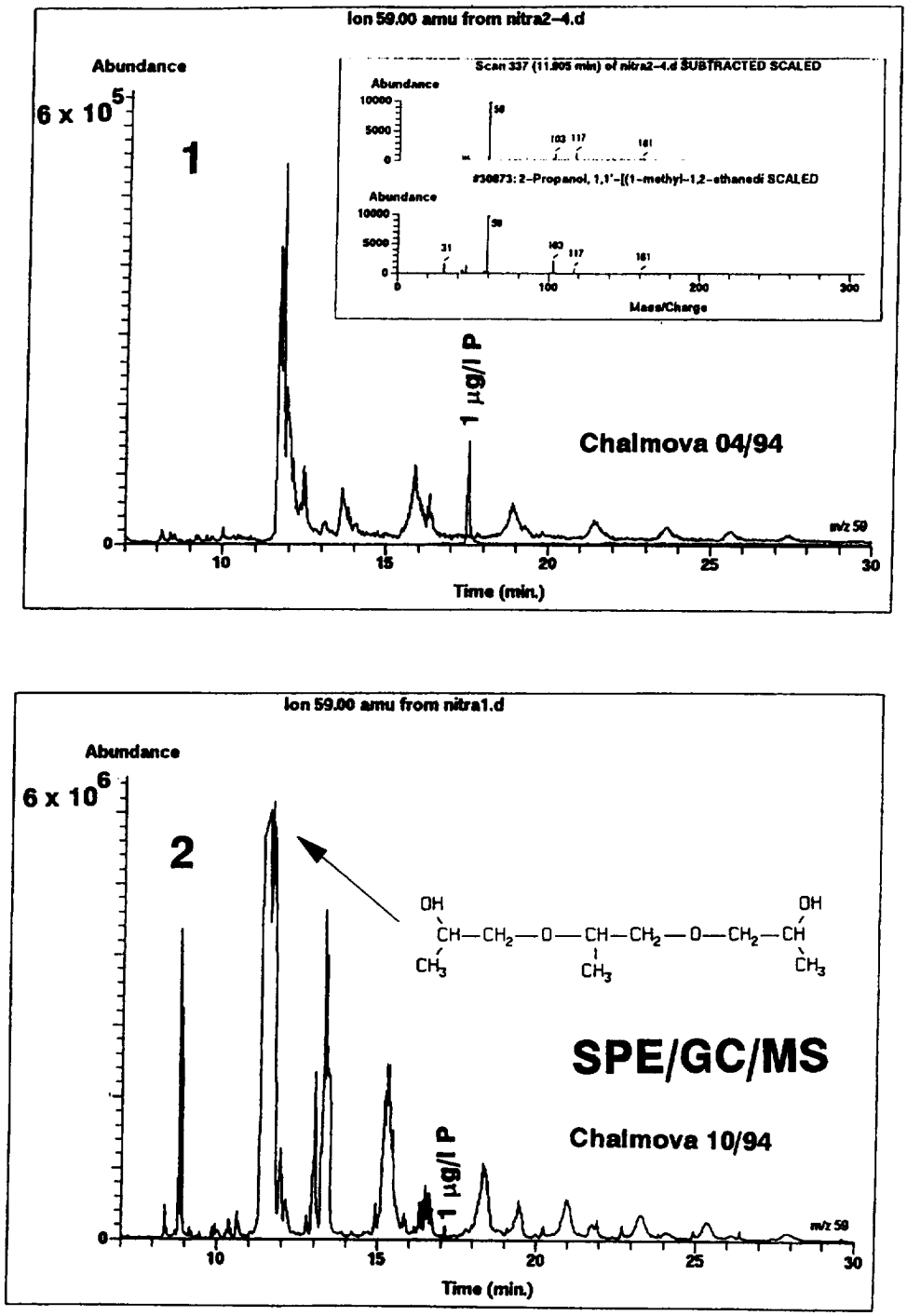

Fig. 11. SPE–GC–MS ion chromatograms (m/z 59) of Nitra river samples collected at Chalmova in (1) April 1994 and (2) October 1994. Enriched volume, 10 ml; P, 1 $\mu g\,l^{-1}$ of internal standard propazine. Inserts, result of library search and proposed structure for compound eluting at 11.9 min. For other conditions, see text.

conditions (see Experimental). For all three methods the R.S.D. values of the retention times varied between 0.1 and 0.9% for each series of samples (n=6–12). The R.S.D. values of peak areas were 1–15% for LC–DAD UV and 10–16% for GC–MS. For PB-MS, the R.S.D. values of peak areas were distinctly less good, viz. 17–31%. This is slightly worse than in a previous report (less than 15% [9,11]), but it should be noted that six different matrices were used in the present study. Library searches of monitored peaks in SPE–LC–DAD UV invariably yielded match factors of at least 997 (out of 1000). In SPE–LC–PB-MS the internal standards were always found as the first hit in the library search, but the match factors were rather low (55–77 out of 100), partly due to spectral interferences from co-eluting compounds. GC–MS spectra of propazine always gave satisfactory match factors well above 90 (out of 100).

SPE–LC–DAD UV showed reliable performance over the whole testing period. Using the set-up and conditions described in the Section 2.2, no real problems were encountered and minimal maintenance was required. The most vulnerable part of the system was the solvent delivery unit of the Prospekt, particularly as regards exact sample volume delivery. Due attention was therefore paid to proper functioning of the pump.

SPE–GC–MS also provided consistent data over the whole period of the study. The results allow us to state that this technique can be considered mature and is ready to be used for routine analyses. The system could handle sequences of 15–20 fully automated runs. The cartridges used for preconcentration could be re-used 50–100 times without any noticeable deterioration. The most common problem was the occurrence of air leaks in the MS vacuum system.

The robustness of SPE–LC–PB-MS was satisfactory but, as expected, not as good as that of the other two systems. Analyte detectability was less good and quantification less reproducible. In order to keep the performance of the system sufficiently stable, a single-point calibration was performed at the start of each day (flow injection of 500 ng diuron [9]). Maintenance consisted mainly of cleaning the PB interface skimmers (once a month), regular exchange of the PB inlet filter and cleaning of the ion source (once every six weeks). Finally, when running the LC–PB–MS unattendedly for more than 6 h, an overall decrease in sensitivity was observed. The PB inlet valve into the MS system was therefore kept closed during the trace-enrichment procedure.

4. Conclusions

Three on-line and automated analytical systems, SPE–LC–DAD UV, SPE–LC–PB-MS and SPE–GC–MS, were successfully used in a 2-year monitoring programme of the river Nitra (Slovakia). In the final stage of the programme, the three techniques were integrated into one system [9].

SPE–LC–DAD UV (100 ml samples) provided useful information on the overall pollution of the water and allowed the low- to sub-$\mu g\,l^{-1}$ determination and provisional identification of pesticides with a laboratory-made UV spectrum library. SPE–GC–MS (10 ml samples) enabled the even more sensitive determination of 50–100 compounds in a single run; many of these compounds were identified by means of an MS library search. SPE–LC–PB-MS (200 ml samples) provided EI mass spectra searchable in conventional MS libraries; quite a number of compounds not amenable to GC could be identified, but detection limits were less satisfactory than with the other two systems, although usually at the acceptable 1–5 $\mu g\,l^{-1}$ level. No relevant maintenance problems were encountered during the whole monitoring period. The samples were transported from Bratislava to Amsterdam within less than 30 h, at a temperature of approx. 10°C. This did not cause any noticeable analyte degradation.

As regards the pollution pattern of the river Nitra, a high number and relatively large concentrations of organic contaminants were found at the sampling site Chalmova (the downstream part of the highly industrialized municipality Prievidza) during all campaigns. Some 100 industrial and agricultural chemicals were tentatively identified by at least one of the techniques. Many of the detected compounds, especially chlorine- and sulphur-containing pollutants, halogen- and alkyl-substituted phenols and aliphatic alcohols were present in the samples throughout the entire period. Concentration profiles showed non-point pollution sources for most pesticides and also

caffeine; point-source pollution was found for many industrial chemicals. With preliminary information on the type and identity of pollutants now available, SPE–LC–TSP-MS and SPE–GC–AED will become more useful. The former technique can be used for the selective and sensitive target analysis of LC-amenable compounds, and the latter for providing additional structural information on suspected GC-amenable pollutants [24]. In summary, the present project has shown the practicality of combining the data from what are often called sophisticated techniques, for the routine monitoring of surface water samples.

Acknowledgments

The authors acknowledge Ir. P.J.M. van Hout and the Rhine Basin Program (Amsterdam/Waldbronn) for providing instrumentation and the EU Environment Programme (Supplementary Agreement No. 1: ERBCIPDCT925102 to contract No. EV5VCT920105) for financial support.

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