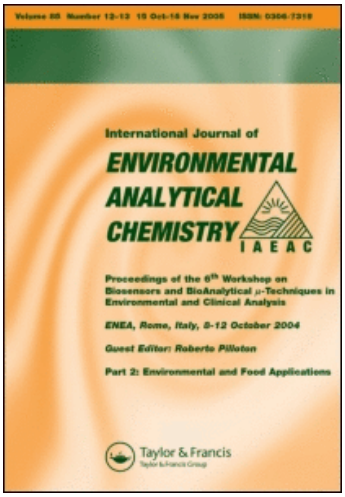

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Rapid Screening of a Large Group of Polar Pesticides in River Water by On-Line Trace Enrichment and Column Liquid Chromatography

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To cite this Article Liska, I. , Brouwer, E. R. , Ostheimer, A. G. L. , Lingeman, H. , Brinkman, U. A. Th. , Geerdink, R. B. and Mulder, W. H.(1992) 'Rapid Screening of a Large Group of Polar Pesticides in River Water by On-Line Trace Enrichment and Column Liquid Chromatography', International Journal of Environmental Analytical Chemistry, 47: 4, 267 — 291

To link to this Article: DOI: 10.1080/03067319208027036

URL: http://dx.doi.org/10.1080/03067319208027036

Intern. J. Environ. Anal. Chem., Vol. 47, pp. 267–291
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RAPID SCREENING OF A LARGE GROUP OF POLAR PESTICIDES IN RIVER WATER BY ON-LINE TRACE ENRICHMENT AND COLUMN LIQUID CHROMATOGRAPHY

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(Received 17 October 1991)

Column liquid chromatography on a reversed-phase (C-18) analytical column using a linear acetonitrile-water gradient and diode-array detection is used in an on-line early-warning system of over 50 pesticides in surface water. It allows the separation of most compounds with detection limits of about 1–5 μg/l after preconcentration of 30 ml of sample. Problems encountered for co-eluting pesticides with similar spectra and pesticides with weak ultraviolet absorption over 230 nm, are discussed. If the system is combined with on-line trace enrichment on a styrene-divinylbenzene copolymer (PLRP-S) precolumn, the overall resolution is only slightly affected, and the qualitative information obtained remains about the same. Chromatograms of spiked (2–10 μg/l) surface (river Rhine) water containing 54 test compounds are shown. Preliminary studies show the stability of several pesticides in water to be rather low. Some degradation products have been detected in 1-month-old methanolic and aqueous solutions.

KEY WORDS: Polar pesticides, on-line preconcentration, column liquid chromatography, diode-array detection, surface water analysis

INTRODUCTION

The increased production and use of pesticides in agriculture has a negative impact on the quality of surface water in the Netherlands. Contamination with those pesticides can take place via the air, surface run-off, by indirect contamination from droplet drift after spraying operation or by leaching and accidental spills. The river Rhine is one of the major tap water sources and it is therefore important to generate information on the water quality of this river both rapidly and continuously. To achieve this goal, an automated early-warning system should be developed which

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[2] To whom correspondence should be sent.

provides qualitative as well as quantitative information for a large group of pollutants.

Early-warning methods for the detection and/or identification of non-polar and volatile pollutants are already in use at several monitoring stations, but as yet no multi-method exists for polar pesticides[1]. It is therefore our aim to design an on-line system suitable for the rapid analysis of this type of compounds which should include trace enrichment and clean-up as well as separation and detection. Trace enrichment of the pesticides is needed since the sensitivity of diode-array detection (DAD) is normally not sufficient to determine real-life concentrations of polar pesticides in surface water. The maximum allowed concentration for pesticides in tap water is 0.1 μg/l[2,3]. In surface water these limits are for the majority of the polar pollutants ca. 2–5 μg/l, because in this case toxicological or ecological standards are used. In case of calamities levels of 3 μg/l are used. In principle both capillary gas chromatography (GC) and column liquid chromatography (LC) can be used as separation technique. GC has the advantage of excellent separation power and easy coupling with mass spectrometry. However, when the determination of polar, ionogenic (weakly acidic and basic compounds) and/or thermolabile pesticides is the main goal, GC loses most of its advantages. Many problems can, admittedly, be solved by introducing derivatisation procedures. This will, however, seriously complicate the total analysis and make an on-line set-up essentially impossible.

The use of LC on alkyl-bonded silica stationary phases for environmental screening purposes has become popular in recent years, viz. since the introduction of sensitive DAD. Today, combining gradient-elution reversed-phase LC (RP-LC) with sophisticated DAD allows one to monitor and provisionally identify a rather large number of solutes in one run, without high polarity and/or thermolability playing an adverse role[4–9]. Additionally, LC can easily be combined with on-line precolumn techniques[5,8,10–20]. The latter techniques are, in particular, suitable for the effective clean-up of surface water samples because they can be used for simultaneous concentration and clean-up of a large variety of compounds, widely differing in polarity or acidic/basic properties[11]. Moreover, these techniques can be used to retain, at least partially, macromolecules (i.e. humic substances) which can clog the analytical column[21].

For the present research project, more than 50 pesticides have been selected which are of distinct environmental concern, cover a wide range of polarities, and occasionally have an acidic or basic character (for structures, see Figure 1). Often, two or three of the analytes selected are useful representatives of a whole class of pesticides. In this communication, attention will be focused on the potential of isocratic and gradient-elution RP-LC-DAD for the separation and detection of the selected pesticides. Aspects such as identification problems encountered with co-eluting peaks, instability of various pesticides in aqueous solution, and preliminary data on on-line trace enrichment are discussed.

The experimental results are used to draw general conclusions about the viability of the on-line precolumn-LC-DAD approach for the rapid monitoring of pesticide levels in river Rhine water in excess of the threshold values.

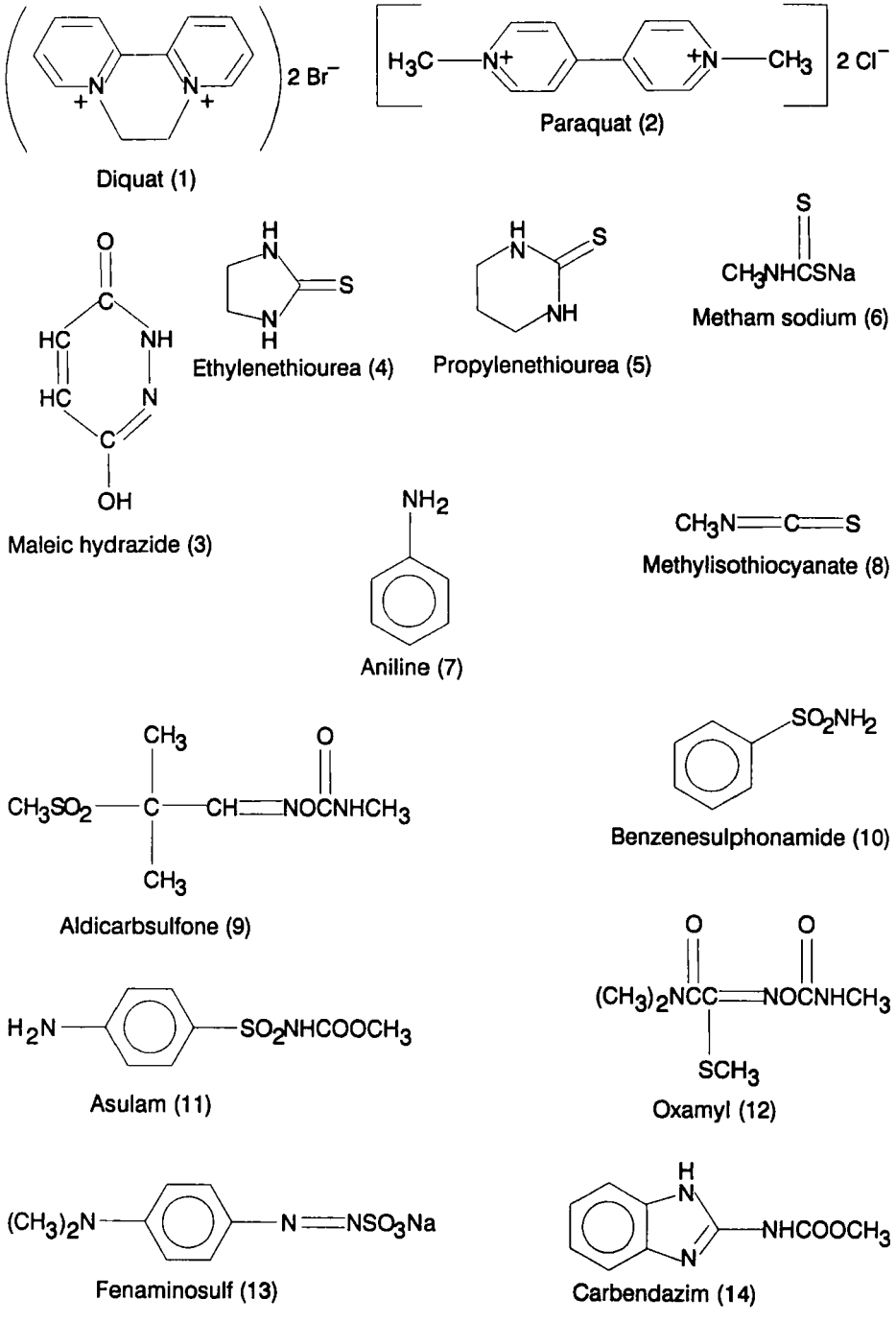
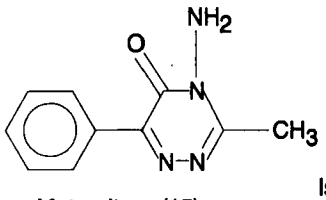


Figure 1 Structures of polar pesticides used as test compounds.



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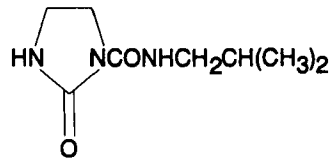
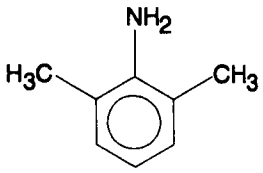
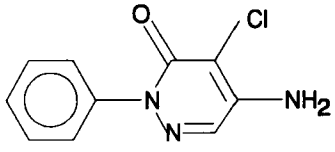


Isocarbamide (16)



2,6-Dimethylaniline (17)



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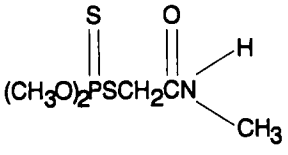
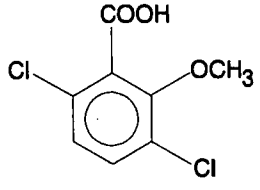
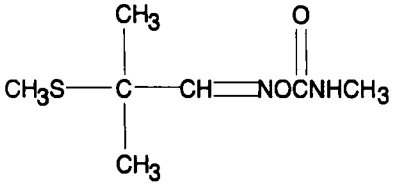


Dimethoate (19)



Dicamba (20)



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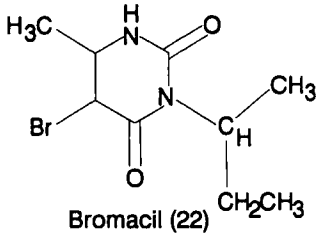

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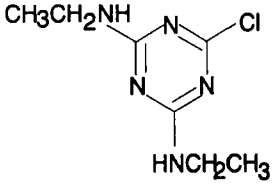

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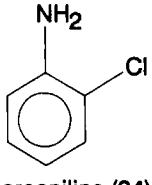
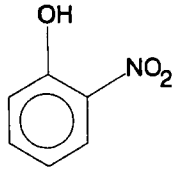
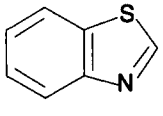


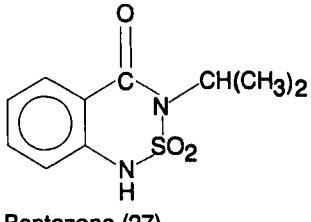
2-Chloroaniline (24)



2-Nitrophenol (25)



Benzothiazole (26)



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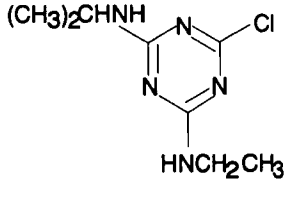
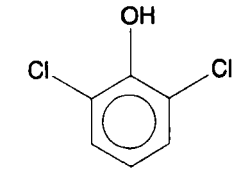
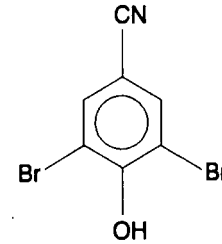


Atrazine (28)

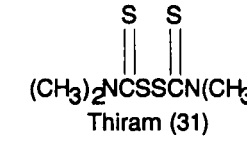
Figure 1 (*continued*)



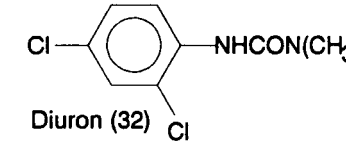
2,6-Dichlorophenol (29)



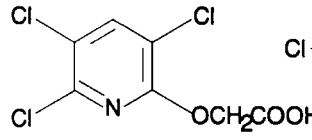
Bromoxynil (30)



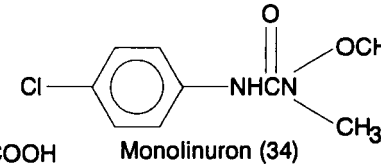
Thiram (31)



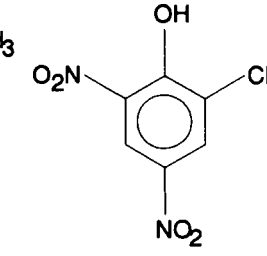
Diuron (32)



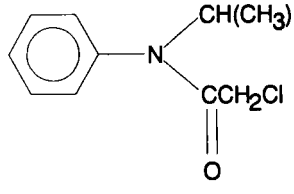
Triclopyr (33)



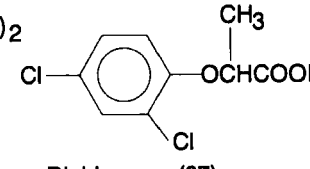
Monolinuron (34)



4,6-Dinitro-ortho-cresol (35)



Propachlor (36)



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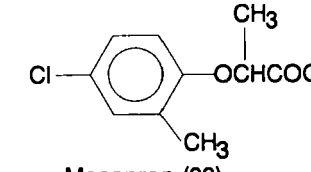
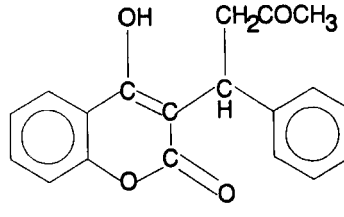
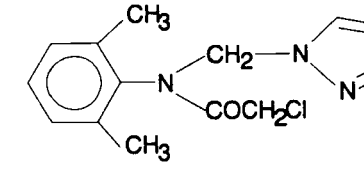


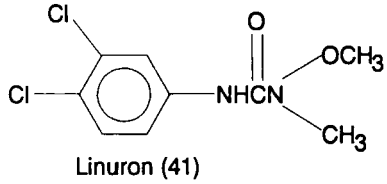
Mecoprop (38)



Warfarin (39)



Metazachlor (40)



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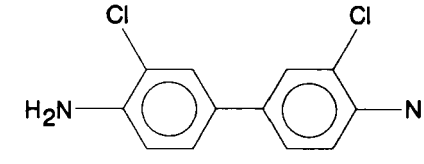
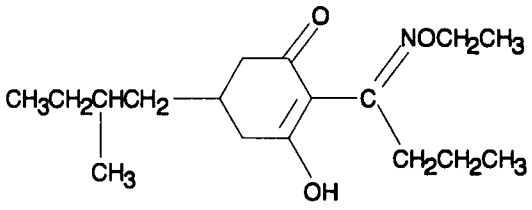
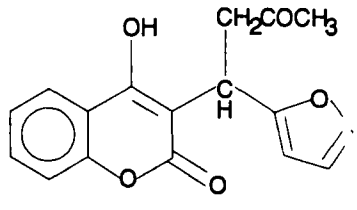


3,3-Dichlorobenzidine (42)

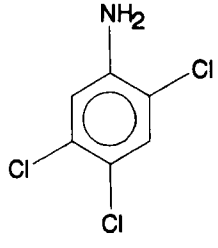
Figure 1 *(continued)*



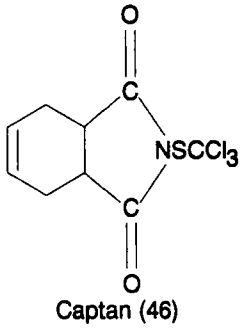
Sethoxydim (43)



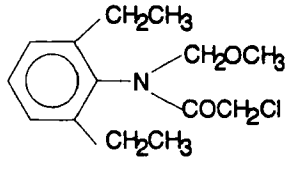
Coumafuryl (44)



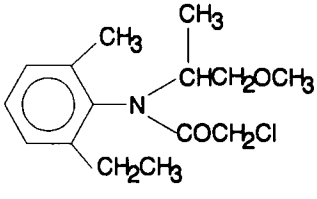
2,4,5-Trichloroaniline (45)



Captan (46)



Alachlor (47)



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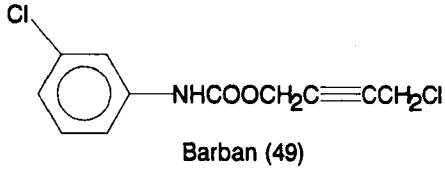
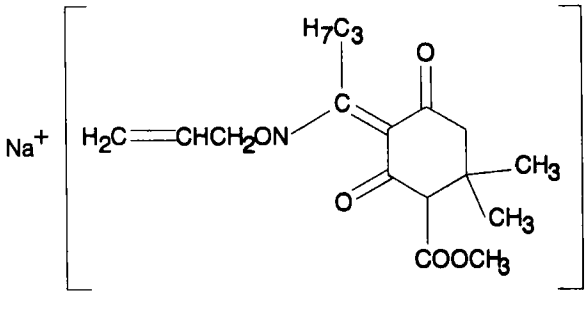
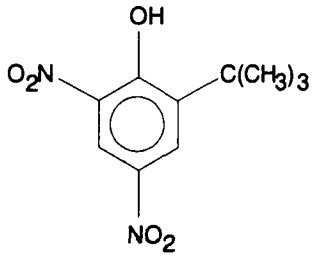


Barban (49)

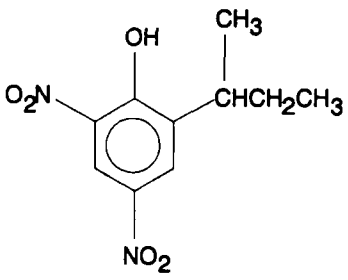


Alloxydim sodium (50)

Figure 1 (*continued*)



Dinoterb (51)



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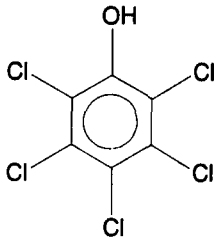
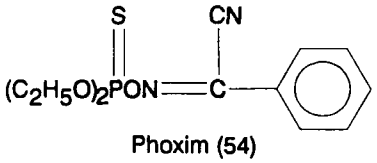
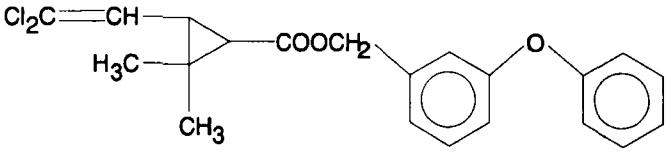


Pentachlorophenol (53)



Phoxim (54)



Permethrin (55)

Figure 1 (*continued*)

MATERIALS AND METHODS

Instrumentation and procedures

The chromatographic analyses were performed with two Applied Biosystems (Ramsey, NJ, USA) Model 400 solvent delivery systems, a Valco (Houston, TX, USA) six-port injection valve equipped with a 30 μl loop, for direct injections, and an Applied Biosystems 1000 S DA detector. The DA detector was set at 230 nm, with a bandwidth of 50 nm. The spectra were recorded in the range of 190–370 nm.

The stainless-steel analytical columns were slurry packed in the upward direction using acetone as the slurry liquid and washed with acetonitrile–water (70/30, v/v). The isocratic runs and some of the gradient runs were performed on a 10 cm × 4.6 mm I.D. column, packed with 5 μm RoSil C-18 (RSL, Eke, Belgium). The gradient runs were performed on a 15 cm × 4.6 mm I.D. column packed with 5 μm LiChrosorb RP-18 (Chrompack, Middelburg, The Netherlands).

The flow rate used during isocratic elution was 0.5 ml/min, while the flow rate during gradient elution was 0.5 ml/min on the 10 cm C-18 column and 1.0 ml/min on the 15 cm RP-18 column. All separations were performed at ambient temperature.

Gradient elution was performed with acetonitrile–0.01 M sodium phosphate buffer (pH 3) (90/10, v/v) as solvent A and acetonitrile–0.01 M sodium phosphate buffer (pH 3) (5/95, v/v) as solvent B. The gradient profile was as follows: initial conditions, 100% B, then linearly to 100% A in 55 min and subsequently back to 100% B in 5 min. Before starting the first gradient, the system was equilibrated during 15 min with solvent B. Isocratic elutions were performed with mixtures of acetonitrile and 0.01 M sodium phosphate buffer (pH 3). All mobile phase solvents were deaerated by ultrasonication during 15 min.

The stainless-steel precolumns (10 mm × 2.0 mm I.D) were slurry packed manually using acetonitrile as the slurry liquid. They contained polymer PLRP-S (15–25 μm) from Polymer Laboratories (Church Stretton, UK) as the sorbent. For trace enrichment on a precolumn a Kontron (Zürich, Switzerland) Model 410 LC pump was used. During breakthrough experiments, fractions of the precolumn were collected by a FRAC-100 fraction collector (Pharmacia, Uppsala, Sweden).

The breakthrough experiments were performed at a flow rate of 2.0 ml/min. During these experiments 2- or 5-ml fractions were collected. A 30-μl aliquot of every fraction was analysed using the described gradient system.

In the trace-enrichment experiments 30 ml of a water sample containing the same total amount of each pesticide as in 30-μl loop injections (i.e. a 1000-fold lower concentration were passed through the precolumn at a flow rate of 2 ml/min.

The on-line set-up for the total analytical scheme is shown in Figure 2.

Materials

Chromatography-grade acetonitrile, HPLC-grade methanol, sodium dihydrogen phosphate, 85% phosphoric acid, and 65% nitric acid were from J. T. Baker

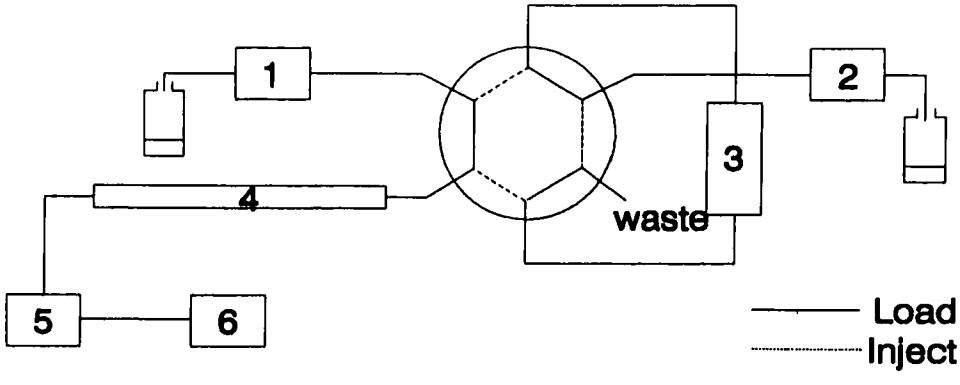


Figure 2 Set-up for on-line preconcentration of water samples. 1 and 2, pumps; 3, precolumn; 4, analytical column; 5, detector; 6, recorder.

(Deventer, The Netherlands). HPLC-grade water was purified in a Milli-Q (Millipore, Bedford, MA, USA) filtration system and was used throughout this study. The various test compounds were at least 95% pure and were a gift of the RIZA (Lelystad, The Netherlands). Rhine water samples were collected at Lobith (river Rhine) and were obtained from the RIZA. The samples were filtered prior to use over a 0.45 μm BA membrane (Schleicher and Schuell, Dassel, Germany).

Phosphate buffers were prepared by adjusting a 1 M solution of sodium dihydrogen phosphate to the required pH with concentrated phosphoric acid followed by 100-fold dilution.

Stock solutions of 500–1000 μl of the test compounds were prepared in methanol. The actual working solutions, used in the stability tests, were obtained by diluting the stock solutions with HPLC-grade water. In all experiments with non-adjusted (non-buffered) water samples, the pH was measured immediately before processing of the samples. In most cases this pH was ca. 6. In order to study the influence of the pH, water samples were acidified with 2 M nitric acid to pH 3.

The decomposition of the pesticides in HPLC-grade water was monitored during 1 month. Similar experiments were carried out with the pesticides dissolved in pure methanol. All solutions used for the stability testing were stored in the dark at ambient temperature during the testing period.

RESULTS AND DISCUSSION

The most important prerequisites for an analytical system that must be used for early-warning purposes of polar pollutants in surface water are: automatability, simplicity, reliability, and robustness. Therefore, first of all the potential of isocratic and gradient elution was compared. Secondly, the potential of DAD was investigated and compared with single-wavelength detection. Thirdly, the preconcentration of the test compounds was optimised, and the overall performance of the system evaluated.

All chromatographic and trace-enrichment experiments were performed at pH 3, unless mentioned otherwise, because this pH seemed to be the optimum pH for the simultaneous separation and trace enrichment of acidic, basic, and neutral compounds, even although the detectability was better at pH values close to neutral[11]. Acetonitrile was chosen as organic modifier because of a somewhat better transparency, compared with methanol, at wavelengths below 240 nm.

Isocratic elution

A number of experiments was performed to investigate if isocratic elution could be used for the separation of the test compounds. Some of the most polar pesticides (e.g., diquat, paraquat, aniline, fenaminosulf) were not included in this study. Screening the usefulness of several acetonitrile–0.01 M phosphate (pH 3) eluents rapidly revealed that a limited number of such eluents—viz., those containing 10, 20, 40, and 60% (v/v) of acetonitrile—suffices to separate a large majority of the 50 pesticides under investigation. The pertinent results of these runs are given in Table 1. In other

Table 1 Retention times of test compounds after isocratic elution*

Compound	t_r (*min*)	*Compound*	t_r (*min*)
Eluent, acetonitrile–0.01 M phosphate (pH 3) (10/90, v/v)			
Etyhylenethiourea	1.4	Methylisothiocyanate	5.5
Maleic hydrazide	1.5	Oxamyl	5.7
Metham sodium	1.6	Linuron	>60
Propylenethiourea	1.7	Metolachlor	>60
Aldicarbsulfone	4.7		
Eluent, acetonitrile–0.01 M phosphate (pH 3) (20/80, v/v)			
Asulam	1.3	Bromacil	12.4
Metamitron	3.1	Benzothiazole	15.1
Chloridazon	4.5	2-Chloroaniline	17.1
Dicamba	6.5	Bentazon	23.2
2,6-Dimethylaniline	10.8	Dinoterb	>60
Eluent, acetonitrile–0.01 M phosphate (pH 3) (40/60, v/v)			
Benzenesulfonamide	1.5	Metazachlor	6.5
Simazine	3.0	4,6-Dinitro-ortho-cresol	7.1
Carbendazim	4.0	Mecoprop	7.3
2-Nitrophenol	4.3	Warfarin	8.8
Atrazine	4.6	Linuron	10.2
Monolinuron	5.1	3,3-Dichlorobenzidine	11.5
Thiram	5.1	Sethoxydim	16.7
2,6-Dichlorophenol	5.4	2,4,5-Trichloroaniline	18.2
Bromoxynil	5.4	Metolachlor	18.2
Diuron	5.6	Alachlor	22.0
Coumafuryl	5.7	Dinoseb	41.0
Propachlor	6.4	Permethrin	>60
Eluent, acetonitrile–0.01 M phosphate (pH 3) (60/40, v/v)			
Isocarbamide	1.2	Dinoseb	5.2
Dimethoate	1.2	Dinoterb	5.8
Asulam	1.2	Pentachlorophenol	6.2
Metamitron	1.3	Phoxim	7.1
Captan	2.9	Permethrin	38.7

* Analytical column, 10 cm × 4.6 mm I.D., 5 μm RoSil C-18; flow rate, 0.5 ml/min; injection volume, 30 μl; detection wavelength. 230 nm; concentration, 1–10 mg/l; t_0, 1.2 min.

words, although isocratic elution cannot be used to separate compounds of so widely different polarity as dealt with in the present study in one or two runs, it certainly is a valuable tool if compounds encompassing a limited range of polarity have to be determined.

Gradient elution

First of all 16 test compounds—which covered the whole range of retention times listed in Table 1—were analysed with the same C-18 column as used in the isocratic study. The potential of gradient elution immediately becomes obvious from the data in Table 2. All test compounds were eluted, and separated, within 45 min using a

Table 2 Retention times of test compounds after gradient elution*

Compound	t_r *(min)*	*Compound*	t_r *(min)*
Maleic hydrazine	1.4	Simazine	14.2
Propylenethiourea	1.6	Atrazine	16.7
Oxamyl	2.9	Coumafuryl	17.3
Benzenesulfonamide	5.0	Diuron	18.5
Chloridazon	6.5	Sethoxydim	23.8
Dicamba	7.9	Metolachlor	27.6
Benzothiazole	13.2	Dinoseb	30.5
Bentazon	13.5	Permethrin	42.1

* Analytical column, 10 cm × 4.6 mm I.D., 5 μm RoSil C-18; flow rate, 0.5 ml/min; gradient system, solvent A-acetonitrile–0.1 M phosphate (pH 3) (95/5, v/v), solvent B-acetonitrile–0.01 M phosphate (pH 3) (5/95, v/v), initial conditions: A = 10% and B = 90%; gradient in 40 min to 80% A and 20% B; for other conditions, see Table 1.

linear acetonitrile gradient. To increase the chromatographic efficiency, the experiment was repeated with a 15 cm × 4.6 mm I.D. C-18 analytical column, and all 55 test compounds. The retention times of the best gradient profile found are given in Table 3. The retention times in Table 3 were only measured in HPLC-grade water. Fenaminosulf was not included in this run and further trace enrichment and chromatographic studies because of stability problems. The repeatability of the retention times was 1–2%; preparation of the mobile phase and equilibration of the system were found to be the two primary parameters determining the precision of the system.

In addition to the compounds listed in Table 3, 11 other compounds (benazolin, dalapon, difenzoquat, chlomequat, endothal, sodium, thiofanox, ethepon, glufosinate ammonium, trichlorfon, glyphosate, dikegulac sodium) were tested. They could not be detected although concentrations of 10–50 mg/l were injected. Limited absorptivity at the chosen detection wavelength of 230 nm is the main reason for this failure.

Detection after gradient elution

From the retention data in Table 3 it will be clear that not all test compounds can be separated in the present gradient-elution RP-LC system. For a number of co-eluting compounds with absorbance maxima between 260 and 330 nm, identity confirmation is possible by recording the absorption spectra, i.e. by using RP-LC-DAD. However, for compounds with no absorbance maximum over 230 nm the potential of spectral resolution is limited. For the sake of convenience, relevant absorption maxima are included in Table 3 (complete spectra are available from the authors upon request). Examples of the possibility of spectral resolution of co-eluting compounds are given in Figure 3, viz. for atrazine and 2,6-dichlorophenol, and sethoxydim and coumafuryl.

The identity of some 35 test compounds, at the 1 mg/l level, could be confirmed by RP-LC-DAD. Exceptions were compounds with low absorptivity over 230 nm (e.g., propachlor, alachlor, metolachlor, metazachlor, captan, aldicarbsulfone, di-

Table 3 Chromatographic and absorbance data of test compounds after preconcentration and/or gradient elution

Compound	t_r *(min)*	λ_{max} *(nm)*	V_B *(ml) in*		*Detection limit (μg/l) in*	
			HPLC water	*Surface water*	*HPLC water*	*Surface water*
1 Diquat	2.0	310	<1			
2 Paraquat	2.6	259	<1			
3 Maleic hydrazide	2.8	207, 303	<1			
4 Ethylenethiourea	2.8	231	<1			
5 Propylenethiourea	2.9	233	<1	<1		
6 Metham sodium	4.1	208, 232	<1	1		
7 Aniline	5.0	<200	<1			
8 Methylisothiocyanate	11.0	242	1		2	10
9 Aldicarbsulphone	11.0	<200	1	1	2	10
10 Benzenesulphonamide	11.1	218, 264	1	1	2	10
11 Asulam	11.3	268	5			
12 Oxamyl	11.4	216	1	1		
13 Fenaminosulf	11.5	262				
14 Carbendazim	14.4	223, 280	<1	1	0.5	5+
15 Metamitron	15.9	309	15	5	0.1	3
16 Isocarbamide	16.5	211	15		0.2	2
17 2,6-Dimethylaniline	16.5	271	5			5
18 Chloridazon	16.8	229, 284	10		0.05	2+
19 Dimethoate	17.2	<200	20		0.4	5+
20 Dicamba	17.5	277	25	5	0.3	5+
21 Aldicarb	20.8	247	30	20	0.4	20
22 Bromacil	21.7	212, 278	50	30	0.1	1
23 Simazine	22.8	244, 263	50		0.05	1
24 2-Chloroaniline	23.2	231, 288	20	20	0.5	0.5
25 2-Nitrophenol	23.8	210, 276, 349	45		2	2+
26 Benzothiazole	23.8	253, 285	35		1	0.5
27 Bentazon	24.7	219, 316	45	35	1	1
28 Atrazine	27.0	222, 263	85	70	0.1	0.5
29 2,6-Dichlorophenol	27.2	280	>100		0.2	1
30 Bromoxynil	27.8	220, 255, 285	65		0.5	1.5
31 Thiram	27.8	215	90		0.5	1.5
32 Diuron	28.2	252	60	70	0.2	0.3
33 Triclopyr	28.6	231, 294	50		0.2	0.3
34 Monolinuron	24.2	247	75		0.2	0.3
35 4,6-Dinitro-ortho-cresol	29.6	210, 267	80		0.3	0.3
36 Propachlor	29.8	<200	65		0.5	1
37 Dichlorprop	29.9	228, 285	60		0.2	0.5
38 Mecoprop	30.1	228, 280	>100	>100	0.4	0.5
39 Warfarin	32.2	281, 305	>100	>100	0.2	0.2
40 Metazachlor	32.9	<200	65			
41 Linuron	33.3	210, 250	>100		0.2	0.2
42 3,3-Dichlorobenzidine	34.1	213, 286	>100		0.2	
43 Sethoxydim	35.4	240	>100	>100	0.5	0.5
44 Coumafuryl	36.5	208, 279, 305	>100			
45 2,4,5-Trichloroaniline	36.2	214, 248, 309	>100		0.2	0.2
46 Captan	36.4	240				
47 Alachlor	36.4	<200	>100		1	1
48 Metolachlor	36.4	<200	>100		0.5	0.5
49 Barban	36.6	205, 237	>100		0.2	0.2
50 Alloxydim sodium	38.8	261	>100		1	1

Table 3 *(continued)*

Compound	t_r *(min)*	λ_{max} *(nm)*	V_B *(ml) in*		*Detection limit (μg/l) in*	
			HPLC water	*Surface water*	*HPLC water*	*Surface water*
51 Dinoterb	39.3	213, 269	>100	>100	0.3	0.3
52 Dinoseb	40.1	211, 269	>100		0.3	0.3
53 Pentachlorophenol	40.5	214, 302	>100	>100	0.3	0.3
54 Phoxim	42.1	221, 284	>100		0.5	1
55 Permethrin	52.5	271	>100			

* Retention times (t_r) measured using only the 15-cm LiChrosorb RP-18 analytical column (t_0 = 1.8 min). Absorbance spectra recorded between 190 and 370 nm. Breakthrough volume in HPLC-grade or surface water at pH 3 (V_B); detection limits after trace enrichment of 30-ml of HPLC-grade or surface water at pH 3 on the PLRP-S precolumn. All further LC and detection conditions are given in "Materials and Methods".

† Detection limit at 215–285 nm: 1–2 μg/l.

methoate) and co-eluting compounds with similar structure and spectra (e.g. dichlorprop, mecoprop).

Depending on the shape of the spectrum and the retention time of the compound, and the background absorbance, the detection limit of most of the test compounds was 0.5–1.0 mg/l using 30-μl loop injections, that is, without trace enrichment of the sample.

Trace enrichment

As mentioned before, considerable trace enrichment is necessary to obtain detection limits of less than 5 μg/l for the test solutes in surface water. A gain of about three orders in sensitivity should be obtainable by trace enrichment of at least 30 ml of sample. Earlier studies on the applicability of hydrophobic sorbents for trace enrichment of polar compounds from water showed that a styrene-divinylbenzene copolymer (i.e. PLRP-S) precolumn in combination with a C-18 analytical column is a good choice. This conclusion was drawn considering the breakthrough volumes of the analytes and the additional band broadening as a result of on-line switching of these two columns which, in essence, are not fully compatible[1].

To evaluate the potential of trace enrichment on PLRP-S the breakthrough volumes of all test compounds were measured (Table 3) using the method of direct analysis of precolumn effluent fractions. This method has the advantage over the classical approach—which uses on-line measurement of the absorption of the precolumn effluent—that several compounds can be tested simultaneously. A disadvantage of the procedure is that the total concentration of the analytes is relatively high to allow DAD. Although this is far from real-life concentrations in surface water, the breakthrough volumes found in this way can be considered as relatively "safe" values because it has repeatedly been observed that the use of low (trace-level) concentrations causes unexpectedly large increases of the breakthrough volumes. For

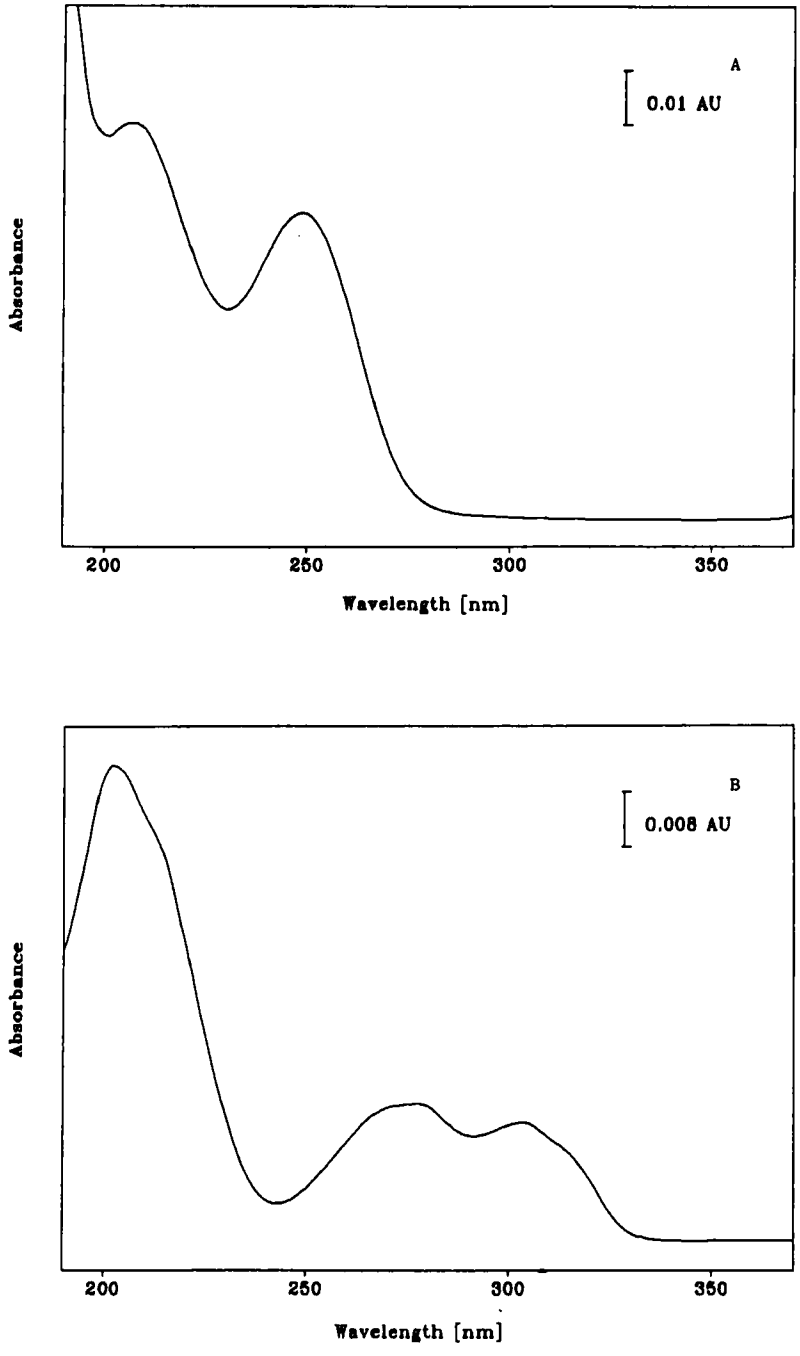
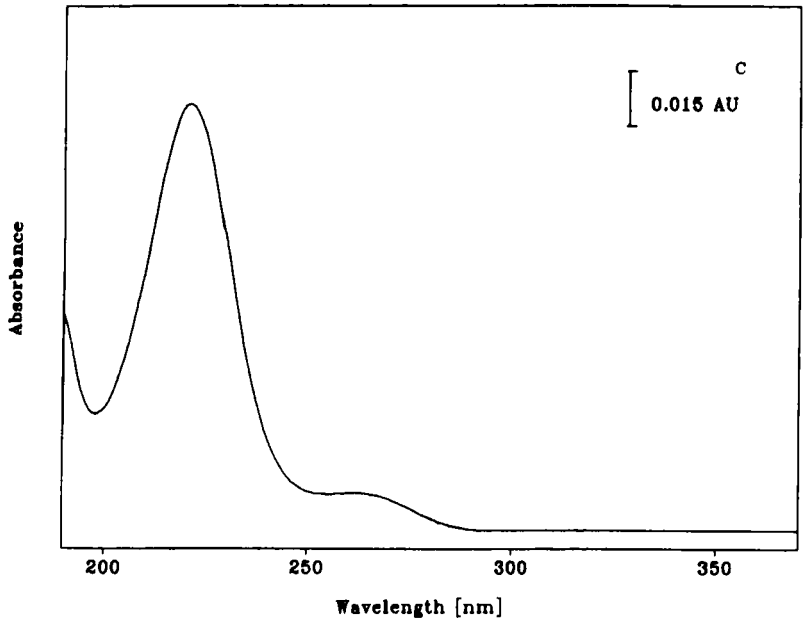


Figure 3 Resolution of co-eluting peaks using DAD. The spectra are taken at the top of the peak. A, sethoxydim; B, coumafuryl; C, atrazine; D, 2,6-dichlorophenol. The concentrations are the same as in Table 3 and the RP-LC conditions are given in "Materials and Methods".



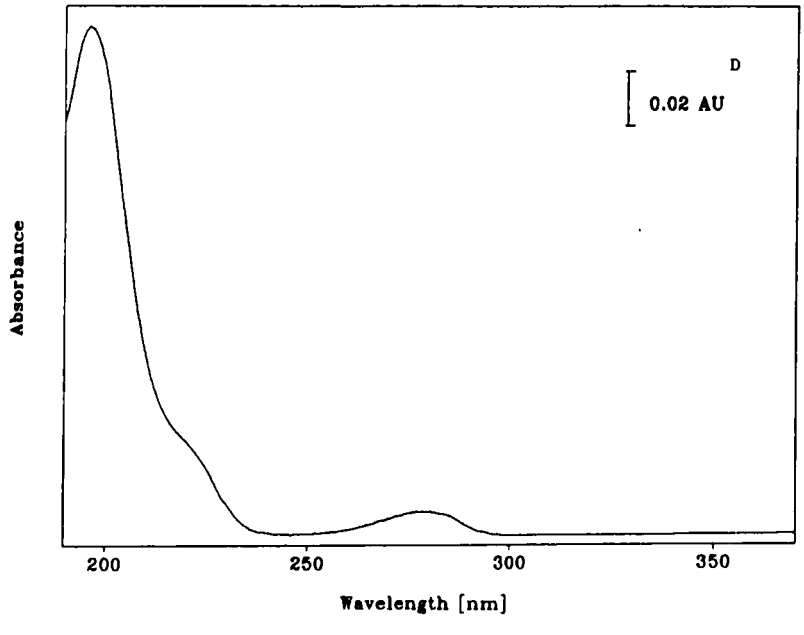


Figure 3 (*continued*)

the rest, the individual concentration of the analytes was 1–10 mg/l and 5–10 compounds were measured simultaneously; that is, the total concentration of organic compounds was 30–50 mg/l, which is near the range of the total organic load of several surface waters.

Since most of the test compounds possess acidic or basic properties, suppression of the ionisation can have a positive effect on the breakthrough volumes. Therefore, the trace-enrichment experiments were not only performed at pH 3—the pH chosen for RP-LC-DAD—but also at pH 6. Comparing the results for all pesticides showed that for only 10 compounds a significant difference exists between the breakthrough volumes at these two pH values (Table 4). For seven compounds the breakthrough volumes were higher at pH 3 and for the three bases (i.e., aniline, carbendazim, 2,6-dimethylaniline) the opposite was true. Although the differences in breakthrough volumes observed for the acidic compounds can only be partly explained on the basis of their pK_a values, it will be clear that for both separation and trace enrichment pH 3 is in many cases preferred over pH 6.

According to their breakthrough volumes at pH 3 the 54 test compounds can be divided into three groups. The first group (diquat to carbendazim and 2,6-dimethylaniline; cf. Table 3) contains compounds with breakthrough volumes of less than 10 ml, which is too low to obtain detection limits of 5 μg/l or less. The second group (metamitron to aldicarb and 2-chloroaniline) consists of moderately retained compounds possessing breakthrough volumes of 10–30 ml, which will, as a rule, be sufficient to obtain the required sensitivity. The other compounds (third group) all have breakthrough volumes of over 30 ml, which means that these compounds can be enriched on PLRP-S with high efficiency.

During the breakthrough experiments a problem was observed for coumafuryl which was due to its limited stability. In this case the breakthrough volumes do not relate to the parent compound, but to one of its degradation products. The

Table 4 Breakthrough volumes of test compounds on PLRP-S after trace enrichment of HPLC-grade water of pH 3 and 6*

Compound	*Breakthrough volume (ml) at*	
	pH 3	*pH 6*
Aniline	<1	5
Carbendazim	<1	25
2,6-Dimethylaniline	5	35
Dicamba	25	<1
Bentazon	45	5
2,6-Dichlorophenol	>100	70
Bromoxynil	65	20
Triclopyr	50	10
Dichlorprop	60	15
Mecoprop	>100	<1
Coumafuryl	>100	1

* Trace-enrichment conditions are given in "Materials and Methods".

absorbance spectrum of the main degradation product was closely similar to that of coumafuryl itself.

To check the influence of surface water on the trace enrichment, 19 selected test compounds were enriched from acidified (pH 3) surface water (Table 3), using the same concentration of the analytes as in the earlier breakthrough experiments. Although the carbon load is much higher in surface water than in HPLC-grade water, the decrease in breakthrough volumes is rather small, or even absent, for almost all analytes. More importantly, the slightly higher values for trace enrichment from acidified HPLC-grade water, as compared with surface water samples, were not observed when non-buffered water samples were enriched (data not shown); in that case, essentially the same breakthrough volumes were found for both types of water samples.

On the basis of the above data, 30-ml samples adjusted to pH 3 as well as non-buffered (pH ca. 6) samples were used in the subsequent trace-enrichment experiments.

Performance of the total analytical system

To determine the detection limits in the total analytical system, 30-ml samples were preconcentrated on a PLRP-S precolumn and, after on-line desorption and transfer to the analytical column, subsequently analysed on the 15-cm LiChrosorb RP-18 column. Using concentrations of 2.5–5 μg/l, the detection limits were determined for all test compounds in HPLC-grade and surface water and pH 3 and 6 (signal-to-noise—peak-to-peak—ratio, 2/1). The data in Table 3 and Figures 4 and 5 show

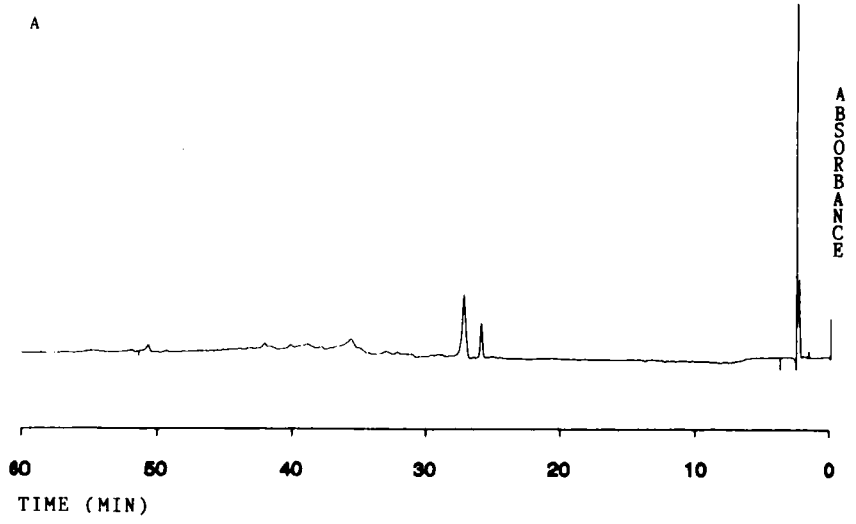


Figure 4 Chromatograms of (A) blank HPLC-grade water (non-buffered), (B) HPLC-grade water spiked with the test compounds at concentrations of 2.5–5 μg/l, and (C) at a 25 times lower concentration; 30 ml of sample were enriched. Detector scale, (A) 0.1 AUFS and (B) 0.01 AUFS. For peak designation, see Table 3; for further details, see text.

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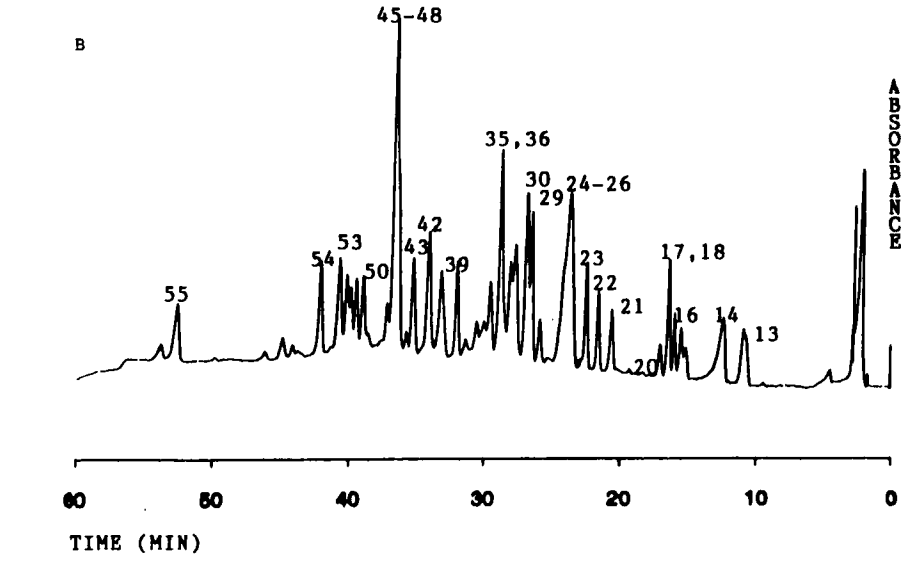

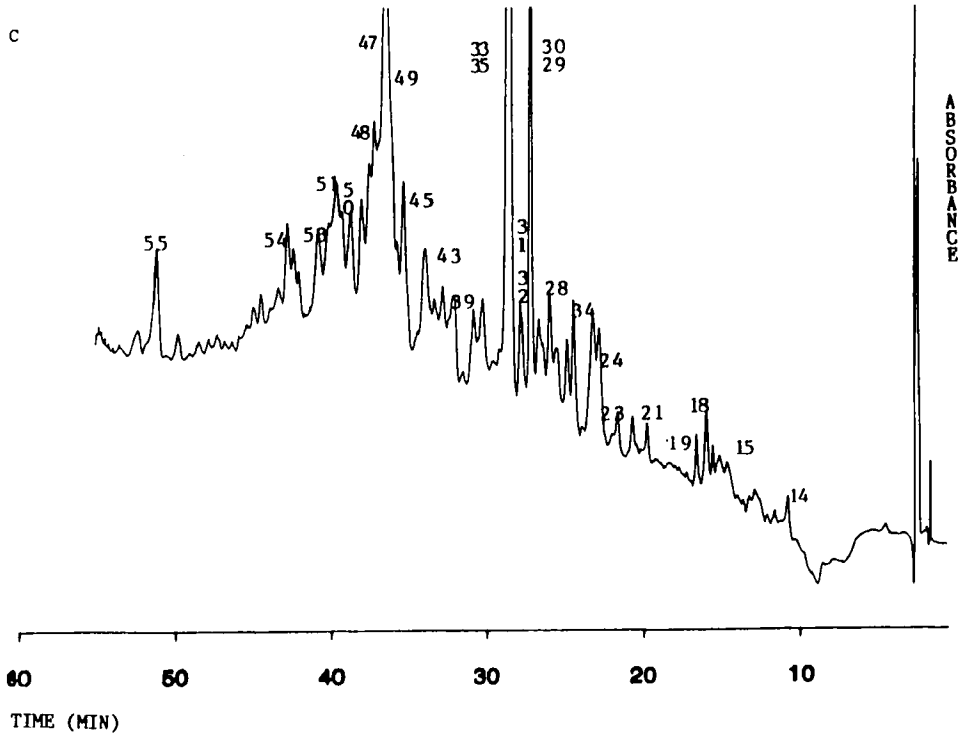

Figure 4 (*continued*)

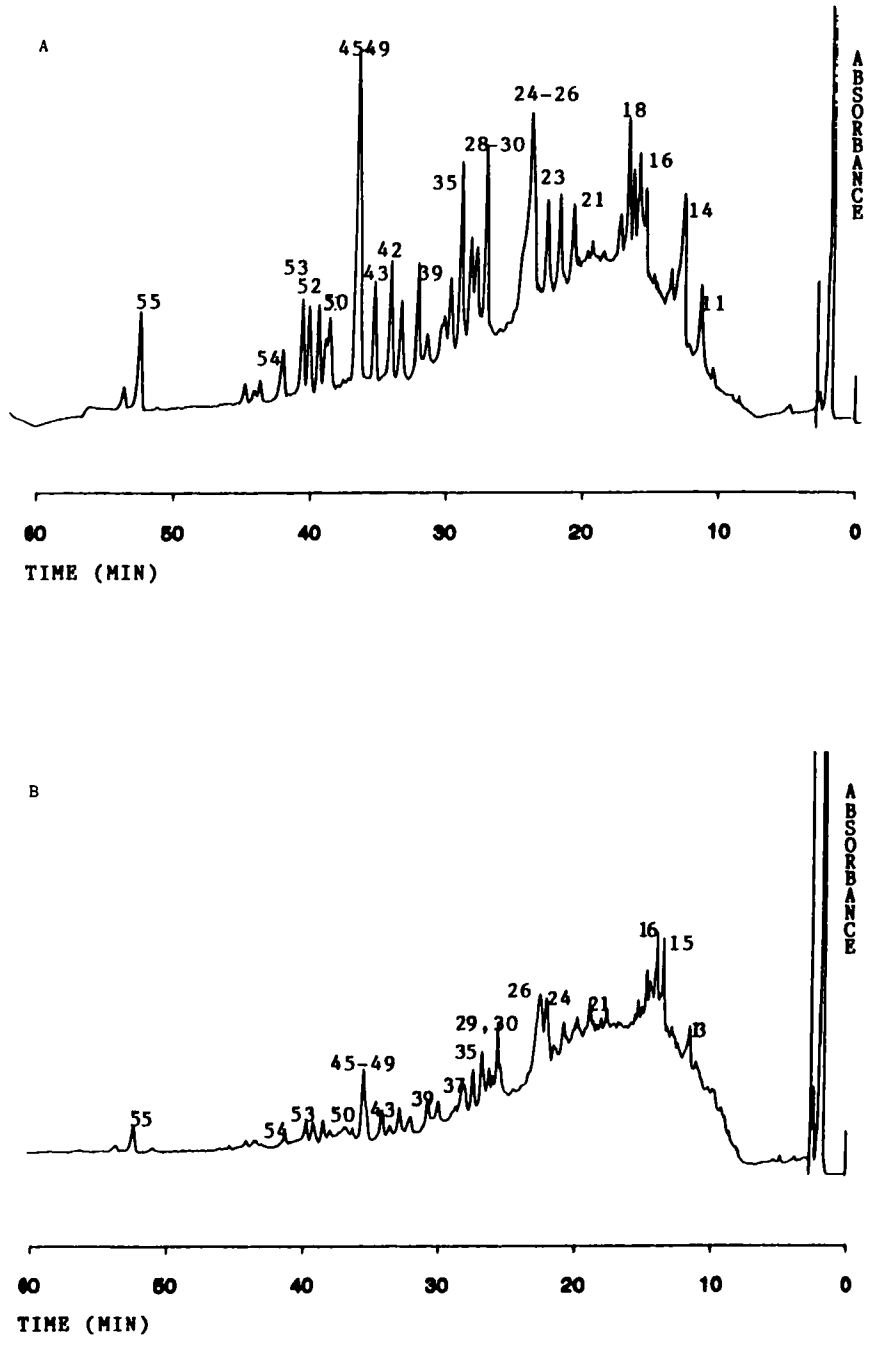

Figure 5 Chromatograms of non-buffered river Rhine water spiked with the test compounds at concentrations of (A) 2.5–5 μg/l and (B) at a 5-fold lower concentration. Detector scale, 0.1 AUFS. For peak designation, see Table 3; for further details, see text.

that for most of the compounds detection limits are at the low μg/l level in HPLC-grade as well as surface water; distinctly higher detection limits in the latter type of water were observed for several early eluting compounds (also see below). Only for compounds with low absorptivity at the detection wavelength (230 nm) and for those with breakthrough volumes lower than 1 ml no signal was obtained. If single-wavelength (230 nm in this study) is preferred, as will today certainly be the case with many only moderately sophisticatedly equipped laboratories, 36 of the test compounds have detection limits of 1 μg/l or less. The pertinent data are included in Table 3. It is interesting to note that even several components with breakthrough volumes of less than 10 ml in our high-concentration studies (e.g., methylisothiocyanate, aldicarbsulfone, benzenesulfonamide) now showed appreciable trace enrichment and could be detected at the 2 μg/l level in HPLC-grade water. Furthermore, it was observed that generally speaking, detection limits in non-buffered (pH 6) surface water were in many cases somewhat better compared with pH 3.

As regards the potential of LC-DAD, reference whould of course be made to what already has been said in the context of Figure 3 above. Secondly, it can be stated that concentration levels which were only 2–3-fold higher than the detection limit normally sufficed to generate useful DAD spectra. A larger increase (3–5-fold) was required for pesticides eluting together with the matrix peak in surface water samples. Last but not least, recording LC-UV chromatograms at or near the wavelength of maximum absorption for each individual pesticide rather than at 230 nm, substantially improved the results for, e.g., carbendazim, chloridazon, dimethoate, dicamba, and 2-nitrophenol. For all these compounds, the final detection limits were at or below the 1 μg/l level for surface water.

Two main parameters negatively influence the detection limits in surface water. Firstly the presence of a number of interfering peaks caused by humic substances complicate the detection. Secondly—due to the low elution strength at the beginning of the gradient—the matrix peak containing all kinds of organic (e.g. fulvic acids) and inorganic compounds, does not appear at the start of the chromatogram, but elutes after approx. 10 min causing a distinct baseline drift (Figure 5).

Stability of pesticides

During our chromatographic studies problems occurred with respect to the stability of some test compounds (Table 5). Subsequent analysis of freshly prepared stock solutions of eight such compounds revealed their relatively rapid degradation, while often simultaneously one or more new peaks showed up (Table 6). It was not possible to identify these decomposition products merely on the basis of the DAD spectra. The only exception was the identification of methylisothiocyanate as the decomposition product of metham sodium.

As shown in Table 5 the stability was tested over a period of 30 days, during which time the solutions were stored in the dark at ambient temperature. The data in this table show that the stability of some compounds in aqueous media, even in the presence of a few percent of methanol originating from the initial stock solutions, is rather low. In real surface water—with a higher concentration of oxygen, irradia-

Table 5 Decomposition of test compounds in aqueous solution*

Compound	*Per cent analyte remaining after*			
	0 days	*2 days*	*14 days*	*30 days*
Coumafuryl	100	0	0	0
Dicamba	100	100	98	80
Fenaminosulf	100	71	7	0
Metazachlor	100	98	85	63
Metham sodium	100	80	20	11
Permethrin	100	90	65	51
Thiram	100	57	55	42
Warfarin	100	84	80	70

* Analyte concentration 50 mg/l in water containing 2.5% methanol. For RP-LC conditions see "Marerials and Methods".

tion with sunlight, and the presence of microbial organisms—the stability will probably even be worse. Therefore, the possibility to detect these pesticides by means of the proposed procedure is limited; still, in order to guarantee the water quality it is important to detect the degradation products as well.

As an example, the DAD spectra of fenaminosulf and its two main degradation products formed in aqueous solution are shown in Figure 6. Because of the formation of these compounds—which elute within the first 3 min of the gradient run—it will be difficult to detect fenaminosulf in the early-warning system, more than approximately 5 days after the pollution. The situation is more favourable for coumafuryl. The parent compound can normally not be detected at all (half-life less than one day). However, the degradation product, in aqueous solutions, is easily identified because of its different retention time and half-life.

Table 6 Retention times of decomposition products of test compounds after 30 days of storage in water and/or methanol*

Compound	t_r *(min) of peaks in solution of*	
	Water	*Methanol*
Coumafuryl	28.8*	28.5*, 36.8
Dicamba	—	—
Fenaminosulf	2.6*, 3.0*	1.6, 2.6*, 3.0*
Metazachlor	—	—
Metham sodium	2.1*, 10.0*	2.1*, 11.0*
Permethrin	53.1*	53.0*
Thiram		9.3, 12.7, 17.0, 21.8, 32.5
Warfarin	28.7	38.1, 39.5

* For gradient elution RP-LC on the 10-cm RoSil C-18 column see "Materials and Methods". The asterisk after the retention times indicates that the degradation products have the same absorbance spectrum in both solutions.

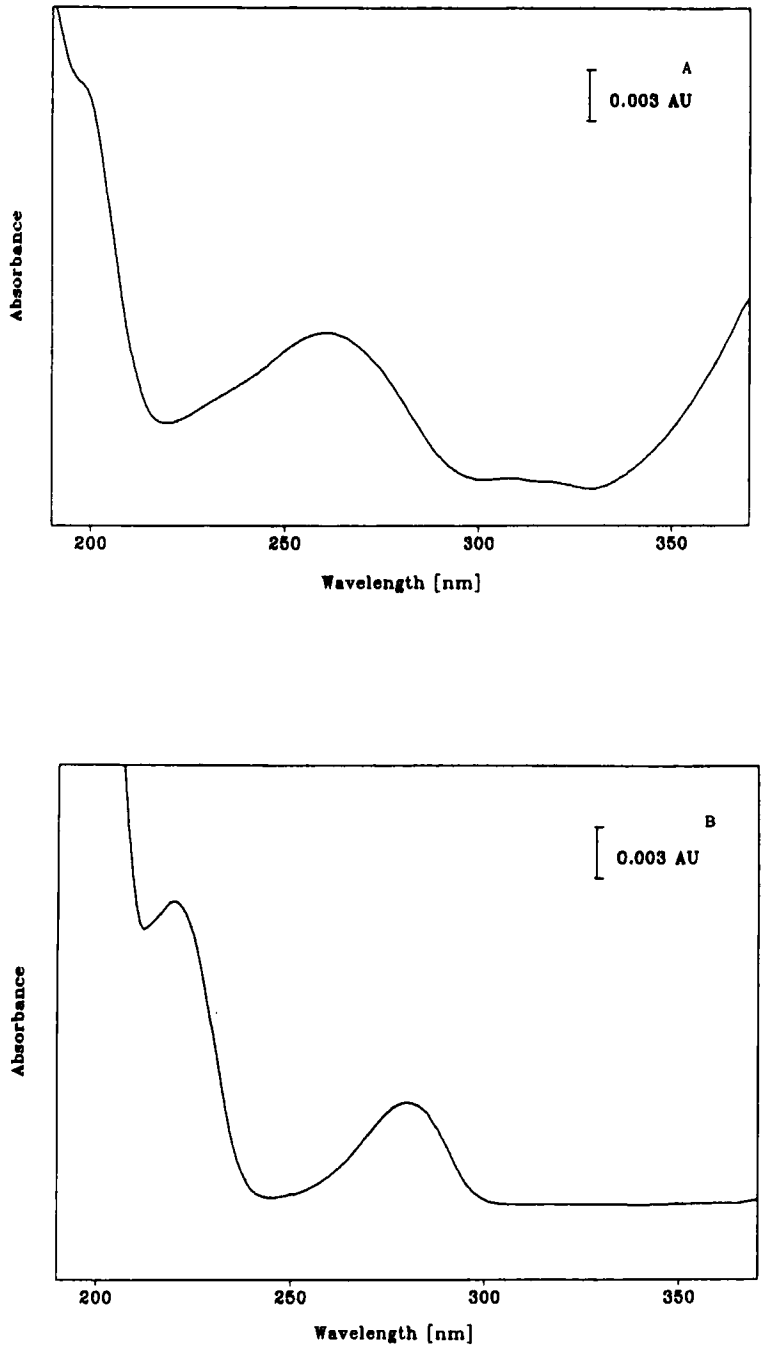

Figure 6 UV absorbance spectra of fenaminosulf (A) and its two main degradation products with retention times of (B) 2.6 min and (C) 3.0 min. Gradient RP-LC is performed on the 10 cm RoSil C-18 analytical column.

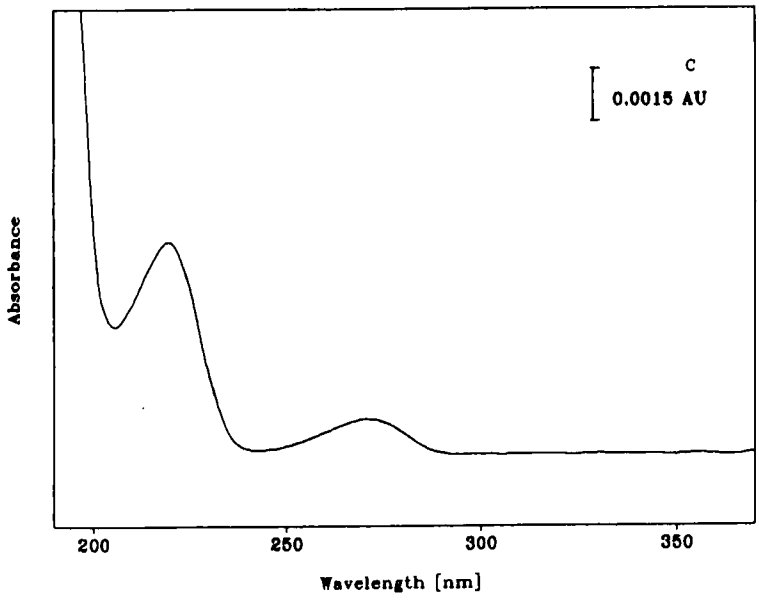

Figure 6 *(continued)*

Since storage of pesticides in non-aqueous stock solutions can also have a negative effect on their stability, as was shown for benomyl in acetonitrile[23], it is important to check the fate of pesticides in organic solvent systems. In this study methanol was used as the solvent and from the data included in Table 6 it can be seen that for most pesticides degradation products were observed after 30 days of storage in the dark at ambient temperature. For some pesticides the degradation process in aqueous and methanolic solutions appears to be the same. For others, however, this is not true. For thiram, for example, a decrease in its concentration was observed in both solvents; however, only the degradation products formed in methanol showed up under the present LC conditions.

CONCLUSIONS

An on-line trace enrichment/RP-LC-DAD system has been developed for the rapid monitoring of a large number of polar pesticides in surface water. Trace-enrichment of 30-ml samples on a PLRP-S polymer-containing precolumn is followed by linear gradient elution with a mixture of acetonitrile and a 0.01 M phosphate buffer (pH 3) on a C-18 analytical column. Both single-wavelength (230 nm) and DAD have been used. Preliminary experiments with river Rhine water samples show that, at the 1–5 μg/l level, a large majority of the 54 test compounds can be detected, and their DAD spectra recorded.

Although first experiences show the present on-line analytical system to be reliable

and robust, several problems still remain. (i) Early eluting ionised (paraquat, diquat) compounds or highly polar (e.g., ethylenethiourea, propylenethiourea, maleic hydrazide) compounds elute essentially with the matrix peak and are easily obscured in the chromatogram. (ii) A few compounds with poor UV absorbance over about 230 nm cannot easily be identified in, e.g., co-eluting peaks.

There are, on the other hand, also aspects which promise well for the future. For example, results obtained with high-concentration and low-concentration trace enrichment of highly polar analytes suggest that breakthrough volumes may in many cases be considerably higher than reported in Table 3. If higher sample volumes can be processed at, possibly, a somewhat higher flow rate, this may effect a noticeable improvement of detection limits without increasing the time of analysis. Secondly, one should realise that, in several cases, only a few test compounds have been included in the present set to represent a whole class of pesticides (e.g., carbamates and phenylurea herbicides). That is, the present method appears to be a promising approach for the trace-level determination of a considerable number of polar compounds in water samples.

Current research already deals with some of the aspects discussed above. In the near future, it will also be attempted to fully automate the systems by inserting a commercially available automated precolumn cartridge exchange system. Besides, a second precolumn will be incorporated in the system in order to increase the number and/or type of analytes that can be handled.

Acknowledgements

This work has been supported by the institute for Inland Water Management and Waste Water Treatment (RIZA; Lelystad, The Netherlands) as part of the Rhine Action Program.

Part of the present research project will be incorporated in the Rhine Basin Program (Amsterdam/Waldbronn).

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