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Fully automated multi-residue method for trace level monitoring of polar pesticides by liquid chromatography

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ABSTRACT

A fully automated liquid chromatographic method using on-line trace enrichment, gradient elution and diode-array detection for the trace level determination of polar pesticides in surface water is described. The automated system uses specially developed software in the form of "user macros", allowing the on-line control of both the automated cartridge exchange unit for sample preparation and the liquid chromatograph with diode-array detector by means of the Pascal Workstation computer of that liquid chromatographic system. The collected data are automatedly evaluated, *i.e.*, pollutants present in the sample at a concentration level above an input treshold level are identified/determined and a report is printed. Parameters such as the sampling interval of the spectra, temperature of the analytical column compartment, wavelength/bandwidth ratios and data handling were optimized. The validation results for 27 pesticides are presented. At an analyte concentration of 1 μg/l the relative standard deviations of the retention times and peak areas in different types of water are in the range 0.2–1.5% and 1–15%, respectively. All calibration graphs are linear in the range 0.1–7 μg/l.

INTRODUCTION

In spite of their adverse effects on the environment, the total amount of pesticides used for agricultural and industrial purposes is still increasing. As a result, pesticides are nowadays present in all compartments of the environment, which explains the great interest in early-warning and monitoring systems for these compounds. The monitoring of these analytes causes substantial analytical problems since, because of recent legislation in many countries, sensitive methods are required [1,2]. For example, in surface water samples, organic micropollutants typically have to be determined at the low- to sub-μg/l level [3,4].

Analysis at these levels requires a concentration step. Solid-phase isolation has proved to be a good alternative to liquid–liquid extractions because of its simplicity, robustness and potential for automation [5–9]. In addition, most modern pesticides are fairly soluble in water and so are less amenable to extraction with organic solvents. As a result, liquid chromatography (LC) in combination with on-line trace-enrichment techniques, using small cartridges [5–7] or membrane extraction discs [7], are frequently preferred over gas chromatographic techniques. Detection is performed by diode-array (DA) detection, providing simultaneously structural information and quantitative data.

The development of a preliminary automated LC system for polar pesticides in various types of water was presented in a previous paper [7]. The aim of this extended study was to investigate a number of parameters to allow the identification and determination of a large group of polar pesticides at sub-μg/l levels in surface water. From the automation point of view the primary purpose was to develop a robust, user-friendly system that is easy to operate. This resulted in a fully automated LC method called SAMOS (System for Automated Measurement of Organic Micropollutants in Surface Water).

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EXPERIMENTAL

Solvents and chemicals

Stainless-steel analytical columns (150 mm × 4.6 mm I.D.) were laboratory packed with 5-μm C_{18} RoSil (RSL, Eke, Belgium) particles using acetonitrile as the slurry liquid. Further, a 150 mm × 4.6 mm I.D. Supelcosil LC-18-DB (Supelco, Bellefonte, PA, USA) column packed with C_{18} DB material (3-μm particles) and a 250 mm × 4.6 mm I.D. Supelcosil LC-18-DB column packed with C_{18} DB material (5-μm particles) were used. HPLC gradient-grade acetonitrile, methanol and water were obtained from J. T. Baker (Deventer, Netherlands). Ultra-pure water was prepared by ultrafiltration with a Milli-Q system (Millipore, Bedford, MA, USA). Disodium hydrogenphosphate, sodium dihydrogenphosphate and orthophosphoric acid (85%) were obtained from J. T. Baker. The various pesticides were supplied by Riedel-de Haën (Seelze, Germany), Promochem (Wesel, Germany), Dr. S. Ehrenstorfer (Augsburg, Germany) and Hoechst (Frankfurt, Germany). As stated by the manufacturers, they were all at least 95% pure. The pesticides used in the study are listed in Table I.

Surface water samples were collected at Lobith and at the 335-km point (River Rhine, Netherlands and Germany, respectively), Eysden and Keizersveer (River Meuse, Netherlands), Toulouse (River Garonne, France), Westminster Bridge, London (River Thames, UK) and the Rivers Uster and Mönchaltdorf (Switzerland).

Instrumentation

The LC analyses were performed with an HP 1090 liquid chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a PV5 ternary solvent-delivery system (SDS), an injection

TABLE I

PESTICIDES [a] USED AND THEIR WAVELENGTHS OF MAXIMUM ABSORBANCE

No.	Name	λ_{max} (nm)
1	Aniline	203
2	Carbendazim	201
3	1-(3-Chloro-4-hydroxyphenyl)-3,3-dimethylurea (CHPDMU)	207
4	Metamitron	201
5	Chloridazon	229
6	Dimethoate	201
7	Monomethyl metoxuron	207
8	Aldicarb	201
9	Bromacil	211
10	Cyanazine	221
11	2-Nitrophenol	213
12	Chlorotoluron	211
13	Atrazine	223
14	Diuron	213
15	Metobromuron	203
16	Metazachlor	201
17	Propazine	223
18	Warfarin	205
19	3,3'-Dichlorobenzidine (DCB)	213
20	Barban	207
21	Alachlor	201
22	Nitralin	229
23	Dinoseb	270
24	Dinoterb	271
25	Phoxim	281
26	Nitrofen	201
27	Trifluralin	207

[a] For structures with *Chemical Abstracts* Registry numbers and molar absorptivities, see refs. 5 and 8, respectively.

valve with a 25-μl loop and an HP 1040 DA detector equipped with a 10-mm flow cell. For single-wavelength monitoring the DA detector was set at 210 nm with a bandwidth of 10 nm. During recording of the absorbance spectra the optical slit of diode width was 4 nm and a sampling interval for recording of the spectra in the All Spectra mode of 1280 ms was used with a peak width of 0.2 min. Absorbance spectra were recorded from 200 to 400 nm. Data from the DA detector were collected and evaluated by the Pascal Workstation (PAWS) computer using Chemstation software.

Trace enrichment was carried out on 10 mm × 2.0 mm I.D. disposable trace-enrichment cartridges of the Prospekt (Spark Holland, Emmen, Netherlands) automated sample preparation unit. The commercially available cartridges (Spark Holland) were packed with PLRP-S, a styrene–divinylbenzene copolymer (Polymer Labs., Church Stretton, UK) with 20-μm particles and a 100 Å pore size, and used only once. Conditioning of the cartridges was performed with a solvent-delivery unit (SDU) from Spark Holland. Surface water was sampled with a Model 300 preparative pump (Gynkotek, Munich, Germany).

Although the SDU itself can be controlled by the Prospekt microprocessor, the Prospekt and the HP 1090 were connected through an auxiliary electronic connection (Fig. 1). The Prospekt and the Model 300 Gynkotek pump were also connected by using another auxiliary electronical connection of the Prospekt and the flow control input of the pump.

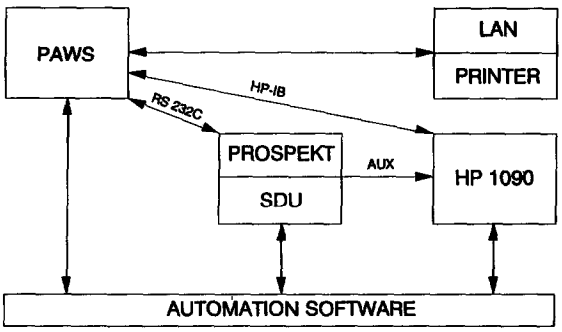

Fig. 1. Schematic diagram of the fully automated SAMOS system. The RS 232C and HP-IB are the communication interfaces and AUX is an auxiliary to connect the Prospekt and the SDU with the HP 1090; PAWS = Pascal Workstation; LAN = local area network. For further explanation, see text.

The PAWS computer controlled the HP 1090 by using both the standard HP-IB communication interface and the Chemstation software. On-line control of the Prospekt and SDU from the PAWS computer was performed via an RS 232C communication interface using additional software in the form of a laboratory-made "user macro" called SAMOSMAC.

Procedures

Fresh stock standard solutions (0.2 mg/ml) of the pesticides in methanol were prepared every 6 weeks. The working standard solutions of the test compounds were prepared by diluting the stock standard solutions daily with Milli-Q-purified, drinking or surface water to a concentration of 0.01–7 μg/l. All stock standard solutions were stored at 4°C in the dark. The stability of the analytes in the stock and working standard solutions, tested in a previous study [5], was sufficient to allow accurate measurements.

The gradient conditions were as follows: solvent B, acetonitrile–0.01 *M* phosphate buffer (pH 3) (90:10, v/v) and solvent A, acetonitrile–0.01 *M* phosphate buffer (pH 3) (5:95, v/v). The gradient profile was 100% A at 0 min, linearly to 100% B in 55 min and subsequently linearly to 100% A in 5 min. The flow-rate was 1.0 ml/min. Prior to use, all eluents were degassed with helium (15 min).

The trace-enrichment cartridges were conditioned with 2 ml of methanol (2 ml/min) followed by 2 ml of 0.001 *M* perchloric acid (1 ml/min), as an additional cleaning step, using the SDU. Thereafter, 100 ml of sample were enriched at a flow-rate of 4 ml/min [7]. Desorption was performed by coupling the cartridge on-line with the analytical column and starting the gradient (Fig. 2). For each analysis a new cartridge was used. In Table II the sample preparation programmes (SPP) are given. Before starting the first analysis, the PAWS computer was switched on, which resulted in a special soft-key set (on the screen) for handling of the macro SAMOSMAC. At a pre-programmed time, the deuterium lamp of the DA detector was switched on and the flow-rate of mobile phase B gradually increased from 0.05 to 1 ml/min in 3 min. Then the programmed sequence of analyses consisting of conditioning of the cartridge, enrichment of 100 ml of sample, a 60-min LC run, qualitative and quanti-

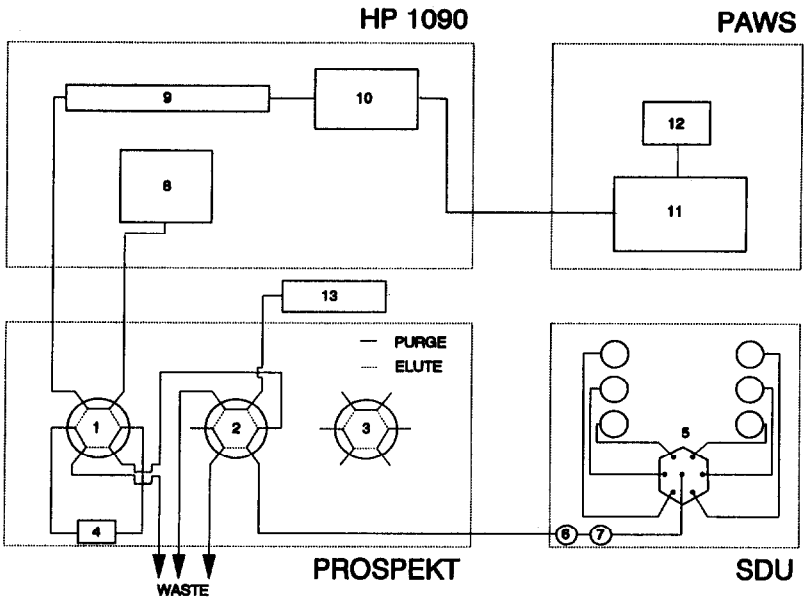

Fig. 2. Automated on-line trace enrichment–LC system for water samples. 1, 2, 3 = High-pressure valves of the Prospekt; 4 = trace-enrichment cartridge of the Prospekt; 5 = solenoid valve; 6 = pulse damper; 7 = purge pump; 8 = solvent delivery system of HP 1090 liquid chromatograph; 9 = analytical column; 10 = diode-array detector (DA); 11 = Pascal Workstation (PAWS) computer; 12 = printer; 13 = preparative pump for sample loading; SDU, solvent delivery unit.

tative evaluation of data obtained and printing of the report started. The system was equilibrated for 31 min during the first enrichment procedure and, in the following enrichment procedures, for 5 min between two successive analyses. No additional time was needed for computing of the data. At the end of a particular sequence of analyses the deuterium lamp was automatically switched off and the flow-rate was decreased gradually from 1.0 to 0.05 ml/min in 3 min.

RESULTS AND DISCUSSION

Compared with previous studies [7], the system configuration was changed by replacing the 1000 S DA detector and the Model 400 gradient systems (Applied Biosystems, Ramsey, NJ, USA) by the HP 1090 system because of the latter's advanced automation potential. If it is preferred to use another configuration, several of the parameters discussed below will have to be optimized again. The general strategy, however, will remain the same.

Sampling interval of spectra and wavelength/bandwidth ratio

In order to obtain sufficient data points for post-run calculations of the chromatograms (*e.g.*, subtraction, extraction of signals at different wavelength/bandwidth ratios), the spectra were taken continuously depending on the settings of the sampling interval. Therefore, the All Spectra option was used in all further experiments. The optical slit of diode width was set at 4 nm. With the average peak width of the test compounds of 0.1–0.4 min, it was possible to take 5–15 spectra of each eluting compound.

A significant increase in the noise level is expected to result from using relatively small sampling intervals. The results in Fig. 3 indicate that the optimum sampling interval should be at least 300–400 ms. In all further experiments a value of 1280 ms was chosen because a smaller sampling interval will require too much computer memory while a larger sampling interval will result in a loss of spectral resolution. The optical slit of diode width was set at 4 nm.

TABLE II

SAMPLE PREPARATION PROGRAMMES

Time (min:s)	Solvent	Valve 1	Valve 2	Flow-rate (ml/min)	Aux2	Aux6	Other
SPP of automated system using SDU for sampling[a]							
00:00		Purge	Elute				
00:01	01			2.0			CHC
02:00			Purge				
03:00	02		Elute				
05:00			Purge	1.0			
07:00	03		Elute	4.0			
08:00			Purge				
33:00				0.0			
33:30	04	Elute	Purge			On	
33:31	01			2.0			
34:31				0.0		Off	
65:00							End
SPP of automated system using additional preparative pump for sampling[a]							
00:00		Purge	Elute				
00:01	01			2.0			CHC
02:00	03		Purge	4.0	On		
03:00	02		Elute	2.0	Off		
05:00			Purge	1.0			
07:00	03		Elute	4.0	On		
32:00				0.0	Off		
32:30	04	Elute	Purge			On	
32:31	01			2.0			
33:31				0.0		Off	
65:00							End

[a] 01, methanol; 02, 0.001 *M* perchloric acid; 03, sample; 04, eluent; valves 1 and 2, positions according to Fig. 1; Aux 6, connection of Prospekt with HP 1090, positions on/off; Aux 2, connection of Prospekt with LC pump, positions on/of; CHC, change cartridge; End, end of programme.

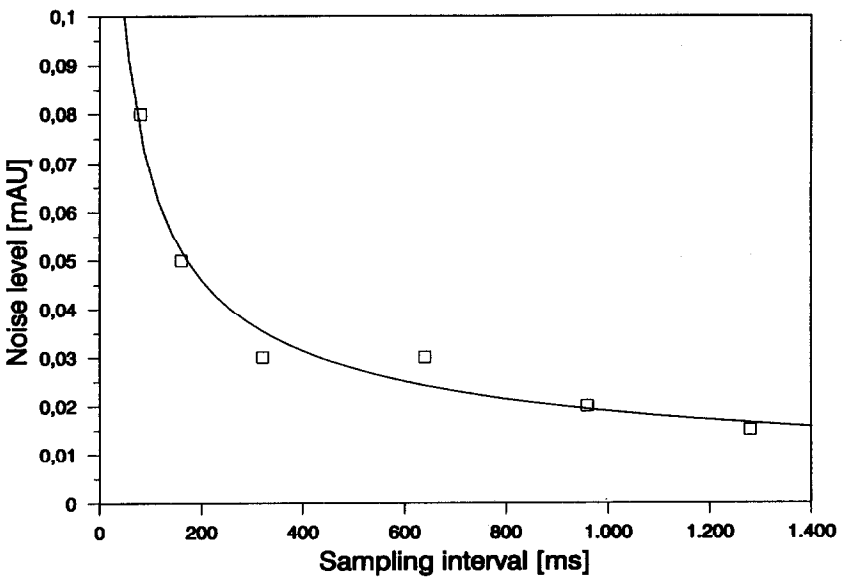

Fig. 3. Noise level as a function of the sampling interval. Optical slit of the diode width, 4 nm; detection wavelength, 210 nm; bandwidth, 10 nm.

In order to obtain a robust method, single-wavelength detection should be performed at an optimised bandwidth in order, to monitor simultaneously a large group of pesticides with high sensitivity. Particularly at the μg/l level, this is a critical choice. Most of the analytes have their maximum absorbance in the range of 200–210 nm (Table I). After testing a number of wavelengths in this range, using bandwidths between 5 and 20 nm, a compromise between the highest and the most selective responses was found with a wavelength of 210 nm and a bandwidth of 10 nm. These values were used in all further experiments.

Analytical column

In order to obtain the optimum sensitivity and selectivity, the choice of the analytical column that has to be used, in combination with a PLRP-S enrichment cartridge, is very important [7]. Two C_{18} columns, one packed with 3-μm particles (150 mm × 4.6 mm I.D.) and one packed with 5-μm particles (250 mm × 4.6 mm I.D.) were tested. In spite of the excellent performance of both columns, the longer C_{18} column was used for all further studies because of its lower back-pressure.

The test mixture of 27 pesticides was analysed at ambient temperature and 40, 45 and 50°C. The best results, with respect to peak shapes and back-pressure, were obtained at 45°C.

Validation of the system

The system was validated for a group of 27 pesticides (Table I). The repeatabilities of the retention times and peak areas were measured using River Rhine water (Table III). The relative standard deviations (R.S.D.s) of the retention times were in the range 0.2–1.5% and those of the peak areas were 1–15%. High R.S.D.s were observed only for analytes eluting between 12 and 25 min. This is partly due to matrix interferences and partly to breakthrough of the more basic compounds on the PLRP-S cartridge [7].

The correlation coefficients of nearly all of the calibration graphs for River Rhine water were over 0.99. The only exceptions were warfarin and phoxim, with correlation coefficients of 0.97. This is caused by small interferent that nearly co-elute with these analytes.

In Table III the identification limits of the individual pesticides in HPLC-grade, drinking and River Rhine waters are also given. The identification limit is the lowest concentration allowing identification of the compound according to its spectrum (see *Data handling*). Of the 27 pesticides, all but two possess identification limits $\leqslant 1.0$ μg/l in surface water and $\leqslant 0.1$ μg/l in HPLC-grade water.

Robustness of the system

The performance of the system was tested in two laboratories, one during a 5- and the other a 7-month period. Within these periods the systems were continuously switched on for 6–8 analyses per day and about once every week a sequence of 14–16 analyses was performed unattended overnight. This means that in total over 1000 samples were analysed, and during this time only two types of problems were encountered: (i) with each system the deuterium lamp had to be exchanged once, and (ii) when using non-filtered surface water for trace enrichment the SDU became clogged twice. Therefore, for the handling of unfiltered samples the SDU was replaced with a preparative pump (Fig. 2). The analytical columns did not deteriorate during the whole test period. The results demonstrated that the present (SAMOS) system is suitable for long-term unattended operation using non-buffered (*i.e.* pH 6–9) and non-filtered surface water samples.

Flexibility of the system

The flexibility of the system was tested for the 27 test compounds extended with an additional number of triazines, phenylurea herbicides and nitrophenols. The analytes were measured without changing any of the preset analytical parameters (see previous sections). In each trial, first a 25-μl loop injection was performed, followed by the enrichment of 100 ml of spiked Milli-Q-purified water. The results of, for example, the group of thirteen phenylurea herbicides, six of which were present in the original set of 27 test compounds and seven additional pesticides, show (Fig. 4) that all of the added analytes can be detected and identified at a level of 0.1–1 μg/l without any modifications to the system. The losses observed for the early-eluting compounds (3 and 44) are due to partial breakthrough [5].

Similar results were obtained for the triazines and nitrophenols. So far, in our department [5–7], about

TABLE III

REPEATABILITY OF RETENTION TIMES AND PEAK AREAS FOR 27 PESTICIDES IN RIVER RHINE WATER, IDENTIFICATION LIMITS IN HPLC-GRADE WATER, DRINKING WATER AND RIVER RHINE WATER AND LINEAR REGRESSION COEFFICIENTS [a]

No.	Name	RT_{RW} (R.S.D.)	PA_{RW} (R.S.D.)	IL_{HP}	IL_{DW}	IL_{RW}	R^2
1	Aniline	–	–	1.0	1.0	2.5	0.999
2	Carbendazim	12.4 (1.5)	270 (15)	0.03	0.1	0.25	0.995
3	CHPDMU	14.3 (1.2)	64 (8)	0.05	0.1	1.0	0.999
4	Metamitron	15.4 (0.9)	228 (5)	0.05	0.1	0.25	0.997
5	Chloridazon	16.2 (1.3)	243 (4)	0.03	0.1	0.25	0.993
6	Dimethoate	17.0 (1.3)	87 (8)	0.1	1.0	1.0	0.996
7	Monomethyl metoxuron	19.0 (1.1)	574 (5)	0.03	0.1	0.25	0.998
8	Aldicarb	21.2 (0.9)	146 (9)	0.05	0.25	1.0	0.997
9	Bromacil	22.4 (0.7)	175 (4)	0.1	0.25	0.5	0.992
10	Cyanazine	23.7 (0.6)	401 (6)	0.03	0.1	0.25	0.999
11	2 – Nitrophenol	24.8 (0.7)	70 (12)	0.25	1.0	2.0	0.990
12	Chlorotoluron	27.0 (0.5)	760 (1)	0.03	0.1	0.25	0.999
13	Atrazine	28.0 (0.4)	489 (1)	0.03	0.05	0.05	0.995
14	Diuron	28.8 (0.5)	502 (4)	0.03	0.05	0.05	0.992
15	Metobromuron	29.8 (0.5)	314 (3)	0.05	0.1	0.25	0.998
16	Metazachlor	30.4 (0.8)	405 (3)	0.03	0.05	0.25	0.999
17	Propazine	32.4 (0.3)	356 (6)	0.01	0.25	0.25	0.991
18	Warfarin	33.1 (0.8)	567 (5)	0.03	0.25	0.25	0.976
19	3,3'-DCB	34.1 (0.6)	664 (6)	0.01	0.1	0.25	0.995
20	Barban	37.4 (0.3)	583 (1)	0.05	0.1	0.25	0.999
21	Alachlor	38.3 (0.4)	336 (4)	0.1	0.1	0.25	0.996
22	Nitralin	40.7 (0.4)	186 (2)	0.1	0.1	0.25	0.999
23	Dinoseb	41.3 (0.3)	114 (7)	0.1	0.5	1.0	0.999
24	Dinoterb	42.0 (0.3)	117 (8)	0.1	0.5	1.0	0.996
25	Phoxim	44.1 (0.3)	108 (8)	0.1	0.25	0.5	0.962
26	Nitrofen	46.5 (0.3)	484 (2)	0.03	0.1	0.25	0.999
27	Trifluralin	49.9 (0.2)	129 (8)	0.1	0.1	0.25	0.995

[a] RT_{RW} = retention time (min) in River Rhine water (n = 18–21); PA_{RW} = peak area (arbitrary units) in River Rhine water (n = 8); R.S.D. = relative standard deviation (%); IL_{HP} = identification limit in HPLC-grade water (μg/l); IL_{DW} = identification limit in drinking water (μg/l); IL_{RW} = identification limit in River Rhine water (μg/l); R^2 = linear regression coefficient in River Rhine water.

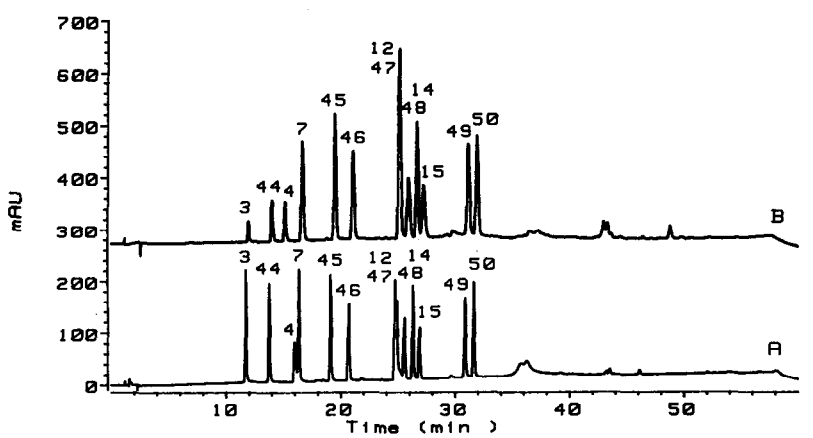

Fig. 4. LC–DAD traces of a mixture of thirteen phenylurea herbicides. (A) Loop injection of 25 μl (10 μg/ml); (B) preconcentration of 100 ml of spiked Milli-Q-purified water (5 μg/l). For peak numbers up to 27, see in Table I; 44 = desmethyl metoxuron; 45 = metoxuron; 46 = monuron; 47 = fluometuron; 48 = monolinuron; 49 = linuron; 50 = chlorobromuron. For further conditions, see text.

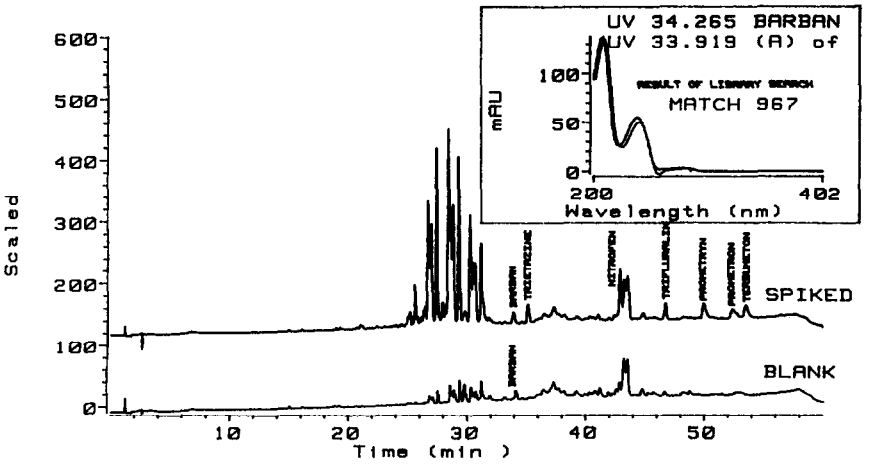

Fig. 5. LC–DAD traces of 200 ml of diluted methanol extracts of suspended particulate matter in River Rhine water (Lobith, Netherlands). A 200-ml volume of sample was spiked with a mixture of six triazines (terbumeton, prometron, prometryn, trietazine, atrazine and simazine) and two nitrophenols (nitrophen and trifluralin) at a concentration of 10 μg/l and filtered through a 0.45-μm filter after 20 h. The filter was extracted with 10 ml of methanol. Thereafter, 5 ml of the extract were dissolved in 200 ml of Milli-Q-purified water, enriched on a PLRP-S cartridge and analysed. In a blank sample treated the same way, barban was found at a concentration of about 0.3 μg/l. For further conditions, see text.

100 polar pollutants have been determined with the system; for 75% of these compounds identification limits of less than 1 μg/l can be obtained. In this context it should be noted that all of the chromatograms shown in this paper represent a compromise situation (see above). It is possible, of course, to optimise the detection conditions for a single or a limited number of analytes if their determination is of particular concern.

The system was also used for the analysis of particulate matter present in River Rhine samples. After off-line filtration (using a 0.45-μm cellulose acetate membrane filter) of 200 ml of sample, the filter was extracted with 10 ml of methanol and 5 ml of this extract were diluted with 200 ml of Milli-Q-purified water which was enriched as described previously on a PLRP-S cartridge. A sample from the same batch of River Rhine water was spiked with a mixture of six triazines and two nitrophenols at a concentration level of 10 μg/l. After 20 h at ambient temperature, 200 ml of the sample were filtered and analysed as described above. Trietazine, prometryn, prometron, terbumeton, nitrofen and trifluralin were found in the extract and are probably adsorbed on the particulate matter (Fig. 5). Atrazine and simazine, which are almost invariably present at low levels in River Rhine water [11,12], were not found in the extract, which suggests that these solutes are not adsorbed. In both blank and spiked River Rhine water samples barban was found, after extraction, at a concentration level of 0.3 μg/l. Obviously barban is also adsorbed on the particulate matter. As these are only preliminary results, this topic will be studied further in the future.

System control of fully automated system

In Fig. 1, an overview of the SAMOS components and corresponding interfaces is given. The HP-IB communication interface between the PAWS and the HP 1090 allows the on-line programming and control of the SDS and DAD. An RS 232C communication interface allows the on-line programming and control of the Prospekt and the SDU using a software package in the form of the "user macro" SAMOSMAC. The possibility of combining the system with a Local Area Network (LAN) will allow the transfer of data to other laboratories and authorities, *e.g.*, in the case of alarm situations.

After the PAWS computer has been switched on, the macro is automatedly loaded into the internal memory. A special soft-key set allows the handling

of the communication between the PAWS and the Prospekt. It is now possible directly to program, edit and save the Prospekt run (RP), SPP and START program on the screen of the computer. All of these can be sent directly to the Prospekt by pressing one of the soft-keys. When the appropriate RP and SPP programs have been loaded, sending of a START program will start the required sequence of analyses. The macro allows the on-line control of all activities of the Prospekt, such as information about the SPP and RP programs in current use with the number of cycles, positions of valves, auxiliaries, solvents used, flow-rates, actual run time and pressure of the Prospekt. In addition, error code messages of the Prospekt are checked at regular intervals and the information is stored in a logbook file. The programming, editing and storage of SPP and RP programs is user friendly and easy to handle. The main advantage is that a system consisting of two non-compatible devices, the Prospekt and the HP 1090, is now acting as a compact unit that can be operated and controlled from one computer.

Data handling

After optimisation of all the relevant data acquisition parameters, the optimum settings for the DAD were found to be a detection wavelength of 210 nm with a 10-nm bandwidth using a reference spectrum at 550 nm with a 100-nm bandwidth. The reference spectrum was taken at 550 nm because at this particular wavelength the absorbance of the analytes is negligible. All spectra were stored with a threshold of 2 milliabsorbance, a peak width of 0.2 min, a sampling interval of 1280 ms and a spectrum range of 200–400 nm with steps of 4 nm. All others conditions are given under Experimental.

Different integration event files were created and tested. With real samples, the best results were obtained using the option "integrate all valleys", where the integration baseline is constructed according to the baseline shape of the chromatograms. To exclude small peaks from the spectral evaluation procedure, the minimum peak area is set at 100, which corresponds to a 0.01–0.05 μg/l concentration level of peaks with a molar absorbance of about 10 000 [8]. A peak width of 0.2 min allows the integration of all peaks except for the 2-nitrophenol peak, which was too broad, at concentration levels below 1.0 μg/l.

Determination of the analytes is based on a calibration function (linear regression using an external standard) of the automated method. This function is used in the range of 0.01-7 μg/l. Three measurements are performed for each analyte at seven concentrations, to test simultaneously the repeatability of the method. The automated evaluation of the analysis of each sample is controlled by a so-called "macro program" run by the PAWS computer operating in the background mode during the analysis of the series of samples. This process is subdivided into three parts. First, the chromatogram is integrated and plotted. Next, the retention times of the peaks are compared with a library containing retention times of standard compounds. If there is a match of retention times within 2.5% the complete absorbance spectrum of the signal is compared with the spectra of the standards in the library. The comparison is based on the spectra recorded at the peak maximum after apex background subtraction.

Report parameters

At the end of the run the chromatogram is automatically evaluated; peaks are identified and quantified and a report is printed. The final report contains the most important results of the analysis and shows for each of the identified compounds: retention time, concentration in μg/l, spectral match factor and peak purity factor. Normally, a spectral match factor between 950 and 1000 and a retention time deviation of less than 2.5% were considered to indicate a positive identification. When analysing compounds with non-selective spectra in the spectral range 210–400 nm, a threshold value of 990 is recommended.

Additional subtract macro

A so-called additional subtract macro is used to subtract two consecutive chromatograms from each other, the result of this subtraction then being printed. This procedure should provide rapid information on changes in the general composition of the surface water between two runs. Results obtained with blank and spiked River Rhine water showed that subtraction of the chromatograms indeed can provide useful additional information (Fig. 6). If the concentration of a pollutant is increasing, the subtracted chromatogram will show an increasing ("upward") response (Fig. 6B and C). A decreasing

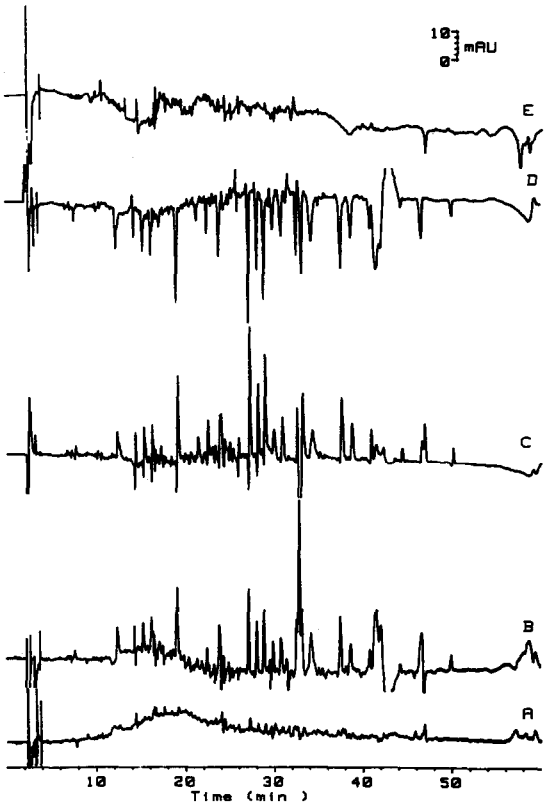

Fig. 6. Subtracted LC–DAD traces of River Rhine water blank samples and samples spiked with a mixture of 27 pesticides at different concentration levels. (A) Subtraction of blanks from two consecutive analyses; (B) subtraction of blank from 0.25 μg/l sample; (C) subtraction of 0.5 μg/l sample from 1.0 μg/l sample; (D) subtraction of 1.0 μg/l sample from 0.25 μg/l sample; (E) subtraction of blanks from two consecutive analyses. For chromatographic conditions, see text.

("downward") response (Fig. 6D) indicates a decreasing concentration level. Obviously, 0.25–0.50 μg/l differences between runs can be easily recognized in most instances.

Applications

Surface water samples were taken from six European rivers and the shape of the matrix interference was similar in all instances (Fig. 7). The samples were analysed by the standard procedure. Except for the sample from the River Mönchaltdorf, in all of them one or more of the pesticides tested in our study were found (Table IV and Fig. 8). The presence of atrazine, simazine and diuron in the River Meuse water samples was confirmed by liquid chromatography–mass spectrometry (LC–MS) [10]. Target analysis of atrazine in another River Rhine sample by means of LC–MS [10] and on-line LC–GC with thermionic detection [11] yielded closely similar concentration levels of atrazine of 0.16 and 0.17 μg/l, respectively. The SAMOS system was installed on a monitoring station on the River Meuse (Keizersveer; RIZA, Lelystad, Netherlands) in June 1992. During that month diuron was found at a concentration level of about 1.4 μg/l (Fig. 8). The results were not completely unexpected because a monitoring study of the River Meuse in 1990 showed that the concentration of diuron was gradually increasing up to about 1 μg/l in June [12]. A general conclusion may be that although LC–MS can provide more structural information, LC–DAD systems are significantly cheaper, and normally provide sufficient data for early-warning purposes.

TABLE IV

PESTICIDES FOUND IN EUROPEAN RIVERS

River	Sampling	Pesticide	Concentration (μg/l)
Garonne	November 1991	3,3'-Dichlorobenzidine	0.3
Meuse (Keizersveer)	June 1992	Atrazine	0.35
		Diuron	1.4
		Simazine	0.65
Rhine (335 km)	April 1992	Atrazine	0.2
		Barban	0.05
		Fluometuron	0.7
Rhine (Lobith)	April 1992	Atrazine	0.2
Thames	February 1992	3,4-Dichloroaniline	0.7
Uster	October 1991	Alachlor	0.25

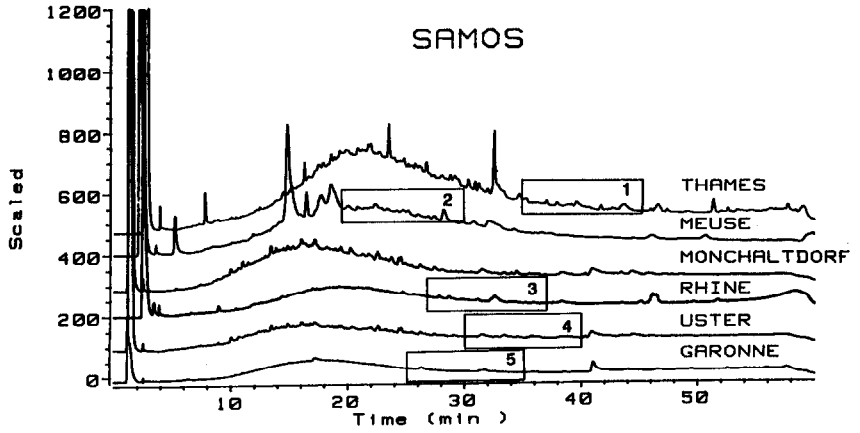

Fig. 7. LC–DAD traces of samples from various European rivers: River Thames (Westminster Bridge, London, UK), River Meuse (Keizersveer, Netherlands), River Monchaltdorf and River Uster (flowing into Lake Greiten, Switzerland), River Rhine (km 335, Germany) and River Garonne (Toulouse, France). The selected windows 1–5 are given in Fig. 8. For chromatographic conditions, see text.

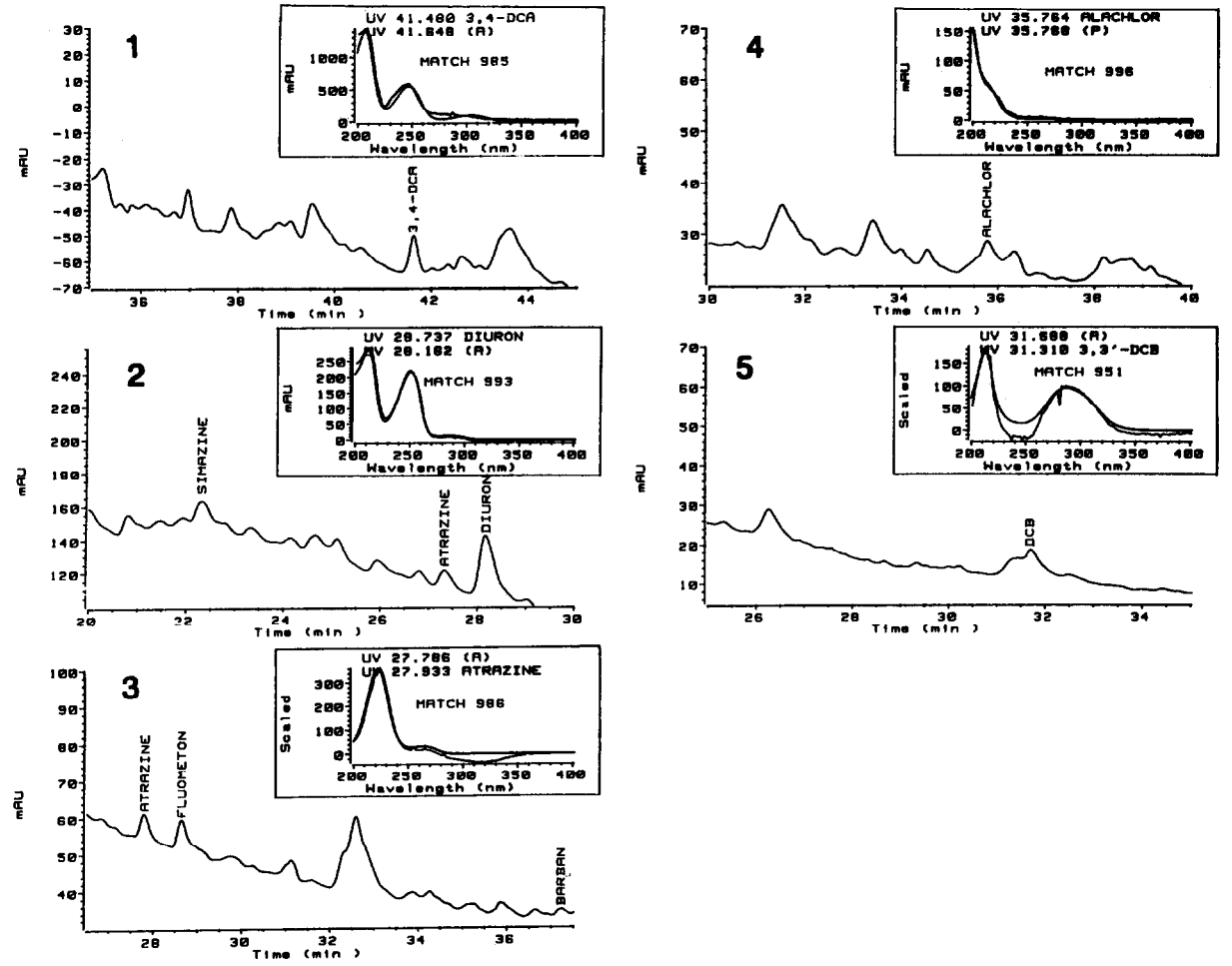

Fig. 8. Blow-ups of parts of the LC–DAD traces shown in Fig. 7, and comparison of UV absorbance spectra of pollutants detected with those stored in the library. The numbers 1–5 refer to the windows in Fig. 7. The concentration levels of the various pesticides are summarized in Table IV.

CONCLUSIONS

An integrated and fully automated LC system has been developed for the monitoring of polar pesticides at trace levels, *i.e.*, low- to sub-μg/l, in surface waters. A cartridge-exchange module (Prospekt) is combined with a gradient LC system (HP 1090) with diode-array detection. The whole system is controlled by the Pascal Workstation (PAWS) of the LC system using a special laboratory-made software package. The data are automatedly evaluated, which means that compounds present at or above a certain concentration level are identified and determined and a report is printed. The SAMOS system has been validated for 27 pesticides and it has since been shown that at least 50–100 organic micropollutants can be determined at concentrations of *ca.* 1 μg/l. The robustness and reliability of the system were tested during several months in two laboratories and at a monitoring station on the River Meuse. This experience showed that the system can be used for monitoring purposes by running it unattended. The method was successfully used for the analysis of samples from six European Rivers; in nearly all instances one or more pesticides were found at concentrations over 0.1 μg/l.

Future research will be devoted to an extension of the range of applicability the system to permanently charged organic pollutants and to obtain lower identification limits by using more selective packing materials.

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