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Determination of phenolic compounds in surface water using on-line liquid chromatographic precolumn-based column-switching techniques

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Abstract

Gradient LC–diode array UV detection, combined on-line with a PLRP-S and an ENVI-Chrom P precolumn in series, enables the on-line trace-level determination and provisional identification of phenol and 13 substituted phenols in surface water. Additional selectivity has been incorporated into the system by properly utilizing the breakthrough properties of phenol over the PLRP-S precolumn. With 50-ml sample volumes, the limits of detection for all analytes are at the low- to sub-μg/l level.

1. Introduction

Phenolic compounds, including phenol, are ubiquitous environmental contaminants. They can be released to the environment by many industrial processes and also as a result of degradation of, e.g., chlorophenoxy acids, a well known group of pesticides. Since many substituted phenols are toxic to aquatic organisms, both the European Union and the US Environmental Protection Agency include many phenolic compounds in their lists of priority pollutants. Consequently, the trace-level determination of these compounds in surface water is of primary concern. In the literature, several procedures are reported which use either capillary gas chromatography (both without and with derivatization) [1–6] or column liquid chromatography [7–10]. In order to comply with tolerance levels typically encountered today for individual pesticides, e.g. 1–3 μg/l in surface water, it is necessary to introduce either an enrichment step or selective derivatization of the analytes [11–13]. At present, solid-phase extraction (SPE) is the preferred method for the enrichment of polar environmental pollutants, because it combines the advantages of convenience, low cost and minimal consumption of organic solvents. Besides, SPE-based procedures can easily be incorporated into fully on-line set-ups which facilitates automation.

Over the years, a wide variety of sorbents, such as graphitized carbon black, ion-exchange materials, octadecyl-bonded silicas, cyclohexyl-bonded silicas and polymer resins, has been used for the isolation of phenols from water samples. Unfortunately, because of a lack of selectivity, several of the reported procedures are not really suitable for the trace-level monitoring of phenols in complex matrices. Therefore, current research aims at using selective sorbents [14] or at instal-

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ling two precolumns in series [10]. Recently a new packing material, ENVI-Chrom P, was introduced. This non-ionic styrene–divinylbenzene copolymer ($d_p = 80–160$ μm), which has excellent chemical and mechanical stability, contains a relatively large number of active aromatic sites which enhances interaction with the aromatic phenols, and, thus, improves the enrichment of many of these analytes. It is well known that the sorption on styrene–divinylbenzene-based sorbents, e.g. the ENVI-Chrom P material in this study, is based on rather selective $\pi-\pi$ interactions. That is analytes not bearing an aromatic ring do not sorb properly on such materials. When using this sorbent, salting out is not necessary anymore. According to Nolan [14], loading 100-ml samples on a tube containing 500 mg of ENVI-Chrom P gave recoveries of 96–106% for 13 phenolic compounds (including phenol). However, off-line methods such as that referred to here have several disadvantages: because an aliquot of the final sample extract is injected only, and losses are prone to occur during (partial) evaporation of the extract, off-line concentration techniques tend to be less sensitive and less reproducible than on-line methods. On-line SPE utilizes small precolumns typically containing 50 mg of the preferred sorbent, from which the analyte can be desorbed and transported directly to the analytical column, either in the forward- or the back-flush mode.

In the present project we studied the use of ENVI-Chrom P as a selective sorbent in an on-line SPE–LC-based analytical system for the sorption of phenols from surface water samples, with final determination by means of diode-array UV detection. Recently we developed several methods for the determination of organic pollutants in surface water within the framework of the international Rhine Basin Program (Amsterdam/Waldbronn) [15–18]. In these studies a polymer-based PLRP-S precolumn was often used because this type of highly hydrophobic sorbent strongly retains many compounds of interest. In the present study the PLRP-S and ENVI-Chrom P sorbents were compared with respect to sorption strength and band broadening of the analytes after on-line desorption. The experimental results led us to construct an analytical system which utilizes two precolumns in series, one containing PLRP-S, the other ENVI-Chrom P material.

2. Experimental

2.1. Reagents and materials

Phenol, 3-nitrophenol, 2,4-dinitrophenol, 2,3-dimethylphenol and 4-chlorophenol were from Fluka (Buchs, Switzerland). 2,6-Dichlorophenol, 2,3,4-trichlorophenol, 2,3,5-trichlorophenol, 2,3,6-trichlorophenol and 2,3,5,6-tetrachlorophenol were purchased from Aldrich (Beerse, Belgium). Dinoseb, dinoterb, 4,6-dinitro-*ortho*-cresol and bromoxynil came from Riedel-de Haën (Seelze, Germany). HPLC gradient-grade acetonitrile, HPLC-grade methanol, and nitric acid were obtained from Baker (Deventer, Netherlands). Buffer solutions were prepared by mixing 1 *M* stock solutions of potassium dihydrogen phosphate and dipotassium hydrogen phosphate (Baker) to the appropriate pH and subsequent dilution to 10 m*M*. All aqueous solutions were prepared with demineralized water, purified with a Milli-Q (Millipore, Bedford, MA, USA) ultrafiltration system.

Stock solutions were prepared by dissolving about 3 mg of the analyte in 15 ml of methanol and stored at 4°C. Surface water samples were spiked by diluting the stock solutions to the appropriate concentration and adjusted to pH 3 with a 1 *M* solution of nitric acid. Prior to use the surface water samples were filtered over a 0.45-μm filter (Schleicher and Schuell, Dassel, Germany). Three 250 × 4.6 mm I.D. stainless-steel analytical columns, i.e., Supelco ABZ, Supelco LC-18 and Supelco LC-18-DB, were a gift from Supelchem (Leusden, Netherlands). Two 250 × 4.6 mm I.D. base-deactivated octadecyl-bonded silica (Chromspher B and Inertsil) stainless-steel analytical columns were a gift from Chrompack (Middelburg, Netherlands). Home-made stainless-steel precolumns (10 mm × 3.0 or 2.0 mm I.D.) were manually slurry packed, using methanol as the slurry liquid, with 15–25 μm PLRP-S (Polymer Lab-

oratories, Church Stretton, UK), 80–160 μm ENVI-Chrom P (Supelchem) or two experimental phases denoted as 1038A and 1038B (Supelchem).

2.2. Set-up and procedures

The LC system consisted of a Gynkotek (Germering, Germany) Model 300 pump to deliver the aqueous sample (3 ml/min) and methanol–acetonitrile (50:50, v/v) for cleaning and wetting of the precolumn. The LC analyses were performed with an HP 1090 (Hewlett Packard, Waldbronn, Germany) LC gradient system equipped with a ternary solvent-delivery system, and an injection valve with a 25-μl loop. For detection an HP 1040 (Hewlett Packard) diode array detection (DAD) apparatus, set at various wavelengths (cf. Table 1), with a 10-mm flow cell was used. Two home-made six-port injection valves were used. The data were evaluated by a Hewlett Packard Pascal workstation using the Chemstation software.

The total analytical set-up is shown in Fig. 1. The final procedure is as follows. Pump P1 delivers the methanol–acetonitrile mixture and the aqueous sample at a flow-rate of 3 ml/min. The precolumn(s) (Pr1 and Pr2) are percolated with 5 ml of the methanol–acetonitrile mixture, to condition the packing material and subsequently 2 ml of Milli-Q water. Prior to loading of the aqueous sample the ENVI-Chrom P precolumn (Pr2) is switched in the analytical system via valve V2 ("inject" position). Subsequently, the PLRP-S precolumn (Pr1) is loaded with 50 ml of sample (at 3 ml/min) by switching valve V1 to the "load" position. When 45 ml of sample have passed the precolumn, valve V2 is switched to the "load" position, and the final 5 ml of sample are loaded with the two precolumns in series. Next, valve V2 is switched to the "inject" position, and the analytes trapped on precolumn Pr2 are desorbed by the LC gradient. After 8.50 min the analytes trapped on the PLRP-S precolumn are desorbed by the LC gradient by switching valve V1 to the "inject" position.

The LC gradient was prepared by mixing an aqueous phosphate buffer (10 m*M*, pH 3; A) and acetonitrile (B). The gradient profile for the desorption of the analytes from both precolumns and the subsequent separation on the analytical

Table 1
Validation data for total SPE–LC–DAD-UV procedure for the determination of phenolic compounds using two precolumns in series

No.	Compound	Detection wavelength (nm)	Recovery (%)	R.S.D. (%) ($n = 6$)	Detection limit (μg/l)
1	Phenol	195	92	5.0	1.0
2	3-Nitrophenol	210	103	8.0	0.8
3	2,4-Dinitrophenol	268	100	2.5	0.8
4	4-Chlorophenol	195	98	3.5	0.05
5	2,3-Dimethylphenol	195	90	3.5	0.1
6	2,6-Dichlorophenol	204	95	3.0	0.3
7	Bromoxynil	210	92	4.5	0.5
8	4,6-Dinitro-*ortho*-cresol	268	94	1.5	0.4
9	2,3,6-Trichlorophenol	204	105	3.0	0.6
10	2,3,4-Trichlorophenol	204	100	2.5	0.3
11	2,3,5-Trichlorophenol	204	93	1.0	0.3
12	2,3,5,6-Tetrachlorophenol	210	96	1.0	0.3
13	Dinoseb	268	105	0.5	0.5
14	Dinoterb	268	103	1.0	0.5

Samples: 50-ml surface water; for other conditions, see text.

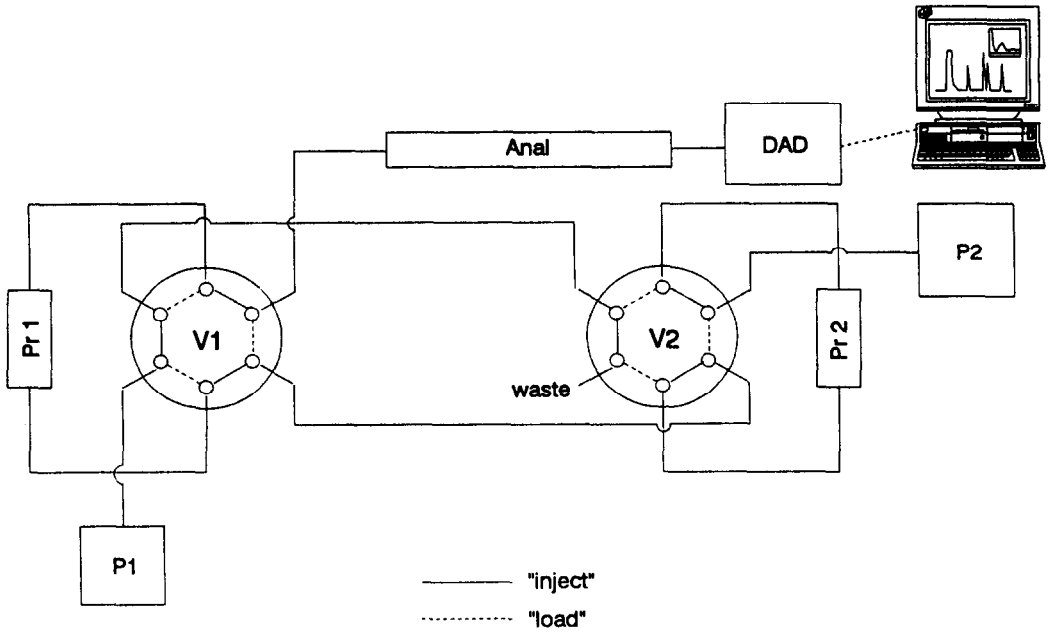

Fig. 1. Set-up of on-line trace enrichment–LC–DAD-UV system; V1, V2: six-port injection valves; Pr1: 10 × 3 mm I.D. precolumn containing 15–25 μm PLRP-S; Pr2: 10 × 3 mm I.D. precolumn containing 80–160 μm ENVI-Chrom P; P1: pump delivering methanol, Milli-Q water and aqueous samples; P2: gradient LC pump; Anal: 250 × 4.6 mm I.D. analytical column; DAD: diode array UV detector.

column was: A–B (80:20) (0 min) which was changed linearly to A–B (39:61) in 45 min, and subsequently to A–B (10:90) (50 min). All separations were carried out at 40°C, using the column oven of the HP 1090 LC system and a flow-rate of 1 ml/min (pump P2).

3. Results and discussion

3.1. Analytical column

In order to obtain optimum sensitivity and selectivity, the selection of the analytical column that has to be used in combination with the enrichment cartridge, is of primary importance. In general alkyl-bonded silica analytical columns provide better separation efficiency than polymer columns and therefore are preferred in most instances. However, it is well known that amines and related compounds often yield very broad non-gaussian peaks as a result of interaction with free silanol groups when non-deactivated silicas are used. In order to solve this problem, a variety of base-deactivated silica columns has been marketed in the last decade. Initially, the separation efficiency of the base-deactivated analytical columns could not compare with that of conventional alkyl-bonded silicas. Today, however, separation efficiencies are similar. Recent experience in our, and also in other laboratories, has shown that problems with non-gaussian peak shapes also occur with phenolic compounds. Therefore, several analytical columns were selected for the present study, viz. four base-deactivated columns (LC-18-DB, LC-ABZ, Chromspher B and Inertsil), along with one non-deactivated column (LC-18). All columns had the same dimensions (250 × 4.6 mm I.D.) and they all contained 5-μm particles.

Since it was our intention to study a wide range of phenolic compounds, the test set included both highly polar analytes such as phenol and several nitrophenols, and apolar phenols such as higher-substituted chlorophenols. Consequently an LC gradient had to be used to effect

the required separation. The final separation conditions, which were the same for the five analytical columns tested, are given in section 2.2. As can be seen from Fig. 2 all selected phenols were baseline-separated when using either the Chromspher B or Inertsil column, although there was a reversal in the order of elution (peaks 6 and 7). However, when the Supelcosil LC-18-DB column, which has successfully been used by us for many applications in the area of polar pesticides [16,17], was tested using the same LC gradient, broad tailing peaks appeared, especially for the late eluting compounds. These broad peaks can not be attributed to working at an unfavourable pH value since the LC gradient was the same as with the two Chrompack columns. In order to test whether the agent used for endcapping the analytical columns caused secondary interactions with the phenols, a non-deactivated Supelcosil LC-18 column was included in the study. However, the peak shapes did not improve. Finally a Supelcosil LC-ABZ column was tested. With this column silanol deactivation is based on electrostatic shielding, a polar group being embedded covalently in the phase. In our hands the LC-ABZ column has been shown to be quite efficient for the separation of neutral, acidic, basic and zwitterionic compounds without the use of additives such as amines or silanol-suppressing LC eluent conditions [19,20]. In the present

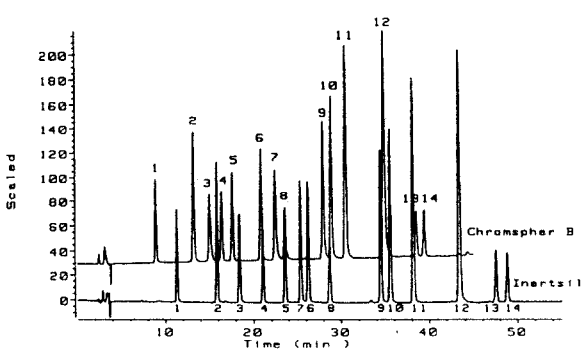

Fig. 2. LC–DAD-UV chromatogram of standard mixture of 14 phenols after loop injection (15 μl; 14 mg/l of each phenol) on 250 × 4.6 mm I.D. Inertsil and Chromspher B analytical columns. For peak assignment, see Table 1; for experimental conditions, see text.

study, the LC-ABZ column provided sharp peaks for most phenolic compounds, but the four dinitrophenols (compounds 3, 8, 13 and 14, Table 1) still gave broad tailing peaks. Since the above data suggest that the non-gaussian peak shapes are not primarily due to the deactivation process, the role of the modifier was also briefly studied. Although the use of methanol instead of acetonitrile did improve the peak shapes for several analytes (e.g., 2,3-dimethylphenol and 2,3,4-trichlorophenol) on the LC-18 and LC-18-DB columns, the results for the dinitrophenols, and also bromoxynil, still were not satisfactory. This observation is in line with data recently published [16].

On the basis of the above results, the Chromspher B column, with its relatively short time of analysis was selected for all further work.

3.2. DAD-UV absorbance detection

In order to determine the optimum wavelengths for DAD-UV detection, UV spectra were recorded for all analytes (data not shown). For most of the test analytes, the wavelength of maximum absorption was selected. It is well known, however, that in surface water analysis co-eluting peaks or bands especially interfere at these low-UV wavelengths. Therefore, for all phenolic compounds featuring a sufficiently intense secondary maximum at a higher wavelength (compounds 3, 8, 13 and 14; Table 1), the latter maximum was preferred. The wavelengths selected for each single analyte are given in Table 1.

3.3. Analyte trace enrichment

In our laboratory several LC-based systems have been developed for the determination of a variety of polar pesticides [15–18]. In most instances, a single precolumn is used to trap the analytes of interest, with a polymer-based sorbent such as PLRP-S being the preferred packing material because of its high and broad-range retention power. Besides, this type of sorbent can be used over the pH range of 0–14. In this study we also tested the recently introduced

ENVI-Chrom P, another styrene–divinylbenzene-based sorbent, which has been modified to increase the retention of polar aromatic compounds. Initially a set-up containing a single precolumn was used; that is, valve V2 (and precolumn Pr2; cf. Fig. 1) was not included. In order to test the retention power and/or selectivity of both sorbents, 50 ml of river Meuse water, spiked with 5 μg/l of all test analytes, was passed through the precolumn at 3 ml/min. Two striking differences are observed when comparing the chromatograms recorded in Fig. 3 (a–d: PLRP-S; e: ENVI-Chrom P). When using the PLRP-S precolumn sharp peaks are obtained at the four wavelengths selected for the efficient detection of the test compounds. Phenol, however, which elutes at $t_R = 8$ min, does not show up. When, instead of PLRP-S, the ENVI-Chrom P material was used, the detection of phenol presented no problems. However, distinctly broader peaks were observed for all analytes and the detectability of especially the late-eluting peaks now left much to be desired (Fig. 3e). The additional band broadening can be explained by the high retention power of ENVI-Chrom P compared with PLRP-S.

For all analytes breakthrough volumes were determined on both sorbents. To this end, increasing volumes of river Meuse water, spiked at the 25 μg/l level, were pumped through the precolumn. For PLRP-S the breakthrough volumes for phenol and 3-nitrophenol were 1 and 50 ml, respectively, while all other analytes had breakthrough volumes of over 50 ml. For ENVI-Chrom P breakthrough of phenol occurred after 5 ml of the sample has been passed through the precolumn. All other analytes had breakthrough volumes over 50 ml, which was the maximum volume tested. Obviously, if analyte trace-enrichment from 50 ml of sample is sufficient to achieve detection limits of ca. 1 μg/l or below (see below), both sorbents behave rather similarly. However, ENVI-Chrom P is to be preferred because of its distinctly higher retention of phenol, while PLRP-S is the better choice in terms of final separation efficiency. It was therefore decided to combine both precolumns in one set-up with the ENVI-Chrom P precolumn inserted between the PLRP-S precolumn and the analytical column (cf. Fig. 1).

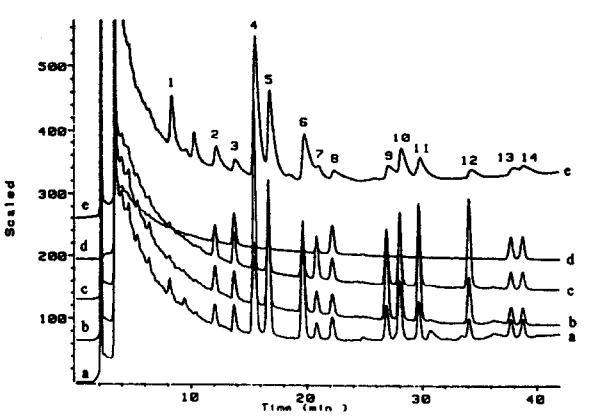

Fig. 3. On-line trace enrichment–LC–DAD-UV chromatogram of 50 ml surface water spiked with 14 phenols (5 μg/l each), using a single precolumn. (a–d) 10 × 3 mm I.D. PLRP-S precolumn, a: $\lambda = 195$ nm, b: $\lambda = 204$ nm, c: $\lambda = 210$ nm, d: $\lambda = 268$ nm; e: 10 × 3 mm I.D. ENVI-Chrom P precolumn, $\lambda = 195$ nm. For peak assignment, see Table 1.

Usually when two precolumns are used in series, the total amount of sample is pumped through both precolumns. However, in the present case this was not the proper solution. In order to prevent breakthrough of phenol only 5 ml of sample can be pumped through the ENVI-Chrom P precolumn. For all other test analytes, however, a larger sample volume is preferred to obtain lower detection limits. Therefore the total, i.e. 50-ml sample was pumped through the PLRP-S precolumn by switching valve V1 to the "load" position and valve V2 to the "inject" position. When 45 ml of the sample had passed the first precolumn —with phenol obviously displaying breakthrough at this point— the second precolumn was inserted in-line by switching valve V2 to the "load" position. In other words, only the final 5 ml of sample were pumped through both precolumns in series, and the recovery is essentially quantitative for all analytes (cf. the data included in Table 1). After trace enrichment of the analytes, the ENVI-Chrom P precolumn was analysed first. To this end, valve V2 was switched to the "inject" position to start LC gradient elution. After 8.50 min, analysis of the PLRP-S precolumn was

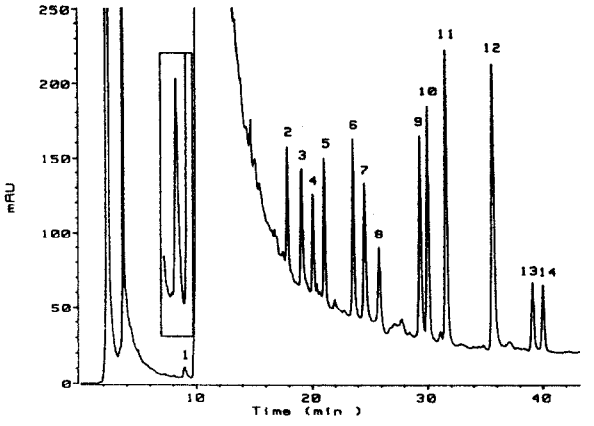

Fig. 4. On-line trace enrichment–LC–DAD-UV chromatogram ($\lambda = 210$ nm) of 50 ml surface water, spiked with 14 phenols (5 μg/l each), using two precolumns in series. The insert shows a blow up of peak 1 (phenol). For conditions, see text. For peak assignment, see Table 1.

started by switching valve V1 to the "inject" position and valve V2 to the "load" position. Comparison of Fig. 4 with Fig. 3 illustrates that (i) the dual-precolumn set-up provides additional selectivity in the early part of the chromatogram, enabling the detection of phenol at 195 nm (!), and (ii) the slightly more complicated set-up does not detract from the performance of the total analytical system.

Analytical performance

Calibration curves were constructed for all analytes in surface water samples over the range 0–25 μg/l using the dual-precolumn set-up of Fig. 1. The plots (six data points; $n = 2$) were linear for all analytes with R^2 values of over 0.99. In order to determine the precision of the total analytical procedure, six consecutive analyses were performed of river Meuse water spiked with a mixture of all test solutes at the 5 μg/l level. Although valve-switching was done manually, the precision for all analytes was quite satisfactory with a mean R.S.D. value of 3% (range 0.6–7.8%; cf. Table 1). Detection limits (signal-to-noise ratio 3:1) in real-life samples typically were ca. 0.5 μg/l (range, 0.05–1.0 μg/l).

In order to test the applicability of the present trace-enrichment–LC–DAD-UV system several surface water samples were analysed. Fig. 5 shows four chromatograms obtained after preconcentration of 50 ml of water from the rivers Meuse (Keizersveer, Netherlands), Rhine (Lobith, Netherlands), Danube (Bratislava, Slovakia) and Nitra (Nizne Krstenany, Slovakia). No phenols were found in these samples at or above the detection limits quoted in Table 1. When an automated library search was performed with spectral match as the only key for peak identification, diuron, a well known phenylurea herbicide, was found to be present in river Meuse water (cf. Fig. 5a). Unfortunately, none of the DAD-UV libraries available to us (polar pesticides, polynuclear aromatics, pyrethroids, sulphonic acids) provided a clue with regard to the nature of the several large peaks showing up in the samples, especially in the river Nitra sample.

4. Conclusions

An on-line trace enrichment–LC system with diode-array UV detection has been developed for the monitoring of a large series of phenolic compounds in surface water at low- to sub-μg/l levels. The procedure involves the preconcentration of 50 ml of surface water on a non-selective and a selective precolumn coupled in series. Because of the early breakthrough of phenol on the first precolumn, only an appropriate 5-ml portion of the total sample is pumped through the second precolumn. This helps to increase the selectivity of the procedure for phenol itself, allowing UV detection even at 195 nm (even for surface water). Besides, compared with literature studies, a wider range of analytes can be analysed using this technique. With the present set-up detection limits are between 1 (phenol) and 0.05 (4-chlorophenol) μg/l; the precision of the total analytical procedure is quite satisfactory. Future research will focus on the use of ENVI-Chrom P material with a smaller particle size than the present 80–160 μm. Two experimental phases, denoted 1038A and 1038B, are currently being tested. First results are quite promising; that is, band broadening

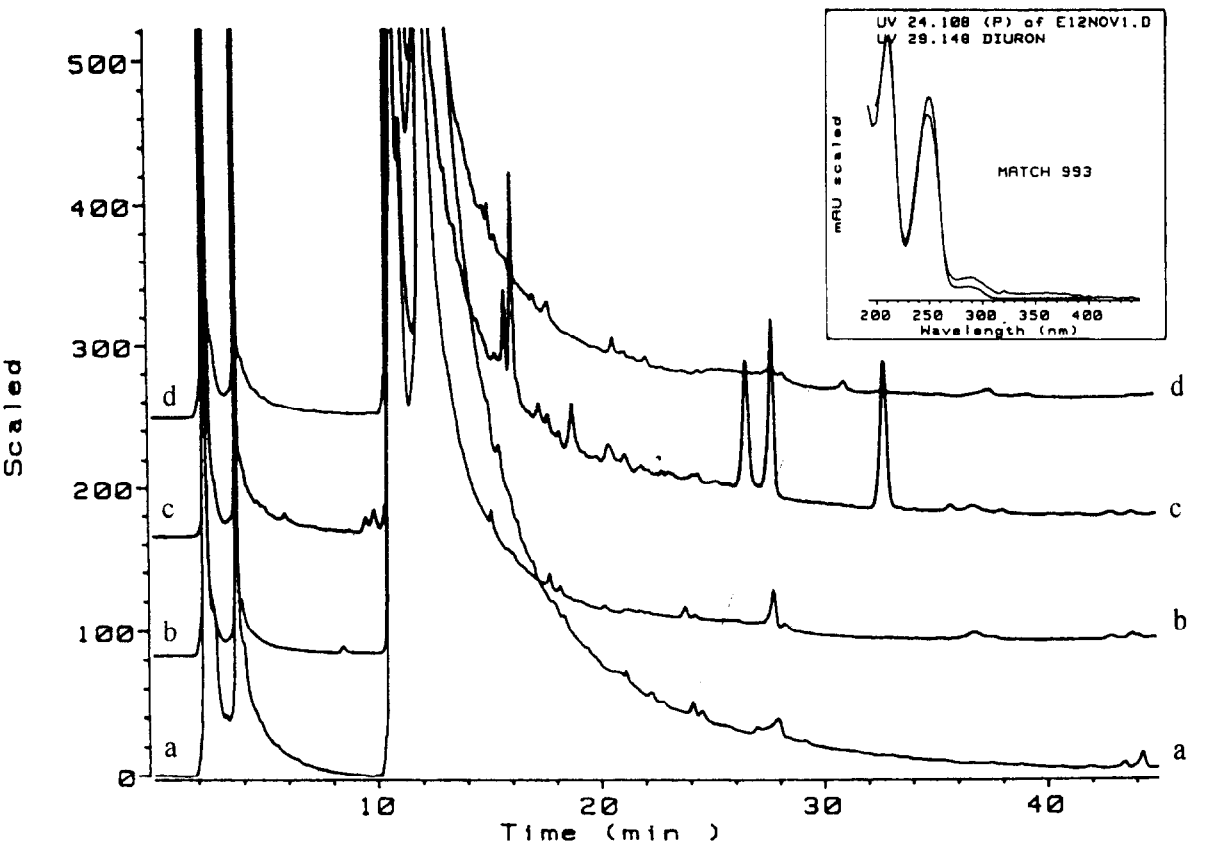

Fig. 5. On-line dual-precolumn trace enrichment–LC–DAD-UV chromatograms ($\lambda = 210$ nm) of 50 ml blank surface water samples. (a) river Meuse; (b) river Danube; (c) river Nitra; (d) river Rhine. The insert shows the library DAD-UV spectrum of diuron and the spectrum recorded for the peak at $t_R = 24.1$ min in trace a. For experimental conditions, see text.

which was the major disadvantage of the ENVI-Chrom P material used in this study, is considerably reduced.

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