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Integrated system for on-line gas and liquid chromatography with a single mass spectrometric detector for the automated analysis of environmental samples

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Abstract

An integrated system has been developed which combines liquid (LC) and gas (GC) chromatographic separation with a single mass spectrometer (MS). On-line solid-phase extraction (SPE) of 10–200 ml aqueous samples on a short (10×2.0 mm I.D.) precolumn packed with a styrene–divinylbenzene copolymer is used for analyte enrichment. The trace-enrichment procedure was automated by means of a PROSPEKT cartridge-exchange/solvent-selection/valve-switching unit. After sample loading, the precolumn is eluted on-line in two subsequent runs, first onto the GC–MS system and, next, onto the LC–MS system using a particle beam (PB) interface. Prior to entering the PB-MS, the LC eluent passes through the flow cell of a UV diode-array detector (DAD). Both GC–MS and LC–PB-MS generate classical electron ionisation (EI) and chemical ionisation (CI) spectra which are useful for the identification of low- and sub-μg/l concentrations of environmental pollutants covering a wide polarity and volatility range. The LC–DAD data provide additional means for quantitation and yield complementary spectral information. All three detection systems (GC–MS, LC–DAD, LC–PB-MS) and the trace-enrichment procedure are fully automated and controlled from the keyboard of the central computer. With such a 'MULTIANALYSIS' system GC–MS, LC–DAD and LC–MS data of the same sample can be obtained within 3 h. The system was optimised with nine chlorinated pesticides in drinking water as test mixture. With 100-ml samples detection limits in GC–MS were 0.0005–0.03 μg/l, and in LC–PB-MS 0.5–7 μg/l, both in the full-scan (EI) mode. Negative chemical ionisation (NCI) with methane as reagent gas improved the sensitivity of six halogenated compounds 3- to 30-fold and provided relevant information for structural elucidation of unknown compounds in real-world samples. LC–DAD detection limits varied from 0.01 to 0.05 μg/l. Relative standard deviations (R.S.D.) of retention times were less than 0.2% in all systems, R.S.D.s of peak areas were 5–15% for GC–MS and LC–PB-MS and less than 5% for LC–DAD. The 'MULTIANALYSIS' system was used to analyse surface water samples and river sediment extracts; several pollutants were detected and identified.

Keywords: Environmental analysis; Water analysis; Automation; Liquid chromatography–mass spectrometry; Gas chromatography–mass spectrometry Pesticides

1. Introduction

Today, several millions of man-made organic substances are known and many of these possess a variety of toxic, carcinogenic and/or mutagenic properties. It is therefore highly important to monitor surface water, groundwater and drinking water in order to verify whether inadmissible levels of toxic microcontaminants and/or their degradation products

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are present. For obvious reasons, mass spectrometry is the key detection technique in such environmental work, because it can be optimised for identification, and quantitation, of target compounds as well as unknowns.

Sample complexity generally requires a separation step prior to MS detection. Capillary gas chromatography–mass spectrometry (GC–MS) is a well established technique which combines a high separation power and sensitive and selective detection [1]. As regards the coupling of column liquid chromatography and mass spectrometry (LC–MS), several interfaces have become commercially available in the past decade. These include thermospray (TSP), particle beam (PB) and atmospheric pressure ionisation (API) interfaces, facilitating the use of various ionisation modes [2,3]. Although API interfaces are considered to have a promising future because of their excellent sensitivity and the possibility of regulated fragmentation in the ion source [4], the PB interface will certainly remain attractive as the only interface which can generate EI and solvent-independent chemical ionisation (CI) spectra.

Analytes of environmental interest are invariably present in samples at very low levels and a concentration step prior to actual analysis is generally required. Off-line concentration generally is laborious and difficult to automate, rather large amounts of organic solvents have to be used and contamination occurs easily. On-line techniques, and especially on-line solid-phase extraction (SPE) probably is the best solution for introducing large sample volumes into GC–MS or LC–MS and several on-line, and automated, systems have been developed in recent years [5–13]. Such systems, often called SAMOS (System for Automated Measurement of Organic Micropollutants in Surface Water), employ SPE on a small precolumn, typically 10×2.0 mm I.D., packed with a highly hydrophobic copolymer or C_{18}-bonded silica [14] in combination with reversed-phase LC and diode-array UV (DAD) detection [6]. In recent studies, TSP-MS detection was introduced for target analysis [8,10], and PB-MS for the detection of unknown compounds [7]. Similar procedures (with an extra step to remove traces of water prior to analyte transfer to the GC part of the system) have been reported for handling 1–10-ml water samples in SPE–GC–MS [11,12].

In this study a unique feature of the LC–PB-MS and GC–MS techniques, that of sharing the same MS ion source, and also a similar analyte-enrichment procedure, is used for their combination in one system. The set-up is extended with a DAD detector which is inserted in front of the PB-MS unit to supply additional spectral information. With SAMOS-type systems already commercially available and operational at several monitoring stations in Europe, one rationale behind the development of a MULTIANALYSIS system would be to use it for the final confirmation of the identity, and concentration level, of a microcontaminant suspected to be present on the basis of the early-warning information supplied by a peripheral laboratory. To this end, SPE cartridges loaded with duplicate samples can be transported to the central laboratory, placed in the cartridge holder of the sample preparation unit and subjected to analysis via the LC–MS and the GC–MS parts of the MULTIANALYSIS system.

2. Experimental

2.1. Solid-phase extraction

Instrumentation

A PROSPEKT automated cartridge-exchange/solvent-selection/valve-switching unit (Spark Holland, Emmen, Netherlands), equipped with an additional automated six-port switching valve (MUST, Spark Holland) was used for sample handling. The solvent delivery unit (SDU) of the PROSPEKT was provided with a six-port solvent selection valve, a pulse damper and a single-piston analytical LC pump. A 10×2.0 mm I.D. stainless-steel precolumn was packed with 15–25 μm copolymer PLRP-S of 100 Å pore size (Polymer Laboratories, Church Stretton, UK). All parts of the set-up were controlled by the SPE/WIN software (version A.03.14B) of the DOS-based HP Vectra 486/66XM computer (Hewlett-Packard, Waldbronn, Germany).

Procedures

All experiments were performed with the set-up shown in Fig. 1 which allows subsequent GC and LC runs of the same sample. Prior to analyte enrichment, the precolumn was conditioned with 4 ml of metha-

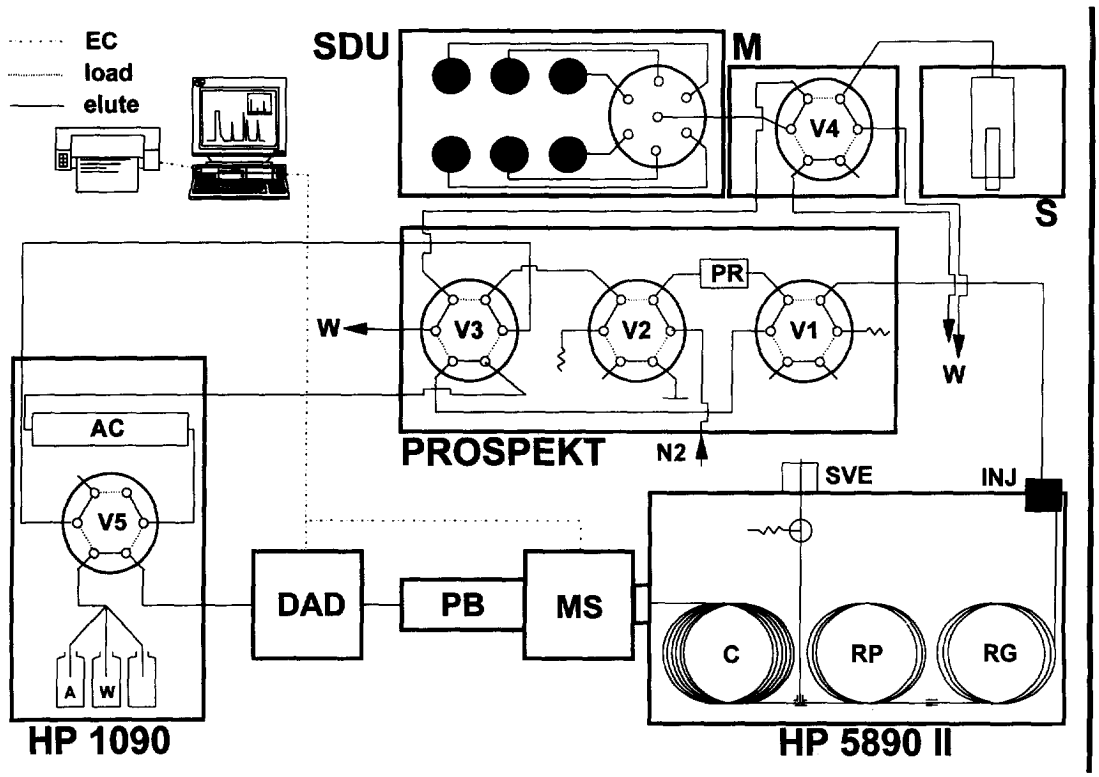


Fig. 1. Schematic representation of the MULTIANALYSIS system which combines SPE–GC–MS and SPE–LC–DAD–PB-MS in one set-up. HP 1090=liquid chromatograph; HP 5890 II=gas chromatograph; MS=mass spectrometer; PB=particle beam interface; DAD=UV diode-array detector; PROSPEKT=automated valve-switching, solvent-selection and cartridge-exchange unit; SDU=solvent-delivery unit of PROSPEKT equipped with six-port solvent selection valve, pulse damper and LC pump; M=MUST, automatic six-port switching valve unit; S=syringe pump; PR=precolumn packed with PLRP-S material; AC=LC analytical column; C=GC analytical column; RP=retaining precolumn; RG=retention gap; SVE=solvent vapour exit; INJ=on-column injector; N_2=nitrogen; W=waste; V1–5=six-port switching valves; EC=electronic connections; load/elute=positions of automatic six-port switching valves; computer and printer. For details, see text.

nol and 2 ml of HPLC-grade water. Next 100 ml of sample were pumped through the precolumn and the analytes of interest trapped. This part of the procedure was identical for LC (Table 1) and GC (Table 2).

LC

After loading, valve V3 (Fig. 1) was switched to the 'elute' position and the trapped analytes were eluted from the precolumn by the LC gradient onto the analytical column (see Section 2).

GC

After trace enrichment, 1 ml of HPLC-grade water was pumped through the precolumn, in order to prevent introduction of salts into the GC system. The precolumn was then dried for 30 min with 40 ml/min of nitrogen. The capillaries were pressurised with ethyl acetate and after switching valve V1 to the 'load' position, the analytes were eluted from the precolumn into the retention gap (see Section 3).

Samples

Nine chlorinated triazine, anilide and organophosphorus pesticides of at least 95% purity were used as test compounds (see Table 3 below). They were obtained from Riedel-de-Haën (Seelze, Germany). Stock solutions of 200 mg/l were prepared in (1) ethyl acetate for GC and (2) methanol for LC, and diluted to a final concentration of 10 mg/l of each compound in a mixture. This was used for direct

Table 1
PROSPEKT sample preparation programme for trace enrichment and analysis of 100-ml aqueous samples by SPE–LC–DAD–PB-MS[a]

Time (min:s)	V3	V4	Solvent	SDU flow (ml/min)	#-AUX	Comment
00:00	load	load	1	0	all-OFF	start position
00:01				5		clean tubings with 5 ml MeOH
01:01				2		
01:02		elute				condition precolumn with 4 ml MeOH
03:02		load				
03:03			2	5		clean tubings with 5 ml HPLC-grade water
04:03				2		
04:04		elute				remove MeOH from precolumn with 2 ml HPLC-grade water
05:04		load				
05:05			3	5		fill tubings with 5 ml sample
06:05				3.5		
06:06		elute				enrich 100 ml sample on precolumn
34:40		load				
34:41				0		stop sample flow
34:45	elute				6-ON	elute precolumn with LC eluent; start HP 1090 time table (see below), DAD and MS data acquisition
90:45	load					end of LC run
90:46						sample preparation end time

HP 1090 time table

Time(min:s)	A (%)	B (%)	V5	Flow (ml/min)
00:00	10	90	load	0.4
45:00	95	5		
55:00	95	5		
56:00	10	90		

[a] Mobile-phase constituents are acetonitrile (A) and HPLC-grade water (B); V1–5, positions of automatic six-port switching valves according to Fig. 1; V1 continually was in elute position and V2 in load position; HP 1090, liquid chromatograph; SDU, solvent delivery unit of PROSPEKT; AUX, auxilliaries for automated control of external devices. For further details see Section 2.

on-column injections and for the preparation of spiked tap and surface water samples.

Surface water samples and river sediments were collected from the Nitra River in Slovakia in October 1994, transported to the Netherlands in a portable refrigerator and stored at 4°C in dark. Prior to analysis, the water samples were filtered through a 0.45-μm acetyl-cellulose filter (Schleicher and Schuell, Dassel, Germany). Sediments were first extracted with 10 ml methanol (J.T. Baker, Deventer, Netherlands). Next, 8 ml of the extract were filtered through a disposable filter holder containing a 0.2-μm pyrogen-free filter (Schleicher and Schuell) and diluted in 250 ml HPLC-grade water. From this solution, 100 ml were used for SPE–GC–MS and another 100 ml for SPE–LC–DAD–PB-MS. Prior to analysis, both surface water and diluted sediment extracts were spiked with metoxuron and propazine (1 μg/l of each internal standard). The internal standards were selected in order to demonstrate the complementarity of the LC and GC techniques. Propazine could be detected with both GC and LC; metoxuron could be detected by the LC procedures only.

2.2. Liquid chromatography

Analyses were performed on a HP 1090 liquid chromatograph (Hewlett-Packard) equipped with an automatic six-port switching valve (Rheodyne, Berkeley, CA, USA). A 250×4.6 mm I.D. stainless-steel column packed with 5 μm C_{18}-bonded silica of 100 Å pore size (Supelco LC-18-DB, Supelchem, Leusden, Netherlands) was used for separation. A HP 1050 UV diode-array detector (Hewlett-Packard) was operated at 210 nm wavelength with 10 nm bandwidth.

A mixture of acetonitrile and HPLC-grade water

Table 2
PROSPEKT sample preparation programme for trace enrichment and analysis of 100-ml aqueous samples by SPE–GC–MS

Time (min:s)	V1	V2	V4	Solvent	SDU flow (ml/min)	#-AUX	Comment
00:00	elute	load	load	1	0	all-OFF	start position
00:01					5		clean tubings with 5 ml MeOH
01:01					2		
01:02			elute				condition precolumn with 4 ml MeOH
03:02			load				
03:03				2	5		clean tubings with 5 ml HPLC-grade water
04:03					2		
04:04			elute				remove MeOH from precolumn with 2 ml HPLC-grade water
05:04			load				
05:05				3	5		fill tubings with 5 ml sample
06:05					3.5		
06:06			elute				enrich 100 ml sample on precolumn
34:40			load				
34:41					0		stop sample flow
34:45				2	5		fill tubings with 5 ml HPLC-grade water
35:45					1		
35:46			elute				remove salts from precolumn with 1 ml HPLC-grade water
36:46			load				
36:47					0		stop HPLC-grade water flow
36:50		elute		1			dry precolumn with N_2 (30 min)
36:51					5		clean tubings with 5 ml MeOH
37:51				2			clean tubings with 5 ml HPLC-grade water
38:51					0		stop HPLC-grade water flow
62:50						4-ON	syringe pump ON, pressurise system with 300 μl ethyl acetate (75 μl/min)
66:50	load	load				1-ON	stop N_2 flow; elute precolumn with 100 μl ethyl acetate; open SVE (2.21 min); start HP 5890 II time table (see below) (solvent delay 6 min)
66:55						1-OFF	start position of AUX 1
68:41	elute						start position of valve 1
69:41						4-OFF	stop ethyl acetate flow
69:42							sample preparation end time

HP 5890 II time table

Time (min:s)	Temperature (°C)	T gradient (°C/min)	He pressure (psi)	SVE
00:00	85		6.0	open
02:21				closed
05:00	85	10		
23:30	270			
24:30	270		12.4	

Temperature of GC–MS interface, 280°C; V1–4, positions of automatic six-port switching valves according to Fig. 1; V3 was continually in load position; HP 5890 II, gas chromatograph; SDU, solvent delivery unit of PROSPEKT; AUX, auxilliaries for automated control of external devices; SVE, solvent vapour exit controlled as PURGE A option of HP 5890 II.

(Riedel-de-Haën) was used as eluent; the gradient conditions are given in Table 1. DAD spectra and retention times of unknown compounds from real-world samples were compared with those stored in a home-made library (ca. 150 entries). If the spectral match was above 950 (arbitrary units; scale, 0–1000), provisional identification was considered positive. PB-MS spectra were used for further confirmation.

2.3. Gas chromatography

Analyses were performed on a HP 5890 Series II gas chromatograph (Hewlett-Packard) equipped with a pressure-programmable on-column injector. The split–splitless injector was replaced by a home-made solvent vapour exit. Separations were carried out on a HP-1 (10 m×0.20 mm, 0.33 μm dimethyl-cross-linked polysiloxane) capillary column (Hewlett-Packard) with high-purity helium (Hoek Loos, Schiedam, Netherlands) as carrier gas. The helium pressure was programmed to increase from ca. 41 to 85 kPa (6 to 12.4 psi) during the run to simulate constant-flow conditions. The injector was connected to a 5 m×0.32 mm retention gap deactivated with diphenyltetramethyldisilazane (DPTMDS, BGB Analytik, Zürich, Switzerland). A 2 m×0.20 mm retaining precolumn contained the same stationary phase as the analytical column. Connections were made with conventional glass press-fits (BGB Analytik).

The analytes trapped on the precolumn were eluted with 100 μl ethyl acetate (75 μl/min) into the retention gap and retaining precolumn; the excess of the eluent was removed through the SVE using partially concurrent solvent evaporation (Fig. 1). After closing of the SVE, the analytes were transferred to the analytical column for separation and MS detection. The GC temperature programme is given in Table 2. For a detailed description of the total procedure, one should consult Ref. [12].

2.4. Mass spectrometric detection

An HP 5989A MS Engine (Hewlett-Packard) equipped with a high-energy dynode and a high-mass option, was used as a detector for both LC–MS and GC–MS. An HP 59880B PB interface was used to introduce the LC eluent into the MS.

The MS was operated in the full-scan mode, using a 45–400 amu scan range for positive-ion EI, and 65–400 amu for NCI detection; the scan rate was 0.5 scan/s and 1 scan/s for LC–MS and GC–MS, respectively. High-purity reagent gas methane (Hoek Loos) was used for NCI experiments; its pressure in the ion source was maintained at 0.8 Torr (ca. 107 Pa). The temperature of the MS ion source was held at 250°C, and that of the quadrupole at 100°C. The PB nebulising gas, high-purity helium (Hoek Loos), was kept at 248 kPa (36 psi, ca. 2.5 l/min); the temperature of the PB desolvation chamber was 70°C. The performance of the PB-MS in EI or CI mode was checked each day with flow injections of 500 ng diuron. The Wiley mass spectral library (130 000 entries) was used for identification of unknown compounds.

2.5. MULTIANALYSIS system

Each sample was first analysed by means of GC–MS (Table 2). Next, the GC-to-MS remote control cable was replaced by that from LC to MS and software packages for handling of LC–DAD (G 1307A, HPLC3D Chemstation) and PB-MS (G 1034C, MS Chemstation) were loaded with appropriate methods. Similarly to GC–MS, a sample preparation programme (SPP) from the SPE/WIN software (for on-line control of the PROSPEKT) started the trace-enrichment procedure with subsequent (DAD and MS) data acquisition. After the run, the LC-to-MS remote control cable was returned to its original position and the GC–MS software was loaded again prior to processing the next sample. Using the sequence option, the unattended operation of three to six LC–MS analyses and ten to twenty GC–MS analyses was repeated without switching between the techniques. If more than six LC–MS runs were performed, a significant decrease in sensitivity was observed. The capacity limits of the unattended GC–MS repetitive analyses were not reached in this study, not even when multiple series of samples were run overnight. Regarding the above results it is recommended to use the MULTIANALYSIS system as it was originally designed,

with a switch between the LC and GC modes after each run. In that case the vacuum part of the LC–MS is not continuously operated at its maximum performance and four to six samples (12–18 h) can be analysed daily by both the LC and GC modes of the system.

Auxiliary output signals of the PROSPEKT were used to control the actions of the MUST six-port switching valve, the syringe pump used to deliver ethyl acetate, the opening and closing of the SVE and the start of GC–MS or LC–DAD–MS data acquisition (Fig. 2). The 'start' signals were transferred from LC or GC to the detectors by means of remote cables. The settings of the HP 1090 and HP 5890 II, the DAD and MS detectors, and the signal/data transfer were on-line commanded by means of HP-IB electronic connections and appropriate software from the central computer. The PROSPEKT was controlled through a RS232C interface and SPE/WIN software.

3. Results and discussion

In the subsequent sections, attention is devoted to the performance of the three separate parts of the total analytical set-up and, next, to the MULTIANALYSIS system. In order to facilitate the comparison of the various modes of operation, most figures combine chromatograms for each of these. As a reference point, sets of three chromatograms obtained for spiked tap water and real-world surface water samples are shown in Fig. 3 and Fig. 4, respectively. The data in Table 3 and Table 4 were obtained with the complete MULTIANALYSIS system.

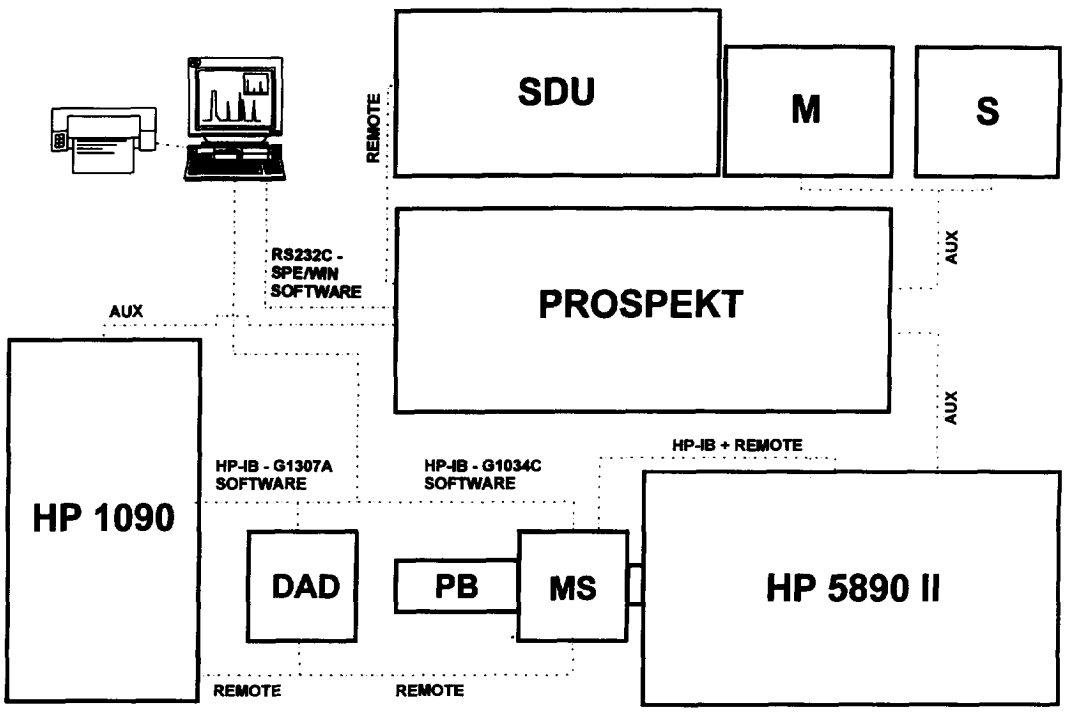
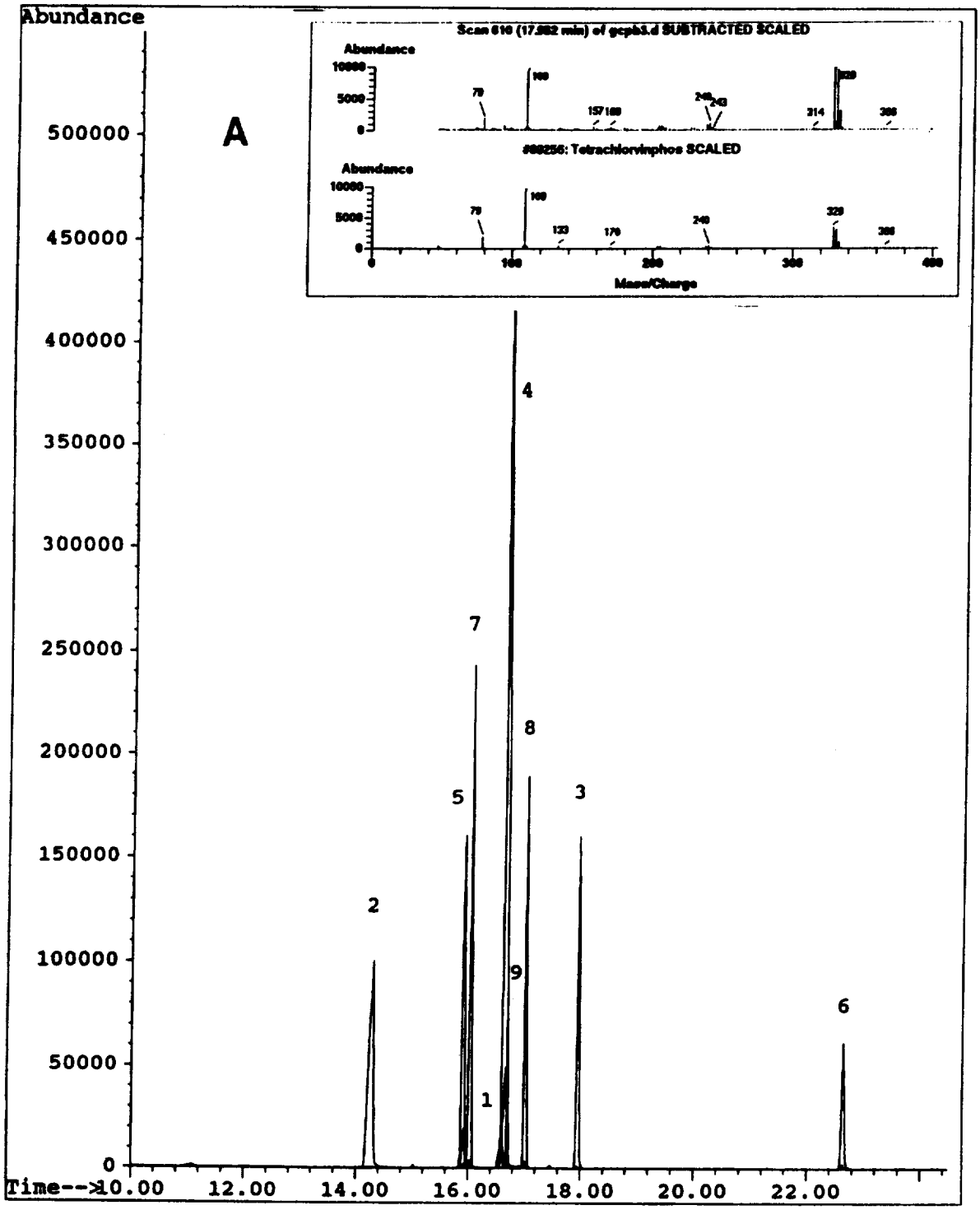


Fig. 2. Schematic representation of the electronical connections of the MULTIANALYSIS system. AUX=auxiliary outputs of PROSPEKT for start of external devices; REMOTE=remote cables for (i) start of GC–MS or LC–DAD–MS data acquisition and (ii) on-line control of SDU from PROSPEKT; HP-IB and RS232C=electronical connections for on-line control of all parts of the system from the central computer. For details, see text.


Abundance
A
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Scan 616 (17.982 min) of gcpb3.d SUBTRACTED SCALED
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Mass/Charge

Fig. 3.

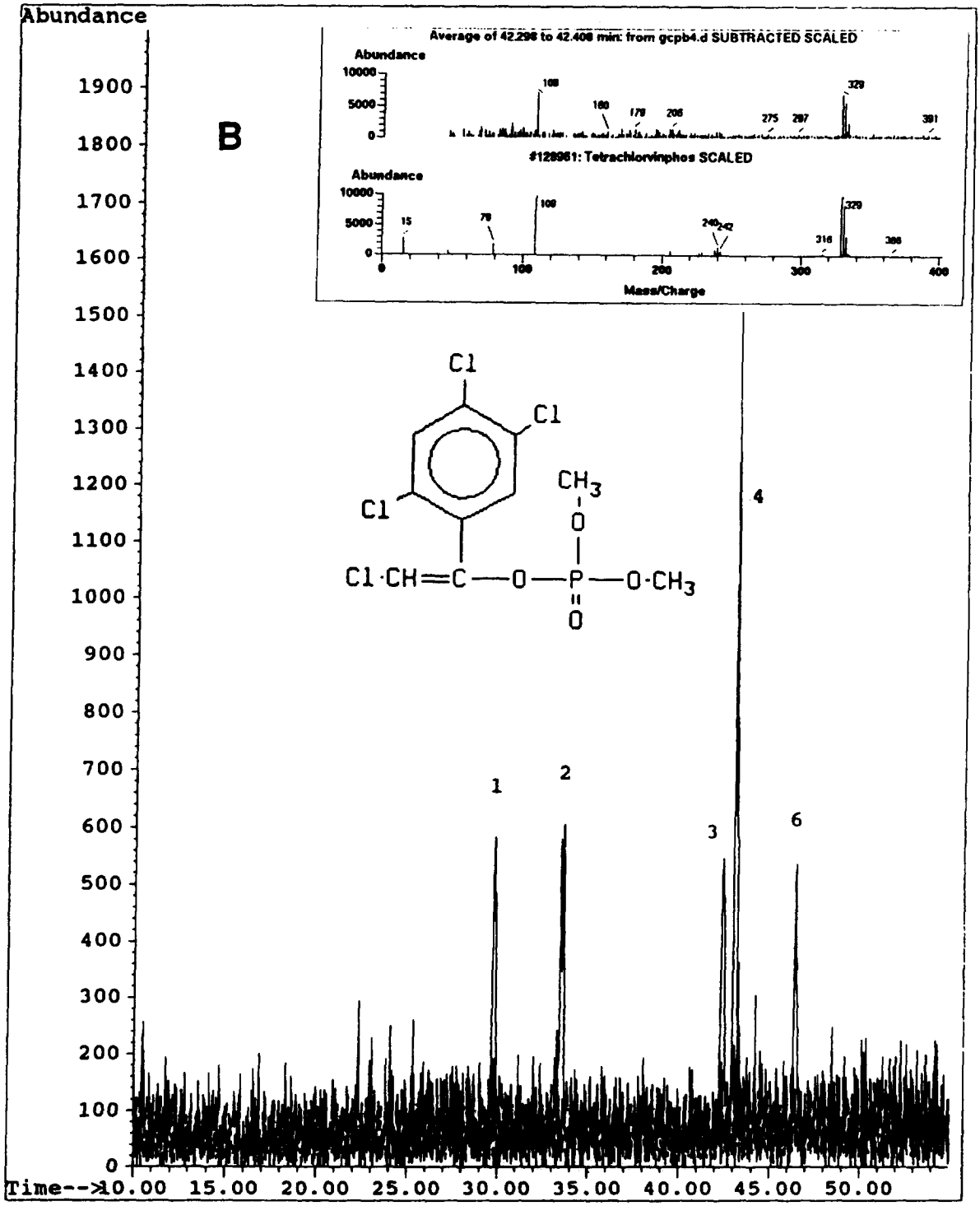

Fig. 3. (*Continued on p. 362*)

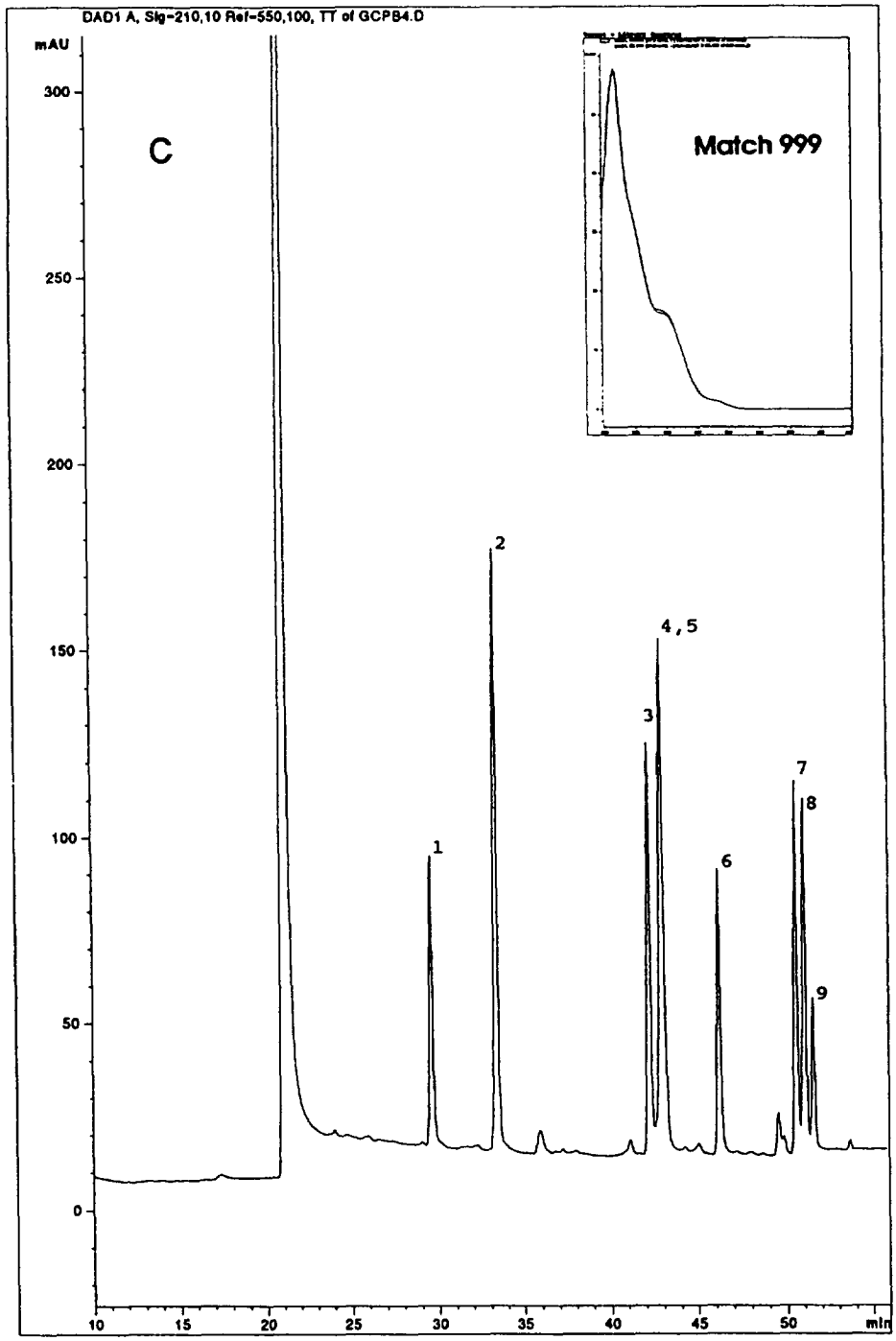

Fig. 3. Chromatograms of nine chlorinated pesticides in tap water obtained by (A) SPE–GC–MS, (B) SPE–LC–PB-MS, and (C) SPE–LC–DAD. Samples were spiked at 1 μg/l of each compound (for numbers cf. Table 3); enriched volume, 100 ml. MS chromatograms (A, B) represent extracted ions obtained at m/z of base peak of each analyte. The DAD chromatogram (C) was obtained at 210 nm wavelength with 10 nm bandwidth. Inserts attached to each chromatogram show results of spectral library searches for EI or DAD spectra of tetrachlorvinphos (No. 3). For more details, see text.

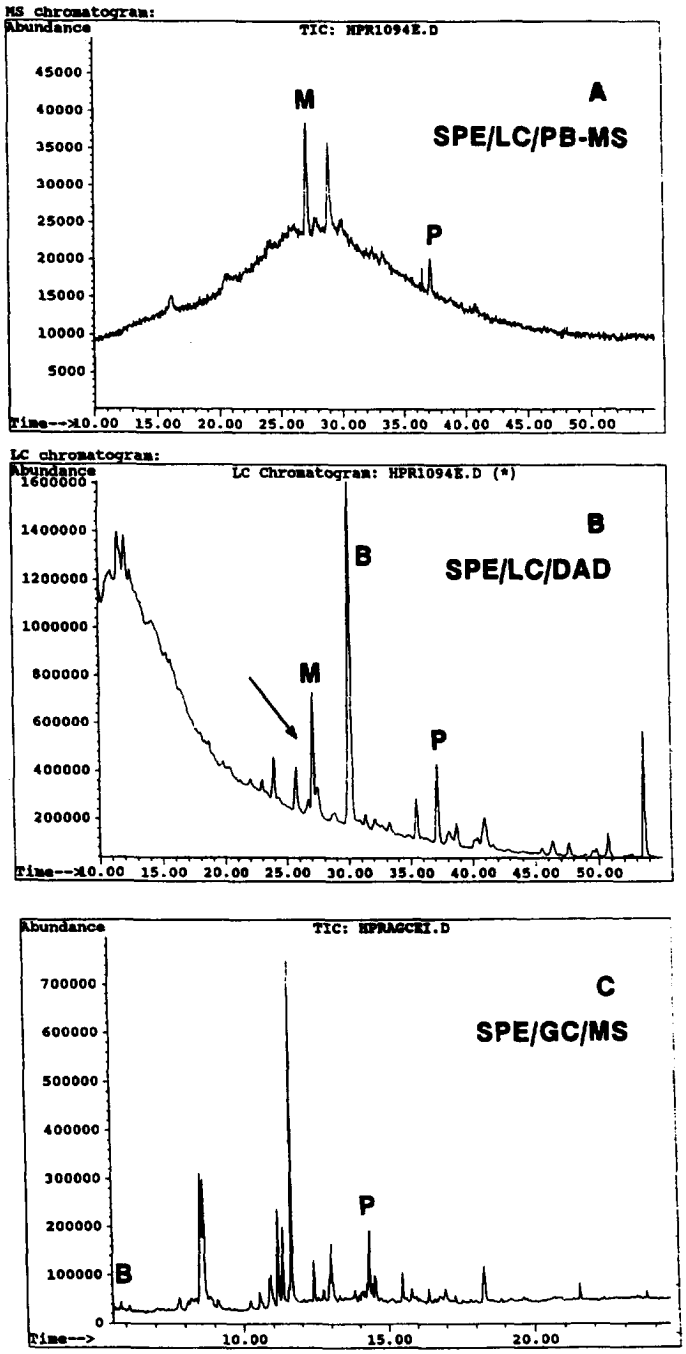

Fig. 4. Chromatograms of Nitra river (Slovakia) water samples obtained by (A) SPE–GC–MS, (B) SPE–LC–PB-MS, and (C) SPE–LC–DAD. Sample volume for LC experiments, 200 ml, and for GC, 10 ml. Sample was spiked at 1 μg/l of internal standards propazine (P) and metoxuron (M). Unknown pollutant benzothiazole (B) was identified by LC–DAD and confirmed by GC–MS. For more details, see text.

Table 3
Detection limits ($S/N=3$) of nine chlorinated pesticides in tap water (enriched volume indicated) obtained with the MULTIANALYSIS system by SPE–GC–MS, SPE–PB-MS and SPE–LC–DAD (for conditions, see text)

No.	Compound	Detection limits (μg/l) with:			
		SPE–GC–MS[a]		SPE–LC–PB-MS[a]	SPE–LC–DAD[b]
		10 ml	100 ml	100 ml	100 ml
1	Cyanazine	0.1	0.03	0.5	0.05
2	Atrazine	0.01	0.001	0.5	0.01
3	Tetrachlorvinphos	0.01	0.001	0.5	0.02
4	Metolachlor	0.005	0.0005	0.5*	0.03*
5	Alachlor	0.07	0.007	7*	0.03*
6	Coumaphos	0.1	0.03	0.7	0.01
7	Fenchlorphos	0.05	0.005	5	0.01
8	Bromophos	0.05	0.005	3	0.01
9	Chlorpyriphos	0.1	0.03	5	0.02

[a] Detection limits obtained from extracted ion chromatograms at m/z of the base peak in spectrum of each compound.
[b] Detection limits obtained at wavelength of 210 nm with 10 nm bandwidth.
* The compounds coelute.

A group of nine chlorinated pesticides was selected as test compounds for this study because of their expected good response in negative chemical ionisation (NCI) experiments.

3.1. SPE–GC–MS

The performance of the GC part of the set-up was tested by the analysis of tap water samples of 10–100 ml spiked with the nine model compounds. The excellent sensitivity and separation power of GC–MS, in the EI mode, combined with the total-sample analysis of rather large water volumes provided full-scan detection limits in the range of 0.5–30 ng/l (Table 3). At those levels all compounds could still be identified from their EI mass spectra by the library search. Obviously, the handling of even 100 ml of sample per run does not detract from the

Table 4
Relevant analytical data on determination of nine chlorinated pesticides using the MULTIANALYSIS system

No.	Compound	Base peak	SPE–GC–MS		SPE–LC–PB-MS		SPE–LC–DAD	
			t_R[a] (R.S.D.)	PA[b] (R.S.D.)	t_R (R.S.D.)	PA (R.S.D.)	t_R (R.S.D.)	PA (R.S.D.)
1	Cyanazine	212	16.57 (0.09)	11 (14)	29.7 (0.1)	73 (7)	29.5 (0.1)	104 (5)
2	Atrazine	200	14.22 (0.04)	64 (12)	33.5 (0.2)	75 (7)	33.2 (0.1)	218 (4)
3	Tetrachlorvinphos	329	17.92 (0.06)	47(7)	42.3 (0.1)	69 (14)	42.2 (0.1)	127 (3)
4	Metolachlor	162	16.65 (0.03)	179 (10)	43.0 (0.1)	110 (9)	42.9 (0.1)	221 (5)*
5	Alachlor	160	15.91 (0.08)	54 (13)	ND	ND	42.9 (0.1)*	
6	Coumaphos	362	22.56 (0.10)	21 (8)	46.3 (0.1)	55 (23)	46.2 (0.1)	98 (3)
7	Fenchlorphos	285	16.02 (0.03)	55 (10)	ND	ND	50.5 (0.1)	110 (5)
8	Bromophos	331	16.99 (0.01)	44 (7)	ND	ND	51.0 (0.1)	104 (5)
9	Chlorpyriphos	197	16,68 (0.07)	16 (9)	ND	ND	51.5 (0.1)	40 (3)

Tap water was spiked with 1 μg/l of each pesticide; 100-ml volumes were enriched on PLRP-S precolumn and eluted sequentialy into, first, the GC and, next, the LC part of the system ($n=6$). MS data were acquired in the full-scan EI mode; the DAD detector was set at 210 nm with 10 nm bandwidth; ND, not detected at 1 μg/l concentration level.
[a] t_R=retention time in min.
[b] PA=peak area in arbitrary units; units different for MS and DAD measurements.
* Peaks coelute; PA of alachlor is included in PA of metolachlor.

performance of the SPE–GC–MS system as is evident from the 10-fold (occasionally, 3-fold) improved detection limits observed upon comparison with 10-ml injections. Actually, the main problem is that one will have to be very careful with regard to memory effects after, e.g., the injection of higher-level standard solutions or when drawing conclusions from selected ion monitoring (SIM) results obtained at or below the 1 ng/l level. For the rest, R.S.D. values of retention times varied between 0.01 and 0.1%, and those of the peak areas ranged from 7 to 14% (n=6 within 4 days; Table 4). All calibration curves were linear within the range 0.1–10 μg/l with linear regression coefficients above 0.998.

A single precolumn was used repeatedly for the trace enrichment of standard and real-world samples. Actually, even after being re-used 100 times, no signs of deterioration, increasing backpressure during sample enrichment or memory effects were observed. The GC–MS part of the MULTIANALYSIS system was tested over a 5-month period, and some 200 analyses were performed during this time. The only problem encountered was clogging of the SVE after 4 months of operation. However, it should be mentioned that this SVE had then been in use for a period of two years as a part of various GC set-ups.

Analyses of real-world surface water (Fig. 4C) and sediment samples revealed the presence of numerous environmental pollutants. More than 50 compounds were detected in each sample. Over 30% of the pollutants could be identified by means of a library search. The water sample was found to contain a wide variety of industrial and agricultural pollutants including chlorinated aliphatic hydrocarbons, aliphatic alcohols, rubber chemicals, pesticides, etc. The sediment extract mainly contained higher aliphatic acids and polyaromatic hydrocarbons and their conversion products. With the methanolic extract of the Nitra river sediment, 10-fold sample dilution or analysis of a smaller volume (10 ml) was needed in order to prevent system overloading: analysis of the concentrated sample resulted in a complete loss of resolution. A major part of the observed peaks yielded EI mass spectra that could not be confirmed by searching the MS spectral library. A more detailed discussion of the results of the sediments and surface water analyses will be given in a separate paper [15].

3.2. SPE–LC–PB-MS

The well-known problem of LC–PB-MS, low sensitivity, which is primarily caused by analyte losses during their transport through the interface is clearly demonstrated by the data from EI experiments reported in Table 3. The full-scan detection limits in the range of 0.5–7 μg/l are about 2–3 orders of magnitude higher than those obtained with GC–MS (cf. Fig. 3). These limits could be improved 5- to 10-fold in cases of target analysis, i.e. when SIM instead of full-scan acquisition was used. Also, for the present set of test compounds, the preconcentrated sample volume could be increased up to 500 ml without any serious loss of analytes; this represents another 3–5-fold gain in sensitivity. As regards the well-known problem of non-linearity of calibration curves, their construction from three data points within the range of interest (0.1–10 μg/l) was found to be satisfactory for semiquantitative evaluation; at least six points were required to achieve precise quantification.

Long-term stability is often quoted as a problem when using a PB interface [16,17], and the present system indeed had to be checked regularly by injecting diuron as a standard compound (see Section 2). Actually, the R.S.D. of the diuron peak area was less than 15% within any one-week period and about 80% over the total five months of the MULTIANALYSIS system operation. As regards spectral recognition, the appearance of numerous small background peaks in the PB-MS spectra requires careful background subtraction to obtain useful EI spectra searchable in standard GC–MS libraries. In the present study, the laborious checking of the significance of major ions in the spectra of detected peaks was automated by means of a simple macro programme. Finally, one should note that with tetrachlorvinphos which is used as an example in Fig. 3, the problem of a high background is mainly related to the fact that the compound had to be identified close to its limit of detection.

The above disadvantages do not seriously detract from the overall performance of PB-MS (the R.S.D. data of Table 4 are rather similar to those obtained in SPE–GC–MS). It remains an attractive technique, primarily because searchable EI spectra can be produced for a wide range of compounds amenable

to GC–MS [18], but also for quite a number of polar, non-volatile and thermolabile compounds. The analysis of the methanolic extract of a Nitra river sediment sample (Fig. 5) is a typical example. Methanol will, of course, preferably extract more polar compounds which are not easily amenable to GC–MS. Fig. 5 shows that both internal standards were readily detected at the relevant 1 μg/l level. Next to the internal standards, some twenty unknown peaks were recorded. One example of an identification, viz. of a degradation product of phenanthrene, is included on the right hand side of the figure. In this particular instance when the standard compound was not available, confirmation could be obtained by means of SPE–GC–MS, but SPE–LC–PB-MS was the preferred method of analysis because of its better sensitivity.

For the rest, as with the SPE–GC–MS approach discussed above, the majority of the compounds detected in the samples did not display spectra that showed satisfactory matches with spectra stored in the Wiley library.

Apart from the daily system check (cf. above) and the usual maintenance tasks, the SPE–LC–PB-MS system proved to be robust, provided that LC eluent was not directed continuously into the MS for more than 6 h (cf. Section 2) and no problems were observed over the whole test period of five months.

3.3. SPE–LC–DAD

The fully automated stand-alone SPE–LC–DAD system referred to above [5,6], has already amply proven its potential for the determination of trace levels of organic microcontaminants in various types of water. However, the present number of about 150

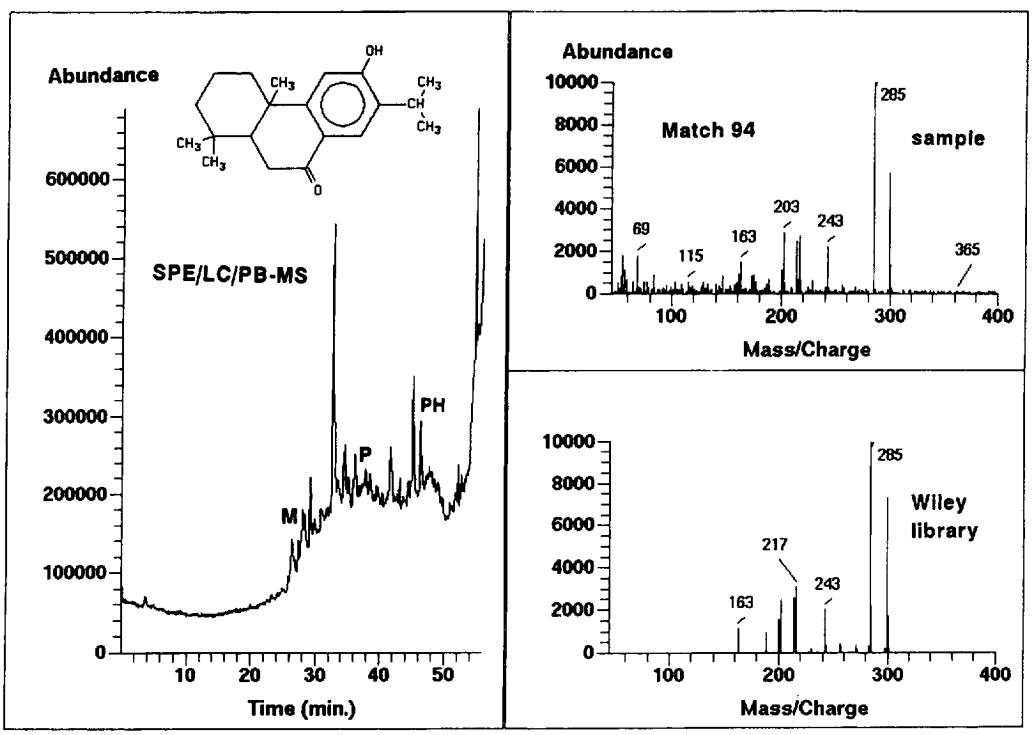

Fig. 5. Chromatogram of diluted extract of Nitra river sediment obtained by SPE–LC–PB-MS. Sediment (1 g) was extracted with 10 ml of methanol, 8 ml of filtered extract were diluted in 250 ml HPLC-grade water and 100 ml of this solution were enriched on PLRP-S precolumn; 1 μg/l of propazine (P) and metoxuron (M) were added to the solution prior to analysis. The unknown pollutant, 9(1H)-phenanthrenone, 2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-, [PH], was identified by the library search. For more details, see text.

compounds stored in the UV-DAD spectral library purposely restricts its use to target analysis. The UV spectra, with their not really high information content, are mainly used for a first screening to find suspect samples or peaks. In other words, on-line combination with a spectroscopic detection device seems to provide an adequate solution, as has recently been demonstrated by Marcé et al. [19].

For the set of nine test compounds, SPE–LC–DAD provided detection limits comparable to those of SPE–GC–MS (with often, however, ca. 100-ml rather than 10-ml samples, Table 3). The R.S.D. values of the retention times were below 0.1% in all cases; those of peak areas varied between 2 and 5% (n=6 at 1 μg/l concentration level, Table 4). The excellent repeatability of the DAD data and the robustness of a UV-absorption-based system make it very useful for quantification. A typical library match of the DAD-UV spectrum of tetrachlorvinphos is shown in the insert of Fig. 3C. Such information, together with retention time characteristics, gives reasonable early-warning information on pollutant identity. As is demonstrated in Fig. 4A and 4B, the DAD chromatograms are a great help when trying to locate the position of a pollutant in the PB-MS chromatogram which significantly speeds up the ion extraction procedure. The very small arrow (indicated by the large one) under the peak of the internal standard metoxuron means that a software option of retention-time alignment was used and that all peaks in the LC–PB-MS chromatogram should now match those in the LC–DAD chromatogram. In this particular instance, a large peak at 30 min in the LC–DAD chromatogram was provisionally identified as benzothiazole. Since there was no matching peak in LC–PB-MS, the GC–MS chromatogram was searched for the presence of a compound with a base peak of m/z 135 (major peak in spectrum of benzothiazole). The analyte was indeed detected and identified on the basis of its EI spectrum at a retention time of 5.7 min. The presence of the antioxidant benzothiazole in the Nitra river can be related to the rubber production industries located in the vicinity of the sampling site.

As was to be expected on the basis of earlier validation studies [6], no technical problems related to operating the SPE–LC–DAD system were encountered during the test period of five months.

3.4. MULTIANALYSIS system

A primary goal of this study was to integrate the three techniques described above, in one system. After evaluating each of the techniques in the present study, the major remaining problem was in testing the simultaneous use of the single mass detector for both LC–MS and GC–MS, and the proper combination of the software packages for on-line control of each part of the MULTIANALYSIS system from the keyboard of the central computer.

In previous experiments, when switching from GC–MS to LC–MS, the analytical column and GC–MS interface were withdrawn from the MS ion source. Now, however, the interface remained in its original position and a constant flow of helium (6 psi, ca. 41 kPa) was maintained also during PB-MS operation. Variation of the helium pressure from 2 to 20 psi (ca. 14 to 140 kPa) did not affect the PB-MS performance. The LC–MS and GC–MS data of Table 3 and Table 4 represent a true comparison of the techniques because the same tuning values of the ion source were used for data acquisition and the two analyses of a single sample were performed within 3 h using identical instrumentation.

The software packages – G1307A (Phoenix) for control of the LC–DAD and G1034C (Mustang) for control of the PB-MS – were operated simultaneously, thus providing a full view and control of each part of the LC–DAD–PB-MS system. Once optimised, the analytical parameters were stored in a single method used by both softwares. Similarly, the SPP programme in SPE/WIN software was used for on-line operation of the PROSPEKT with its SDU, and other external devices. After activation of the LC–DAD–MS method, a single keystroke started the PROSPEKT run and all subsequent events as described in Table 1.

After a simple change in the configuration file, the G1034C software was also used for control of all GC parameters. In combination with another SPP programme of the SPE/WIN software a procedure identical to the above was used for starting the events described in Table 2. Both LC–MS and GC–MS could be operated unattendedly by means of sequence programming.

An important aspect of the set-up (cf. Fig. 2) is that all modules of the system are electronically

connected. This allows their automated control by the three independent software packages. Since the softwares can communicate with each other through common methods, there is essentially no possibility of the failure of an individual part of the system not being noticed, and methods can be called 'fully automated'. It should be added that this rather complicated system was designed for routine operation and that the 'net' of connections and software is hidden in a 'black box'. An operator is basically responsible only for placing the samples in the SDU and starting up a single method/sequence from the keyboard of the computer.

Finally, the data obtained for the nine test compounds by all three techniques clearly show that SPE–GC–MS is the most powerful part of the system, that is, provided the compounds are GC-amenable (Table 3, Fig. 3). However, when analysing real-world samples, the value of LC–DAD and LC–PB-MS increases and numerous compounds which are not detectable by GC–MS, can be determined. Two examples are the detection of the phenylurea metoxuron (Fig. 4), used as an internal standard, and the identification of the degradation product of phenanthrene (Fig. 5).

Negative chemical ionisation

With the present ion source both LC–PB-MS and GC–MS can also be operated in the chemical ionisation mode with positive (PCI) or negative (NCI) ion detection [20]. In order to further demonstrate the potential of the MULTIANALYSIS system, the efficiency of the NCI mode of operation, with methane as a reagent gas, was briefly studied. An interesting example is shown in Fig. 6. Whereas atrazine, metolachlor and alachlor were not detected in LC–PB–NCI-MS even at a 10 μg/l concentration, five of the remaining six halogenated compounds gave 10- to 30-fold higher responses than under EI conditions. An about 3-fold improvement was observed for cyanazine; its EI and NCI PB-MS spectra are shown as inserts of Fig. 6. They were rather similar to the spectra observed in GC–MS (data not shown). Generally, the NCI spectra obtained throughout this study by three different operators were consistent and reproducible despite several indications of problems with their appearance published previously [21]. The practicality of the combined EI–NCI operation is demonstrated in Fig. 7. The peak patterns of the SPE–GC–EI-MS and SPE–GC–NCI-MS traces recorded for the Nitra river sample are seen to be rather different, one of the peaks which responds better in the NCI mode being the peak eluted at 12.4 min (No. 1). Interpretation of the EI- and NCI-MS spectra shown as inserts revealed the presence of (2-methylthio)-benzothiazole, presumably a transformation product of benzothiazole found previously in the same sample (cf. above). Identification of the two (or three) compounds co-eluted as peak No. 2 is presently being attempted and, together with detailed discussion on the NCI operation of the MULTIANALYSIS system, will be addressed elsewhere [22].

4. Conclusions

An integrated system has been developed which combines SPE–LC–DAD, SPE–LC–PB-MS and SPE–GC–MS with a single mass detector. All three techniques use the same trace enrichment of 10–100 ml aqueous sample on a small copolymer-packed precolumn. Analytes trapped on the sorbent are on-line eluted into the GC–MS with 100 μl of ethyl acetate for GC–EI-MS or GC–NCI-MS analysis. Next, after another enrichment of the same sample, the analytes are desorbed with the LC gradient into the LC–DAD–PB-MS part of the system. All parts of this so-called MULTIANALYSIS system are on-line controlled from the keyboard of the central computer and analyses can be performed unattendedly in a fully automated way. It is possible to obtain LC–DAD, LC–PB-MS and GC–MS chromatograms of the same sample within 3 h. During a 5-month test period, no significant maintenance problems were encountered.

Detection limits of nine test compounds (a mixture of triazines, anilides and organophosphorus pesticides) in tap water were at or below 0.1 μg/l for SPE–GC–MS (10-ml sample) and 0.5–7 μg/l for SPE–LC–PB-MS (100-ml sample) in the full-scan mode and about 0.05–1 μg/l in the SIM mode for target analysis. With all three techniques R.S.D. values of retention times generally were below 0.1%. For peak areas the values were 2–5% for SPE–LC–DAD, and 5–15% for SPE–GC–MS and SPE–LC–

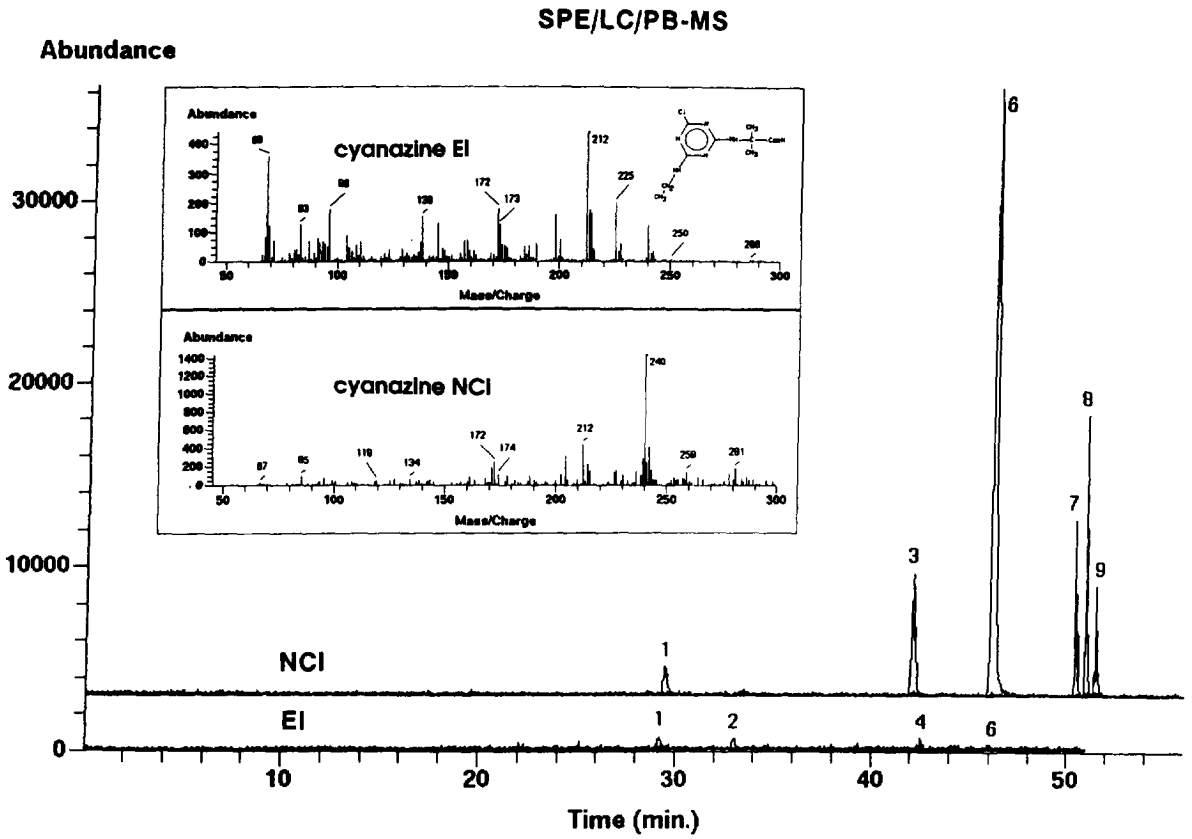

Fig. 6. Chromatograms of nine chlorinated pesticides in tap water obtained by SPE–LC–PB-MS in the EI and NCI mode. Samples were spiked with 1 μg/l of each compound (for numbers, cf. Table 3); enriched volume, 100 ml; reagent gas, methane. MS chromatograms represent extracted ions obtained at m/z of base peak of each analyte. The corresponding spectra of cyanazine in the (A) EI and (B) NCI mode are shown as inserts. For further details, see text.

PB-MS. For each compound methane-NCI analysis provided identical spectra in LC–MS and GC–MS. The use of CI-based detection caused a 3- to 30-fold increase in detectability for six of the nine halogenated pesticides. For the rest, it is obvious that the detection limits of LC–PB-MS do not match those obtained from LC–DAD and GC–MS. There is an urgent need to improve the transfer efficiency of the PB interface, which is a topic currently under investigation in our group.

The performance of the MULTIANALYSIS system was tested by analysing surface water and sediment extract samples. In both cases numerous unknown pollutants were detected and several of these could be identified on the basis of their EI mass spectra. NCI spectra were used for molecular mass determination of unknown pollutants in surface water and, together with EI, for provisional identification of compounds not contained in spectral libraries. LC–DAD provides useful early-warning information on overall pollution of a sample, and reliable quantitative data, and indicates peak positions in co-acquired LC–MS chromatograms. The complementary nature of the present techniques will be further discussed in subsequent papers on the information that will be provided by the results of a monitoring programme of a small river [15] and the added NCI-MS of real-world studies [22].

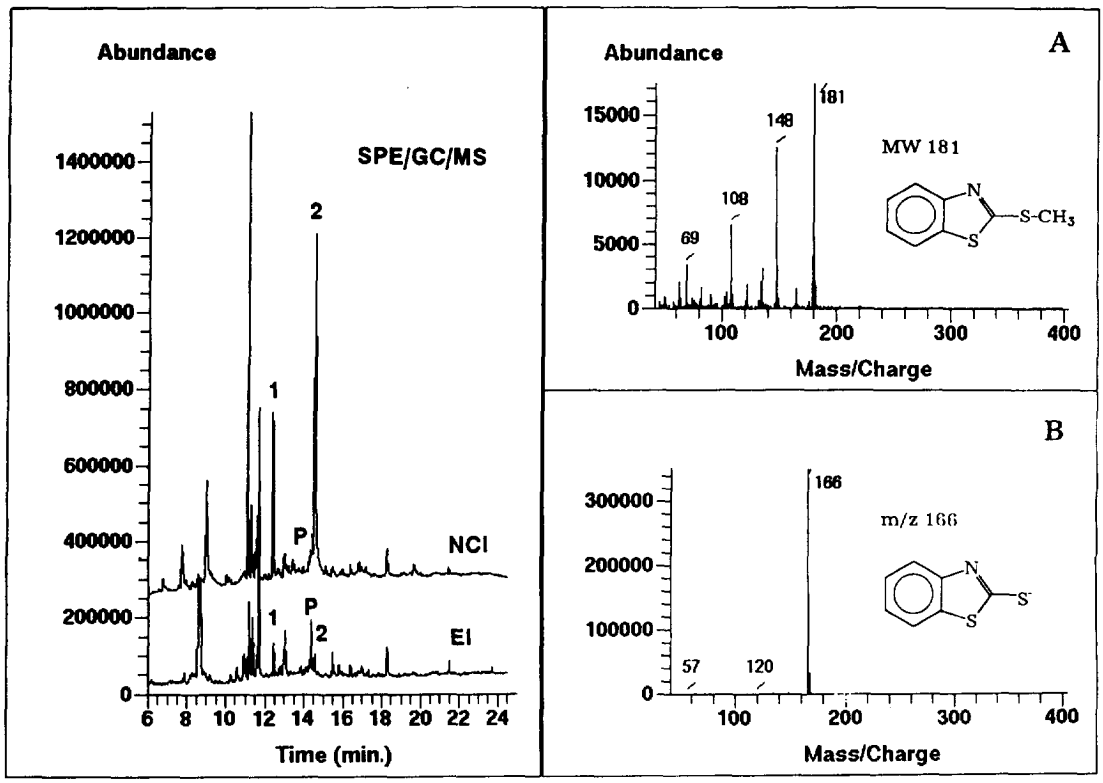

Fig. 7. Chromatograms of a Nitra river (Slovakia) water sample obtained by SPE–GC–MS in the full-scan EI and methane-NCI mode; sample volume, 10 ml. The sample was spiked with 1 μg/l of the internal standards propazine (P) and metoxuron (M). (A) EI and (B) NCI spectra of the compound eluted at 12.4 min are shown as inserts. For more details, see text.

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