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Development of a semi-automated high-performance liquid chromatographic–diode array detection system for screening pesticides at trace levels in aquatic systems of the Axios River basin

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Abstract

A semi-automated HPLC–diode array detection (HPLC–DAD) system associated with an on-line sample enrichment device was developed for the analysis of a wide range of pesticides in water samples of the Axios River basin. The system was optimised with respect to the analytical column, the on-line trace enrichment device, the mobile-phase composition and gradient duration, the sample volume and pH, and the chromatographic profile of the background dissolved organic material. The system developed was applied for the tentative identification and quantitation of 46 target analytes including parent pesticides and major conversion products in field water samples. The limit of detection (LOD) for the majority of the compounds was in the range 0.10–0.02 μg/l level; the LOD for three target analytes, the aliphatic carbamate esters aldicarb sulfone, oxamyl and methomyl, was in the range of 1.0–0.5 μg/l. In addition, stability studies of all analytes in field water samples stored either under ambient or refrigerated conditions were conducted to optimise field water transport and sample storage conditions.

Monitoring residue data derived from the HPLC–DAD system were validated with off-line solid-phase extraction (SPE) of field water samples and analysis of extracts by independent GC–NPD–ECD and GC–MS systems.

Keywords: Axios River basin; Trace enrichment; Diode array detector; Solid-phase extraction; Pesticides

1. Introduction

In recent years the presence of pesticides, parent compounds and major metabolites or conversion products in surface and ground waters has become a serious environmental concern. In addition, the European Community (EC) directive 80/778, concerning the quality of water destined for human consumption, mandates the maximum permissible level of each pesticide in water to be 0.1 μg/l level and the total amount of pesticides to be not higher than 0.5 μg/l. Therefore, analytical methods adequate to identify and accurately quantitate pesticides and their major conversion products in the aquatic systems and adaptations to the regional situation are needed.

In the determination of trace levels of pesticides in water samples much attention has thus far been paid to the analyte preconcentration techniques to achieve the EC 80/778 directives. Traditional liquid–liquid partition methods for pesticide extraction from water

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samples are laborious, requiring the handling of large volumes of chlorinated solvents and therefore solid-phase extraction (SPE) techniques are presently preferred for both off-line [1–4] and on-line analyte preconcentration HPLC [5–13] and GLC [14–17] associated systems. On-line SPE set-ups are gaining preference over off-line systems mainly due to the possibility of system automation for unattended operation, improvement of the limits of determination, and the decrease of human error in sample handling affecting the reproducibility and precision of the measurements to be made. An automated system has been already developed [18,19] which is now commercially available as SAMOS (System for Automated Measurement of Organic Micropollutants in Surface Waters).

Over the last four years, surveys of land and pesticide use in the Axios River basin as well pesticide residue monitoring studies in soils and aquatic systems [20,21] of this basin indicated that about 150 pesticides, including some of their major conversion products, are of main environmental concern. In the present communication a semi-automated HPLC–DAD system associated with on-line trace enrichment for screening pesticides in the aquatic systems of the Axios River basin is reported.

2. Experimental

2.1. Materials

All solvents used were of HPLC grade purchased from Merck (Darmstadt, Germany) and Lab-Scan (Dublin, Ireland) unless stated otherwise. Pesticide analytical standard materials were purchased from Promochem (Augsburg, Germany), Chem Servis (West Chester, PA, USA), and Riedel-De Haen (Seelze-Hannover, Germany). Atrazine, deisopropyl-atrazine (G-28) and deethylatrazine (G-30) were gifts from CIBA (Basel, Switzerland). Stock solutions of individual pesticides were made in acetonitrile at 1 mg/ml and stored frozen in aluminum/Teflon-lined capped vials (−23°C). Mixed working solutions of standard pesticides in acetonitrile at 50, 25, 5, 1, and 0.5 μg/ml were used for the construction of calibration curves, computer-operated pesticide UV-spectra libraries and the preparation of fortified water samples. Mixed standard solutions were stored under refrigerated conditions and were renewed every two months.

2.2. Instrumentation

A schematic diagram of the chromatographic system used is shown in Fig. 1. The HPLC system consisted of a SSI (State College, PA, USA) Model 222D pump associated with a SSI Model 232C gradient controller, a SSI Model 02-0296 six-port valve (20-μl loop) injector, a reversed-phase (RP) C_{18}, 250×4.6 mm I.D., 5 μm analytical column (LiChrospher, AZ-Analytical, Amtsgericht Mainz, Germany), and a Waters (Milford, MA, USA) Model 996 photodiode array detector controlled by the Millennium (Waters) software operated on an IBM compatible computer connected to a Laserjet 4P (Hewlett-Packard, Palo Alto, CA, USA) printer. The photodiode array detector was operated in the range 190–350 nm at 1.2 nm spectrum resolution. The analytical column was operated at ambient temperature.

For the on-line sample enrichment a 20×3 mm I.D. cartridge packed with a polymeric RP material, PRP-1, 10 μm purchased from Hamilton (Reno, NE, USA) was used. The cartridge was installed in the loop mode of a 7000 Rheodyne (Cotati, CA, USA) valve and was interfaced between the SSI injector and the analytical column. A Waters Model M-45 preparative pump operated at 5 ml/min was used for sample loading. On-line to the analytical column a 30×4.6 mm I.D., RP-18, 10 μm guard column (Brownlee Labs, Santa Clara, CA, USA) was installed. On top of the guard column holder an additional 2-μm pore frit (Whatman, Clifton, NJ, USA) was installed which was periodically replaced.

For validation of the HPLC–DAD system, two Hewlett-Packard Model 5890 gas chromatographs equipped with nitrogen–phosphorous detector (NPD)–electron capture detector (ECD) (system A) and NPD (system B), respectively, and a Tremetrics (Austin, TX, USA) Model 9001 gas chromatograph equipped with a Model 850 quadrupole MS (system C) were used. System A was equipped with a DB-17 (Alltech, Deerfield, IL, USA) 30 m×0.25 mm I.D., 25 μm film thickness capillary column connected via a glass splitter to the two detectors. System B was

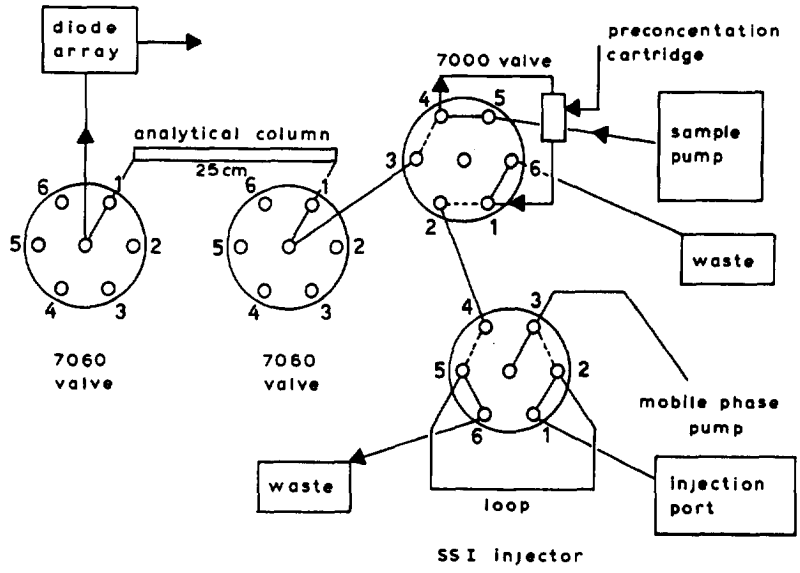

Fig. 1. Diagram of the on-line SPE HPLC–DAD system.

equipped with a PAS-1701 (Hewlett-Packard) 25 m×0.32 mm I.D., 25 μm film thickness capillary column and system C with a DB-5 (Alltech) 30 m×0.25 mm I.D., 25 μm film thickness capillary column. All three columns were connected to split/splitless injectors, respectively, and were operated in the splitless injection mode. The detectors of systems A and B were connected to respective HP 3396 Series II integrators (Hewlett-Packard).

All GC injectors were thermostated at 230°C, the NPDs at 300°C, the ECD at 320°C and the GC–MS transfer line of system C at 250°C. In all three systems helium was used as carrier gas at a constant flow-rate of 1 ml/min by use of EPC control. For operation of the NPD systems the hydrogen flow was set to 3–4 ml/min and the air to 120 ml/min. Helium was used as make-up gas for the NPD systems and nitrogen for the ECD. Both the DB-17 and PAS-1701 columns were operated under a temperature gradient regime from 80°C to 260°C at 20°C/min (final hold-time 5 min). The DB-5 column installed in the GC–MS system was operated under the following temperature regime: initial 80°C, hold for 1 min, increase at 6°C/min to 200°C, hold for 3 min; increase at 8.0°C/min to 260°C, hold for 5 min.

2.3. Procedure

The mobile phase of the HPLC system consisted of distilled tap water, pH 5.8 (solvent A) purified via a Norganic cartridge (Millipore, Bedford, MA, USA) and acetonitrile (solvent B, HPLC far-UV grade). The mobile-phase gradient regime is given in Table 1. The mobile phase components were filtered through 0.45-μm membrane filters (Millipore) in an all glass filtration apparatus operated under reduced pressure and degassed for 15 min in an ultrasonic

Table 1
Mobile-phase gradient regime

Time (min)	Solvent A[a] (%)	Solvent B[b] (%)	Flow-rate (ml/min)
00.00	95	5	1.0
60.00	10	90	1.0
64.00	10	90	1.0
65.00	59	5	1.5
70.00	59	5	1.5
75.00	95	5	1.0

[a] Distilled tap water, pH 5.8, purified via filtration through a Norganic cartridge and filtered through 0.45-μm membrane filter.
[b] Acetonitrile HPLC far-UV grade filtered through 0.45-μm membrane filter.

bath before use. Periodically, during operation of the HPLC system, solvents were degassed by purging with helium for 5 min.

For optimisation of the HPLC–DAD system all experiments were made with Axios River water samples filtered through 0.45-μm filters (Millipore) as previously described, and fortified at either a 5 or 1 μg/l level depending upon the water solubility [22] of the sought analytes. One-liter samples were fortified by addition of 1 ml of the 5 or 1 μg/ml mixed working standard solution in acetonitrile, respectively. Aliquots of 100 ml were introduced into the pre-concentration cartridge at 5 ml/min and the on-line cartridge was eluted with a forward flow of mobile phase onto the analytical column. At the end of each run the cartridge was washed in a back flush mode with 5 ml distilled water followed by 30 ml 0.1 *M* sodium citrate acidified at pH 2 with H_3PO_4, 10 ml water, 5 ml acetonitrile, 10 ml of CH_2Cl_2–hexane (50:50, v/v) or 10 ml ethyl acetate followed by 5 ml acetonitrile. Before use each newly installed cartridge was washed with 10 ml distilled water.

Quantitative measurements were made at 210 nm by use of four-point external standard calibration curves. The calibration curves were linear over a 1000-ng range (1–1000 ng) with respective correlation coefficients higher than 0.999.

The system was realised with Axios River basin water samples collected in 2.5-l amber glass bottles. Samples were filtered through 0.45-μm membrane filters, as previously described, and 100-ml aliquots were introduced into the sample enrichment cartridge at 5 ml/min.

To validate the system, field water samples were also analyzed by the gas chromatographic systems after analyte off-line extraction by SPE (solid-phase extraction). In this case, 1-l filtered water aliquots were introduced by suction onto three tandem C_{18} Sep-Pak (Millipore) cartridges previously washed with methanol followed by distilled water. The retained organic materials on the SPE cartridges were eluted with 30 ml of ethyl acetate (Residue grade, Merck). The ethyl acetate extract dried over anhydrous sodium sulphate previously rinsed with 20 ml of ethyl acetate, was concentrated to dryness by use of a nitrogen stream and the residue dissolved in 1.0 to 0.1 ml of ethyl acetate (Residue grade, Merck) was analyzed by the GC systems. Filtered Axios River water samples fortified at 5, 1, 0.1 and 0.01 μg/l level were processed as previously described and analyzed by GC to evaluate the analyte recovery performance. Quantitative measurements were made by use of external standard calibration curves.

3. Results and discussion

3.1. Method development

The analytical column used (LiChrospher) in the proposed system was selected from three RP-C_{18} columns (the others being Partisil and Spherisob) of the same dimensions, carbon content and particle size. A test standard solution containing 14 pesticides at 10 μg/ml was chromatographed on each column and parameters such as the mean peak width, peak height and resolution were calculated. The mean peak widths derived from the analysis of the 14 test analytes on the Partisil, Spherisorb and LiChrospher columns were 1.63, 1.45 and 0.63 min, respectively; the respective mean peak heights were 3.66, 4.00 and 11.44 cm and the mean resolutions were 1.77, 2.17 and 5.55, respectively. Based on these data the LiChrosphere analytical column was selected. The same parameters were also used to optimise the mobile-phase gradient. Increase of the gradient duration from 45 to 90 min did not substantially improve analyte resolution while the respective mean peak width increased by 57% and the mean peak height decreased by 60%. Therefore, a 60-min linear gradient was adopted.

The on-line sample enrichment cartridge Hamilton PRP-1 (20×3 mm) was selected among other commercially available RP guard columns including the BrownLee C_{18} (30×4.6 mm), Hamilton C_{18} (20×3 mm), and in-house LiChrolut (Merck) dry-packed (20×3 mm) cartridges. The sorbent materials were evaluated with respect to target analyte recovery, repeatability of recovery as determined from the respective R.S.D. (relative standard deviation) values, and the durability affecting the overall performance of the system during consecutive runs (data not shown). The best data for the majority of the target analytes were obtained on cartridges packed with PRP-1 resin and therefore this material was selected

for the on-line sample preconcentration device. The same material was selected by Liska et al. [11] from ten different sorbents for the analysis of five test compounds including aniline, 2-chloroaniline, 2-nitrophenol, atrazine and diuron. This type of cartridge can be used for the processing of 8–10 100-ml field water samples before being discarded. The only reason necessitating cartridge replacement is the progressive backpressure increase after each run caused by deposition of microparticulate material (<0.45 μm) on the head of the cartridge. A small percentage of these deposits can be removed by backflushing the cartridge. Alternatively, filtering each field sample through a 0.2-μm filter can prolong the cartridge life; however, this is an extra time-consuming step and as expensive as the replacement of the cartridge every 8–10 samples.

Preliminary work on the limits of detection of the diode array detector used in the proposed system indicated that for the analytes of interest, except for aldicarb sulfone, 2–10 ng had to be injected for a solute to be recognised; for aldicarb sulfone 15 ng/injection was required. Taking into account the detection limits of the target analytes and the magnitude of the background interference from the dissolved organic matter present in the various field water samples, the sample volume was optimised independently of the breakthrough volumes of the analytes of interest. Data from the sample volume optimisation procedure are shown in Table 2. The recoveries increased as the sample volume increased up to the respective breakthrough volume for each analyte; however, the absolute amount of each analyte retained still increased even when the sample volume exceeded the respective breakthrough volume as long as the cartridge was not overloaded. This approach to optimise solute preconcentration was demonstrated first by Subra et al. [23] who also proposed a theoretical model for such conditions. Therefore, to allow for the analysis and tentative identification of the wide chemical range of target analytes the sample volume was set to 100 ml.

The low recovery of the lipophilic compounds (compounds with water solubilities in the range 2–0.002 mg/l) [22] – represented in the test mixture by fluazifop butyl, pirimiphos ethyl and deltamethrin, with deltamethrin being the least polar – was further investigated. The low recoveries (Table 2) were attributed to adsorption of these analytes onto the glass sample container and the different transfer lines during sample enrichment. Silanization of the sample container with 5% dimethyldichlorosilane in toluene did not improve these recoveries. However, when methanol was added to the sample to 10% (before loading), the recoveries of the more polar analytes (compounds with water solubility higher than 50.000 mg/l), as expected, decreased; the recoveries of the analytes with medium polarity (compounds with water solubility in the range 50.000–9 mg/l) were only slightly affected; the recoveries of fluazifop butyl and pirimiphos ethyl increased significantly while the recovery of the least polar analyte, deltamethrin, was only slightly affected. Apparently the presence of a higher percentage of methanol in the sample was required for recoveries of the lipophilic compounds in the 70–80% range, although with the risk of loosing a wide range of early eluting analytes. With 10% methanol present in the sample, the recovery of oxamyl was reduced to a non-detectable level and the recovery of metamitron – being 88.4% in the absence of methanol – decreased to 45.6%. Therefore, to accommodate in a single run the wide chemical range of target analytes, samples were analyzed without methanol addition.

Data on retention times and recoveries with the

Table 2
Analyte recoveries[a] on PRP-1 on-line cartridge with sample volume varied from 15 to 1000 ml

Pesticides	Sample volume (ml)						
	15	30	50	75	100	500	1000
Oxamyl	71	73	49	31	21	ND	ND
Metamitron	66	78	86	88	88	30	11
Aldicarb	70	81	89	90	89	92	49
Simazine	68	80	90	93	99	86	88
Chlortoluron	65	81	90	93	95	96	89
Diuron	64	81	90	93	95	95	93
Metobromuron	65	80	87	90	94	101	85
Linuron	70	84	91	95	96	102	99
Metolachlor	59	77	86	90	93	97	92
Fenitrothion	56	72	80	86	87	93	87
Diazinon	45	77	92	98	100	90	84
Fluazifop butyl	20	29	39	43	53	72	82
Pirimiphos ethyl	26	33	48	53	65	81	85
Deltamethrin	35	38	47	39	44	49	57

[a] Recoveries were determined with Axios River water samples fortified at 5 μg/l level.
ND=not detectable.

respective R.S.D. values for all the analytes of interest are presented in Table 3. The repeatability or the precision of the system with respect to retention times and recoveries – the respective R.S.D.s being less than 1 and 10%, respectively, for most of the analytes – is good. Only for oxamyl, methomyl, dimethoate and prometryne the R.S.D.s of their retention times were 2, 2 and 8%, respectively. The decreased precision with respect to the retention time of prometryne is due to the fact that this triazine herbicide is the most basic compound among the target analytes and therefore the suitability of the selected analytical column is marginal. The retention times of the target analytes were slightly lower when stock solutions were chromatographed after direct loop-injection; however, the respective R.S.D.s were in the same range as those of the samples introduced via the on-line preconcentration device.

Also shown in Table 3 is the limit of detection (LOD) of each analyte of interest. The LOD was considered to be the fortification level in μg/l of a certain pesticide in a field water sample at which this pesticide was recognised by the library search menu of the system in at least three consecutive chromatographic runs of 100-ml aliquots. Based on this limitation the LOD of most pesticides was in the 0.02–0.1 μg/l range. The LODs of the aliphatic carbamate esters oxamyl, methomyl, and aldicarb sulfone were 0.5, 0.5 and 1.0 μg/l, respectively.

The pH of surface and ground waters of the studied area is in the range 7.8–8.2. Therefore, acidification of the water samples at the site of sampling was considered necessary to avoid alkaline hydrolysis of pesticide esters. However, as will be discussed later, field water samples can be stored for up to 4 days under refrigerated conditions without significant change in the concentration of most of the analytes of interest. Moreover, since no highly acidic or basic compounds were included in this study a sample pH ranging from 3.0 to 8.2 did not affect the recoveries of the analytes of interest; however, the recovery of the background humic acids was significantly affected (Fig. 2). At high pH (8.2) the recovery of the humic acids decreased by 70% compared to the recovery from samples with the pH adjusted to 3.0; thus cleaner chromatograms were obtained and background interference in the identification and quantitative measurement of the sought analytes was minimal. Therefore, samples were analyzed without previous pH adjustment.

The effect of the mobile-phase pH on the chromatographic behaviour of the sought analytes was also investigated. Sample data are presented in Fig. 3. Since, as mentioned above, neither highly acidic nor basic compounds were among the target analytes, the retention times of the analytes were not affected by changes in mobile-phase pH. However, the effect of mobile-phase pH on the chromatographic profile of the background humic materials in field water samples was very profound (Fig. 3). When the mobile-phase pH was adjusted to 5.8, the humic background eluting between 6 and 16 min interfered with the analysis of a few very polar analytes while at pH 3 the humic acid background eluting at approximately the middle of the gradient run (12–29 min) interfered with the analysis of a larger number of analytes. Therefore, a non-buffered mobile phase with pH 5.8 was selected for the proposed HPLC–DAD system.

3.2. Analyte stability studies

Since a substantial number of the sought analytes are carbamate, organophosphorus, thiocarbamate, and carboxylic esters, stability studies of these pesticides at the natural pH range (7.8–8.2) of the field water samples were considered necessary to optimise transport and storage conditions of field water samples. Analyte stability studies were conducted at both ambient (23°C) and refrigerated (5°C) conditions. Therefore, two series of 5-l field water samples filtered and fortified at the 5 or 1 μg/l level, depending upon the water solubility of the different pesticides, were kept under ambient and refrigerated conditions, respectively, and 100-ml subsamples taken at different storage periods were analyzed. Data from the stability studies of the target analytes under ambient and refrigerated conditions are shown in Table 4 and Table 5, respectively.

Under ambient storage conditions only two analytes, i.e. the carbamate esters aldicarb sulfone and phenmedipham, were depleted to non-detectable levels within an 8-h storage period. However, after storage for 6–24 h the recoveries of the carbamate esters oxamyl, 3-OH-carbofuran and carbofuran, the polycyclic alkanoic esters diclofop methyl and

Table 3
Repeatability of retention times (t_R)[a] and recoveries[b] and limits of detection (LOD)[c] of pesticides in Axios River fortified water samples

Pesticide	Mean t_R (R.S.D.) (min)	Mean recovery (R.S.D.) (%)	LOD (μg/l)
Aldicarb sulfone	11.6 (1.4)	14	1.0
Oxamyl	11.8 (2.0)	21 (3)	0.5
Methomyl	12.8 (2.3)	19	0.5
G-28 atrazine[d]	13.9 (1.1)	22	0.1
Metamitron	17.5 (0.9)	88 (5)	0.1
3-OH-Carbofuran	17.7 (1.2)	85	0.1
G-30 atrazine[d]	18.5 (0.7)	89	0.04
Dimethoate	19.7 (1.6)	82	0.1
Aldicarb	23.8 (0.3)	89 (11)	0.1
Simazine	25.6 (0.2)	99 (3)	0.05
Paraoxon methyl	26.8 (0.4)	91	0.05
Carbofuran	29.0 (0.3)	102	0.05
Chlortoluron	30.4 (0.1)	95 (1)	0.02
Atrazine	30.8 (0.2)	80	0.05
Fluometuron	30.7 (0.8)	90	0.05
Metalaxyl	31.3 (0.7)	92	0.05
Diuron	32.6 (0.1)	95 (1)	0.02
Prometryne	33.2 (7.5)	83	0.02
Paraoxon	33.5 (0.2)	81	0.05
Metobromuron	34.0 (0.1)	94 (1)	0.05
Propanil	36.5 (0.2)	87	0.05
Terbuthylazine	37.2 (0.5)	89	0.02
Phenmedipham	38.0 (0.2)	80	0.05
Linuron	38.4 (0.1)	96 (2)	0.02
Molinate	39.9 (0.4)	87	0.05
Parathion methyl	41.6 (0.3)	92	0.05
Ethofumesate	41.6 (0.6)	90	0.05
Metolachlor	42.4 (0.1)	93 (1)	0.05
Alachlor	42.7 (0.3)	82	0.05
Chlorothalonil	43.4 (0.2)	76	0.05
Fenitrothion	43.5 (0.1)	87 (2)	0.05
Propiconazole	44.2 (0.4)	88	0.05
Parathion	47.2 (0.1)	76	0.05
Diazinon	47.5 (0.1)	100 (3)	0.05
Chlorpyrifos methyl	49.9 (0.1)	86	0.05
Chlorobenzilate	49.4 (0.3)	76	0.05
Pirimiphos methyl	50.3 (0.7)	76	0.05
Diclofop methyl	53.1 (0.2)	63	0.05
Fluazifop butyl	54.0 (0.2)	53 (2)	0.1
Trifluraline	55.5 (0.1)	59	0.1
Pirimiphos	55.8 (0.7)	65 (1)	0.1
Pendimethalin	55.2 (0.1)	51	0.1
Cypermethrin[e]	59.8 (0.1)	40	0.1
DDT[e]	60.7 (0.2)	29	0.1
Deltamethrin[e]	60.9 (0.3)	44 (7)	0.1
Fenvalerate[e]	61.2 (0.2)	59	0.1
p,p'-DDE[e]	62.8 (0.2)	30	0.1
trans-Permethrin[e]	62.2 (0.1)	37	0.1
cis-Permethrin[e]	63.6 (0.2)	56	0.1

[a] Retention times of analytes with respective R.S.D.s when fortified water samples were analysed.
[b] Recoveries were determined with Axios River water samples (100 ml) fortified at 5 μg/l level unless indicated otherwise.
[c] LOD (limit of detection) is the fortification level of a field water sample when a certain pesticide was recognised by the library search during three consecutive runs.
[d] G-28 and G-30 atrazine metabolites denote deisopropylatrazine and deethylatrazine, respectively.
[e] Recoveries of these pesticides were determined when water samples were fortified at 1 μg/l level.

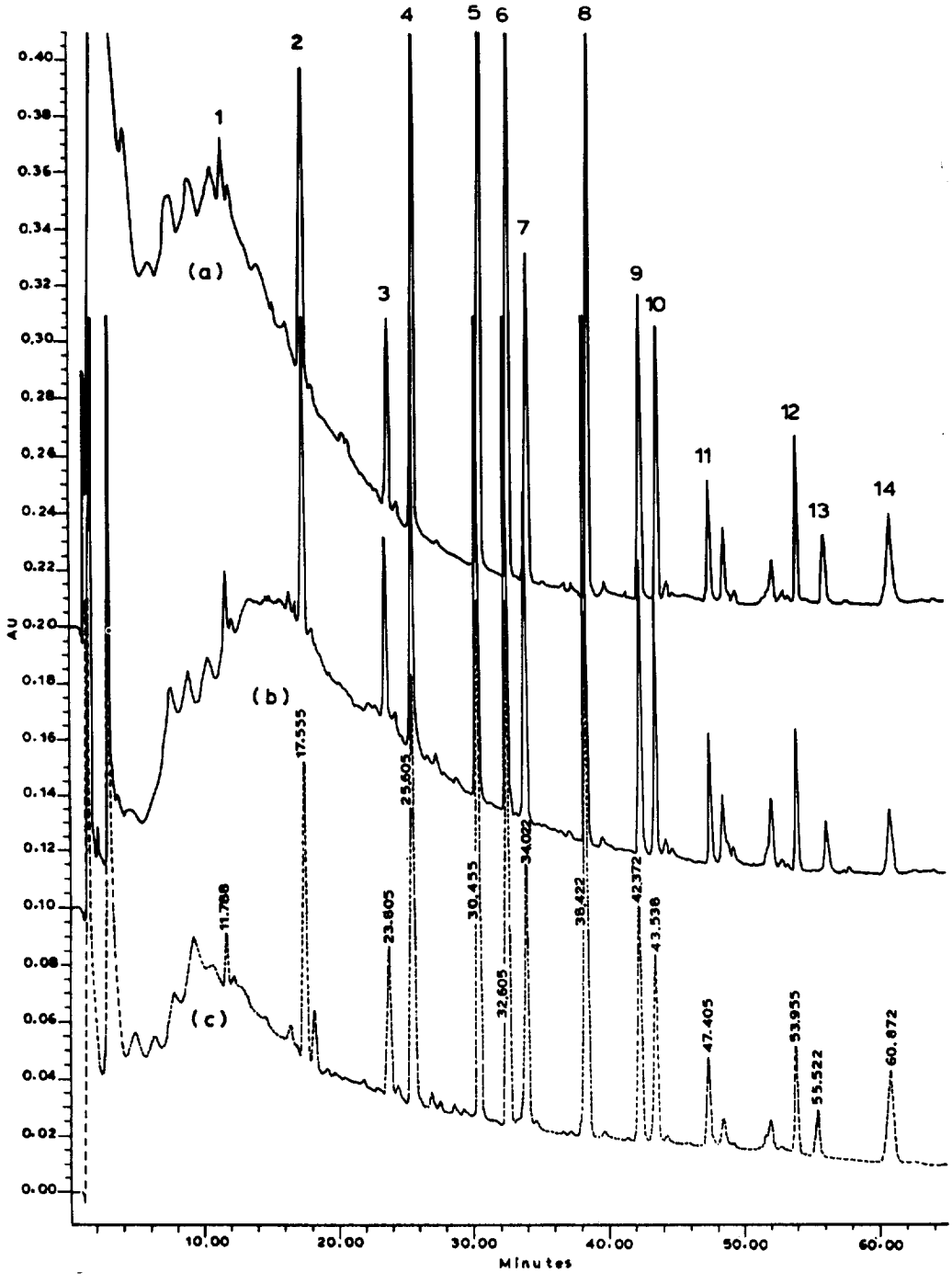

Fig. 2. Sample chromatograms from the analysis of fortified (5 μg/l) Axios River water samples (100 ml) by on-line SPE–HPLC–DAD when the sample pH was adjusted to 3 (trace a), 6 (trace b), and 8.2 (trace c). The other chromatographic conditions were the same as described in the Experimental section. Peaks: (1) oxamyl, (2) metamitron, (3) aldicarb, (4) simazine, (5) chlortoluron, (6) diuron, (7) metobromuron, (8) linuron, (9) metolachlor, (10) fenitrothion, (11) diazinon, (12) fluazifop butyl, (13) pirimiphos ethyl, (14) deltamethrin.

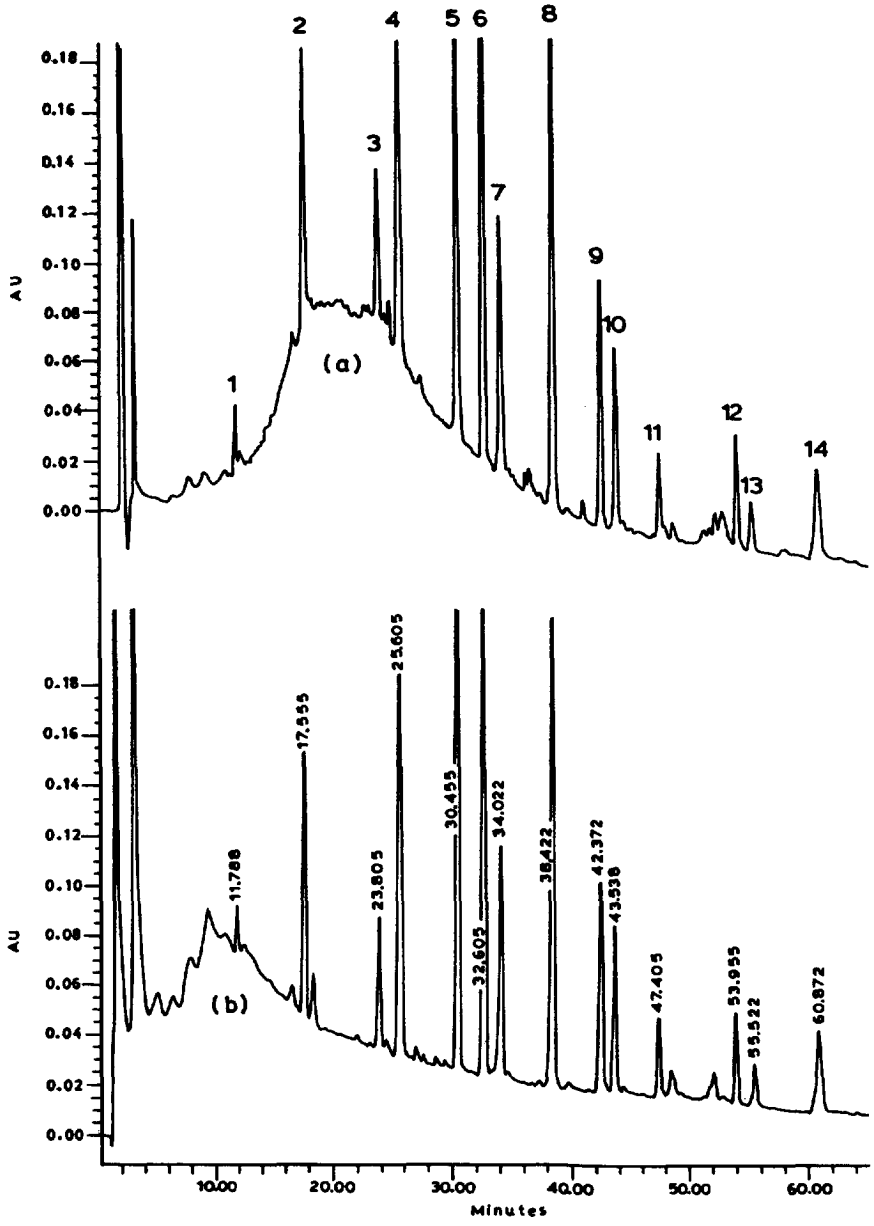

Fig. 3. Sample chromatograms from the analysis of fortified (5 μg/l) Axios River water samples (100 ml, pH 8.2) by on-line SPE–HPLC–DAD when the pH of the mobile phase was adjusted to 3 (trace a) and 5.8 (trace b). The other chromatographic conditions were the same as described in the Experimental section. Analyte identification as in Fig. 2.

fluazifop butyl, and the pyrethroid esters cypermethrin, deltamethrin, fenvalerate and *cis/trans*-permethrin were significantly reduced (Table 4).

Under refrigerated storage conditions the rate of ester hydrolysis was reduced significantly (Table 5). However, after an 8-day storage period, phenmedipham was reduced to a non-detectable level while the recoveries of a few other compounds (aldicarb sulfone, oxamyl, methomyl, diclofop methyl and fluazifop butyl) were only slightly affect-

Table 4
Analyte stabilities expressed as recoveries[a] from fortified Axios River water samples (pH 8.0–8.2) stored under ambient temperature (23°C)

Pesticide	Recovery (%)					Mean recovery[b] (R.S.D.) (%)
	0 h	4 h	8 h	16 h	24–26 h	
Aldicarb sulfone	14	6	ND	ND	ND	–
Oxamyl	19	15	12	5	6	11 (53)
G-28 atrazine[c]	20	17	17	19	19	18 (7)
Metamitron	88	89	77	89	82	85 (7)
3-OH-Carbofuran	81	75	70	43	32	60 (35)
G-30 atrazine[c]	83	78	73	78	78	78 (4)
Dimethoate	99	88	82	85	96	90 (8)
Aldicarb	87	71	87	–	80	81 (9)
Simazine	96	88	92	87	88	90 (4)
Paraoxon methyl	93	90	83	83	82	86 (6)
Carbofuran	86	82	95	69	69	80 (14)
Chlortoluron	88	87	89	86	87	87 (1)
Atrazine	84	89	89	91	92	89 (4)
Fluometuron	124	110	116	113	109	115 (5)
Flutriafol	74	81	79	82	82	80 (4)
Metalaxyl	84	85	88	88	86	86 (2)
Diuron	88	90	87	88	84	88 (3)
Paraoxon	87	85	85	88	87	86 (1)
Metobromuron	86	90	86	87	87	87 (2)
Propanil	89	89	88	90	88	89 (1)
Terbuthylazine	84	87	82	87	87	85 (3)
Phenmedipham	62	4	ND	ND	ND	–
Linuron	86	88	84	87	88	87 (2)
Molinate	84	84	86	87	86	85 (1)
Parathion methyl	88	89	87	85	85	87 (2)
Ethofumesate	89	89	87	81	81	85 (5)
Metolachlor	81	82	79	84	85	82 (3)
Alachlor	83	85	86	87	85	85 (2)
Chlorothalonil	95	94	89	85	89	90 (5)
Fenitrothion	82	80	76	82	83	81 (4)
Propiconazole	100	100	102	102	100	101 (1)
Parathion	83	86	83	82	82	83 (2)
Diazinon	87	82	88	93	90	88 (4)
Chlorpyrifos methyl	79	78	76	80	73	77 (4)
Chlorobenzilate	80	77	72	72	73	75 (5)
Pirimiphos methyl	75	76	77	80	74	76 (3)
Diclofop methyl	69	63	57	45	31	53 (28)
Fluazifop butyl	68	59	54	29	12	44 (52)
Trifluraline	61	61	60	61	60	60 (1)
Pirimiphos	72	68	67	68	69	69 (3)
Pendimethalin	53	55	57	58	51	55 (5)
Cypermethrin[d]	31	24	26	34	19	27 (23)
DDT[d]	36	36	31	31	30	33 (9)
Deltamethrin[d]	29	22	22	23	21	23 (13)
Fenvalerate[d]	37	30	24	33	26	30 (17)
p,p'-DDE[d]	33	28	29	27	29	29 (8)
trans-Permethrin[d]	19	18	21	18	13	18 (16)
cis-Permethrin[d]	44	36	29	30	32	34 (18)

[a] Recoveries were determined for 100-ml aliquots of fortified at 5 μg/l Axios River water samples.
[b] Mean recovery and respective R.S.D. values derived from all measurements made during the entire storage period.
[c] G-28 and G-30 denote deisopropylatrazine and deethylatrazine, respectively.
[d] Fortified samples were made at 1 μg/l level.
ND=not detectable.

Table 5
Analyte stabilities expressed as recoveries[a] from fortified Axios River water samples (pH 8.0–8.2) stored under refrigerated conditions (5°C)

Pesticide	Recovery (%)				Mean recovery[b] (R.S.D.) (%)
	0-day	2-day	4-day	8-day	
Aldicarb sulfone	14	10	11	8	11 (24)
Oxamyl	21	23	23	27	24 (11)
Methomyl	19	26	20	22	22 (13)
G-28 atrazine[c]	22	22	24	21	22 (5)
Metamiron	88	89	85	87	87 (2)
3-OH-Carbofuran	85	79	78	74	79 (6)
G-30 atrazine[c]	89	96	95	94	93 (3)
Dimethoate	81	80	88	82	83 (4)
Aldicarb	88	88	81	87	86 (4)
Simazine	99	89	92	92	93 (5)
Paraoxon methyl	91	89	77	84	85 (7)
Carbofuran	102	97	93	96	97 (4)
Chlortoluron	95	90	89	92	92 (3)
Atrazine	80	89	87	88	86 (5)
Fluometuron	90	89	90	89	90 (1)
Metalaxyl	92	89	88	84	88 (4)
Diuron	95	92	91	91	92 (2)
Paraoxon	81	84	88	80	83 (4)
Metobromuron	94	88	79	89	87 (7)
Propanil	87	89	89	88	88 (1)
Terbuthylazine	89	88	88	89	89 (1)
Phenmedipham	80	13	3	ND	–
Linuron	96	92	89	92	92 (3)
Molinate	87	88	91	88	89 (2)
Parathion methyl	92	90	89	88	90 (2)
Ethofumesate	90	91	91	85	89 (3)
Metolachlor	93	89	88	89	90 (3)
Alachlor	82	83	85	87	84 (3)
Chlorothalonil	76	80	80	82	79 (3)
Fenitrothion	87	83	74	84	82 (7)
Propiconazole	88	89	89	90	89 (1)
Parathion	76	79	72	78	76 (4)
Diazinon	100	89	90	91	93 (6)
Chlorpyrifos methyl	86	71	78	77	78 (8)
Chlorobenzilate	76	75	74	76	75 (1)
Pirimiphos methyl	76	72	62	65	69 (9)
Diclofop methyl	63	57	46	24	48 (37)
Fluazifop butyl	53	51	51	27	45 (27)
Trifluraline	59	55	55	51	55 (6)
Pirimiphos	65	64	69	62	65 (4)
Pendimethalin	51	56	55	50	53 (5)
Cypermethrin[d]	40	47	42	43	43 (7)
DDT[d]	29	23	20	19	23 (20)
Deltamethrin[d]	44	53	51	48	49 (8)
Fenvalerate[d]	59	54	52	47	53 (9)
p,p′-DDE[d]	30	23	23	21	24 (17)
trans-Permethrin[d]	37	47	42	41	42 (10)
cis-Permethrin[d]	56	53	50	44	51 (10)

[a] Recoveries were determined for 100-ml aliquots of fortified at 5 μg/l Axios River water samples.
[b] Mean recovery and respective R.S.D. values derived from all measurements made during the entire storage period.
[c] G-28 and G-30 denote deisopropylatrazine and deethylatrazine, respectively.
[d] Fortified samples were made at 1 μg/l level.

ed. The decreased recovery of DDT and DDE during storage under refrigerated conditions (Table 5), while both compounds are considered among the most environmentally stable chlorinated hydrocarbons, was apparently due to their marginal water solubility and hence their precipitation under cold storage conditions. Continuous stirring of the fortified solutions during storage by use of magnetic stirrers did not entirely prevent their precipitation.

From the data presented in Table 4 and 5 it appears that water samples of the studied area have to be transported for analysis stored under refrigerated conditions and that they should not be stored for more than 2–4 days before analysis. Under such conditions the recovery variations for most analytes should be in the ±10% R.S.D. margin of the repeatable recovery range of this method. Under ambient conditions, samples should be analyzed within less than 4 h of sampling to avoid recovery decreases due to ester hydrolysis. However, the latter is impossible in practice and therefore on-site associated with on-line sample analysis is the alternative option under investigation.

3.3. Application of the HPLC–DAD system for the analysis of field water samples of the Axios River basin

The on-line sample preconcentration HPLC–DAD system was used for the analysis of field water samples collected from the Axios River basin. A sample chromatogram from the analysis of a water sample taken from the phreatic horizon of a corn field is shown in Fig. 4. Atrazine and deethylatrazine

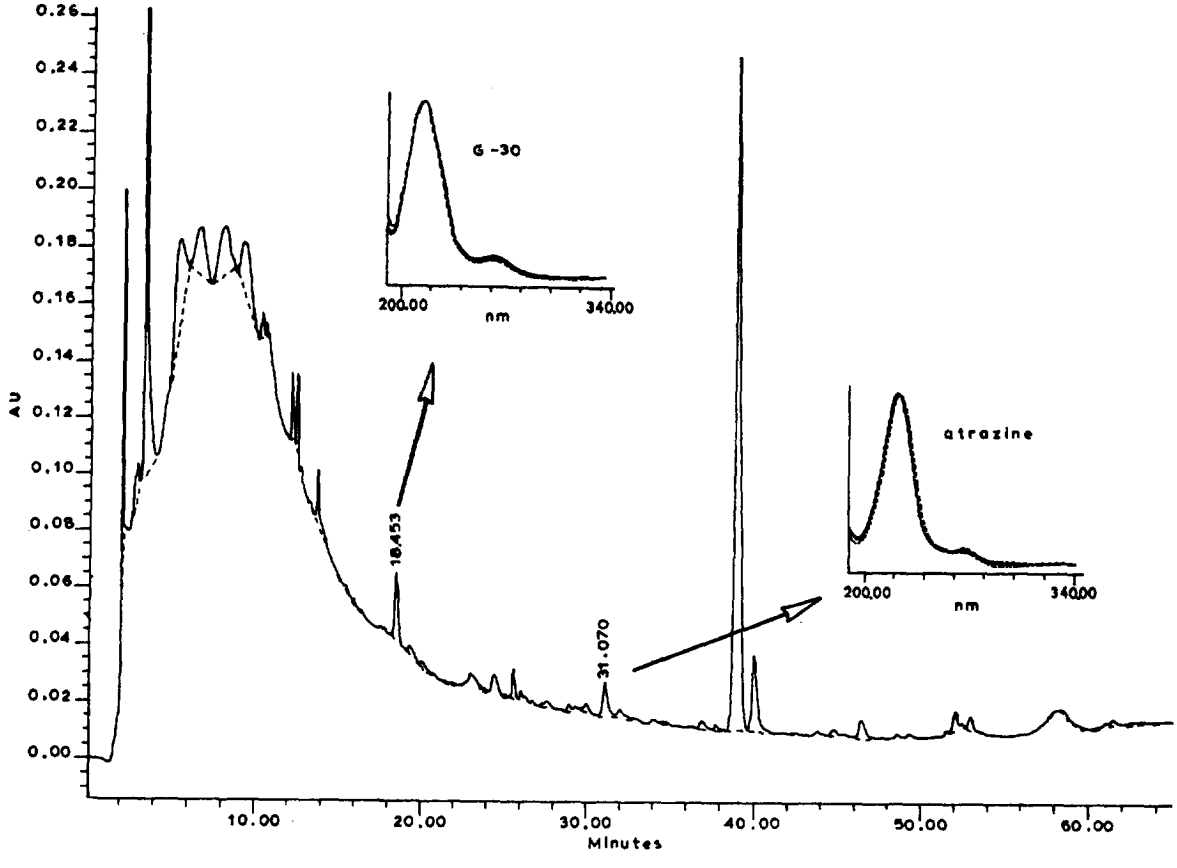

Fig. 4. Sample chromatogram from the analysis by on-line SPE–HPLC–DAD of a field water sample (100 ml, pH 7.8) taken from the phreatic horizon of a corn field. Chromatographic conditions were the same as described in the Experimental section. Deethylatrazine (G-30) and atrazine were found at 0.57 and 0.48 μg/l level, respectively.

(G-30) were tentatively identified and determined at 0.57 and 0.48 μg/l, respectively. Fig. 5 shows a sample chromatogram from the analysis of a water sample taken from the phreatic horizon of a rice field. In this sample atrazine, carbofuran and deethylatrazine were tentatively identified and determined at 0.09, 0.09 and 0.04 μg/l, respectively.

To evaluate the accuracy of the proposed system and confirm the identity of the analytes, aliquots of field water samples analyzed by the on-line HPLC–DAD system were also analyzed by GC after analyte extraction by off-line SPE. Comparative data from the analysis of two field water samples are shown in Table 6. There is good agreement between the two analytical systems and thus the semi-automated HPLC–DAD system bearing all the advantages of on-line sample processing is also as accurate as the rest of the analytical systems and can be reliably used for screening pesticides in aquatic systems of the studied area.

4. Conclusions

A simple semi-automated system involving on-line sample SPE associated with HPLC–DAD was developed to accommodate for the analysis of a wide chemical range of target pesticides in the aquatic systems of the Axios River basin located in Macedonia, Greece. The system, in spite of the low recovery values for the very polar and least polar analytes, is convenient for screening the analytes of interest at the 1.0–0.02 μg/l or higher levels and is

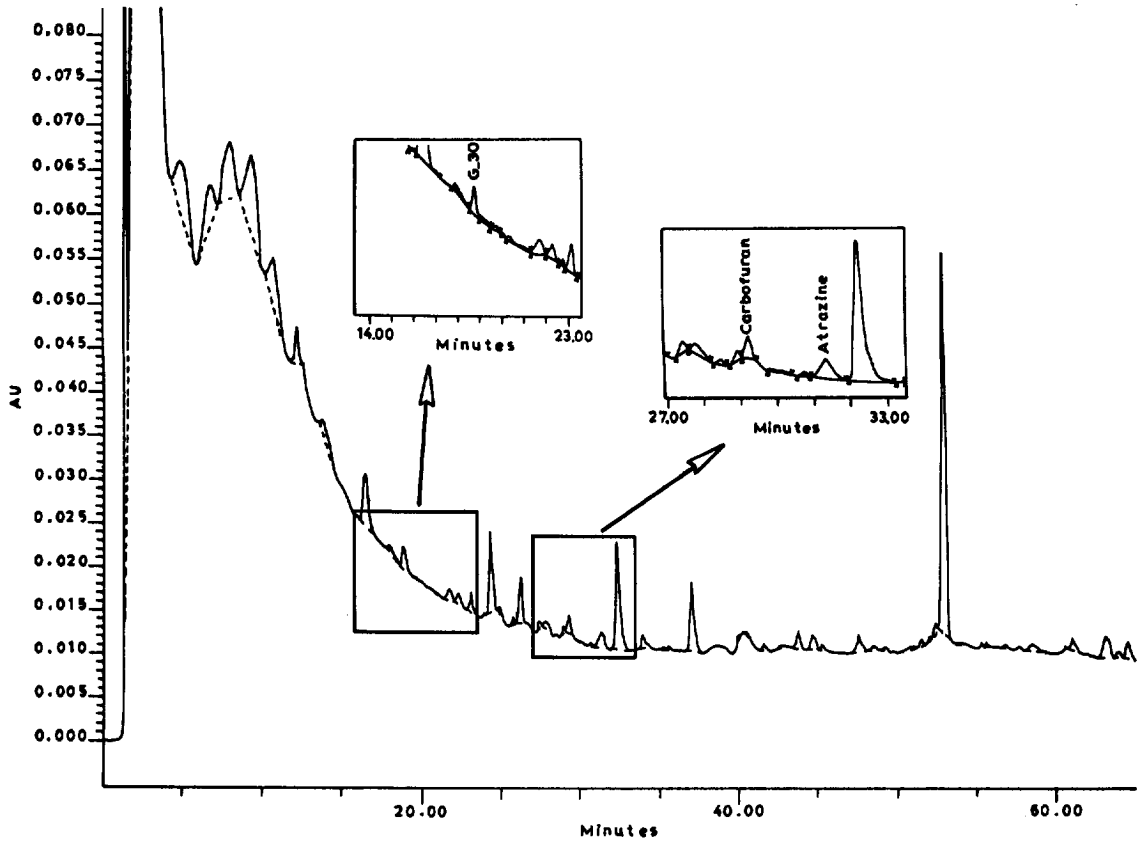

Fig. 5. Sample chromatogram from the analysis by on-line SPE–HPLC–DAD of a field water sample (100 ml, pH 7.8) taken from the phreatic horizon of a rice field. Chromatographic conditions were the same as described in the Experimental section. Deethylatrazine (G-30), carbofuran and atrazine were found at 0.04, 0.09 and 0.09 μg/l, respectively.

Table 6
Comparative residue[a] data derived from the analysis of field water samples of the Axios River basin by both HPLC–DAD and GC systems

Pesticide	HPLC–DAD	GC–NPD–ECD DB-17	GC–NPD PAS-1701	GC–MS DB-5
Sample A[b]				
G-30	0.77	–	–	–
Atrazine	0.75	0.84	0.90	1.0
Molinate	0.42	0.30	0.24	0.28
Prometryne	0.02	0.04	0.03	<0.05
Terbufos	–	0.02	ND	ND
Carbofuran	ND	1.92	ND	ND
Sample B[b]				
Atrazine	ND	0.02	0.02	0.02
Terbufos	–	0.05	ND	ND
Prometryne	0.30	0.27	0.27	0.30
a-BHC	–	0.05	–	0.15
Lindane	–	0.03	–	0.12

[a] Residue data are expressed in μg/l and these are not corrected for recovery. The recovery data for the HPLC–DAD system are shown in Table 3; the recoveries of these analytes when samples were subjected to off-line SPE followed by GC analysis ranged from 80 to 90%.
[b] Samples A and B are ground and surface water samples, respectively, taken from the Axios River basin.

as accurate as other chromatographic methods of analysis.

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