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Short communication

Rapid liquid chromatographic screening of organic micropollutants in aqueous samples using a single short column for trace enrichment and separation

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Abstract

A single short (20 mm × 4 mm I.D.) column packed with C_{18}-bonded silica is used for both trace enrichment and liquid chromatographic (LC) separation of organic micropollutants in aqueous samples. The column can be used for at least twenty real-life samples without any decrease of performance. On-line short-column LC–diode array UV detection enables the detection of down to about 1 μg/l of organophosphorus and other polar pesticides in surface water, using 15-ml samples. The total time of analysis is ca. 25 min. Preliminary results are shown for short-column LC–particle beam mass spectrometry.

1. Introduction

In recent years much attention has been devoted to the design and optimisation of liquid (LC) and gas (GC) chromatography-based systems for the on-line, and preferably automated, screening and early-warning of water samples such as surface, ground and tap water. In essentially all such set-ups trace enrichment using solid-phase extraction (SPE) is combined on-line with the separation-cum-detection method of choice [1–7]. For rather obvious reasons (aqueous samples; wide applicability), the most popular option is SPE–LC–diode array UV detection (DAD). In most instances these and other related systems are designed so that analyte detectability, i.e. trace enrichment, is adequate for the goal in mind, and that selectivity, i.e. primarily separation efficiency, is as high as possible. In many real-life applications, however, the general situation is that in a large majority of all samples no micropollutants are present above the threshold limit of interest. In other words, if rapid general screening or early-warning is the main goal, too much selectivity is often introduced into such systems, making these unduly complicated and expensive, and causing a decrease of sample throughput.

In view of the above, it is obviously of interest to decrease chromatographic —in the present instance, LC— resolution by decreasing the length of the analytical column, while simultaneously maintaining sensitivity: for the goals outlined above, a limited number of false positives is allowed, but false negatives are not! In the end, one should attempt to rely for both trace enrichment and sample separation on a high-pressure (not a manually)-packed 10–30

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mm precolumn only. Preliminary results of such a study, which was initiated by another current research project [8], using commercially available modules which can be applied in routine analysis will be presented below.

2. Experimental

2.1. Materials

HPLC-gradient grade acetonitrile was purchased from Biosolve (Barneveld, Netherlands). HPLC-grade water was prepared from distilled Milli-Q water (Millipore, Bedford, MA, USA) which was subsequently distilled over a quartz bed. pH adjustments were made using phosphate buffers which were prepared from 85% orthophosphoric acid and sodium dihydrogenphosphate, both obtained from J.T. Baker (Deventer, Netherlands), or ammonium acetate (99%) (Merck, Darmstadt, Germany). HPLC-grade methanol was obtained from J.T. Baker. All pesticides were over 95% pure and were purchased from Riedel-de Haën (Seelze, Germany).

Stock solutions of the pesticides of 200 or 1000 μg/ml in methanol were used to prepare mixtures. These mixtures were further diluted with acetonitrile to a concentration of 2.5 or 5 μg/ml. For calibration purposes these solutions were directly injected (12-μl syringe) into the LC system. Spikes were made by adding a known volume of these solutions to a known volume of a water sample. Prior to spiking, the river samples were filtered over a 0.45-μm membrane filter (Schleicher & Schuell, Dassel, Germany).

2.2. Instrumentation

The LC system consisted of a Hewlett-Packard (Waldbronn, Germany) 1090 II liquid chromatograph equipped with a ternary solvent delivery system, a six-port switching valve and a HP 1040 A diode array detector. The system was controlled by a Pascal Workstation (PAWS) using Chemstation software (Hewlett-Packard). The Chemstation was also used for data evaluation.

Trace enrichment and separation were performed on a 20 mm × 4 mm I.D. column packed with 5-μm Hypersil ODS or on a 60 mm × 4.1 mm I.D. column packed with 3-μm Hypersil ODS. A schematic of the system is given in Fig. 1.

Mass spectrometric (MS) detection was per-

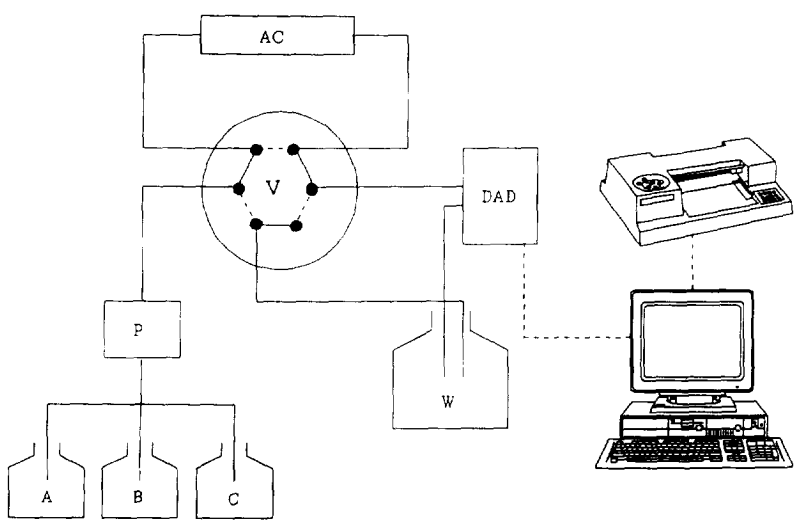

Fig. 1. Experimental set-up of the LC system. P = Ternary gradient pump; A, B and C = eluent bottles containing phosphate buffer, acetonitrile and sample; V = six-port switching valve; AC = 20 mm × 4 mm I.D. analytical column; DAD = diode array detector; W = waste flask. With the MS experiments, after the diode array detector, a second six-port switching valve was inserted which led to the PB-MS system.

formed using an HP MS Engine equipped with a HP 59980 particle beam (PB) interface and a "high energy dynode" detector (Hewlett-Packard, Waldbronn, Germany). All data were collected and evaluated using the Hewlett-Packard Chemstation software (Hewlett-Packard, Böblingen, Germany) running under Microsoft Windows (version 3.1) on the HP 486/66X computer. Optimisation of the PB-MS parameters was done according to Bagheri et al. [1]. A filter photometric detector was inserted between the LC and the PB-MS parts of the system for monitoring purposes.

In order to avoid clogging of the PB interface by sample constituents not retained during sample loading, an additional six-port valve was inserted between the UV detector and the PB-MS unit. During column conditioning and sample loading the PB-MS was switched off-line; 3 min after sample loading the valve was switched manually, and the LC effluent was now led to the PB-MS system.

2.3. Analytical procedure

Prior to separation the 20 or 60 mm long LC column was conditioned with subsequently 10 ml of acetonitrile, and 10 ml of a 10 m*M* phosphate buffer (pH 3), and loaded with 15 ml of sample; the flow-rate during these steps was 2.5 ml/min. Next, separation was performed, at 1.5 ml/min, with a linear gradient of 100% aqueous 10 m*M* phosphate buffer (pH 3) to 100% acetonitrile. The gradient was run in 15 min, unless is otherwise indicated. The total analytical procedure is schematically presented in Table 1.

The DAD signal was invariably monitored at 210 nm (bandwidth, 4 nm); 254 nm was selected as an additional wavelength because it provided better selectivity and/or sensitivity with, e.g., the phenylurea herbicides and pyrazophos. UV spectra were recorded from 200 to 400 nm for library identification; integration was performed at 210 nm unless otherwise indicated.

3. Results and discussion

In the past few years, we have developed a fully automated SPE–LC–DAD system that enables the detection and quantification of 60–100 micropollutants in surface water at or below the alert (1 μg/l) and alarm (3 μg/l) levels typically in use today [2]. The practicality of this so-called SAMOS (system for automated measurement of organic micropollutants in surface water) LC system has been demonstrated for a variety of samples. If now, for the reasons presented above, the feasibility of a simplified SAMOS

Table 1
Time schedule of the total analytical procedure

Time (min)	Flow (ml/min)	% A (PO_4 buffer)	% B (acetonitrile)	% C (sample)
0	2.5	100	0	0
0.01	2.5	0	100	0
4.01	2.5	0	100	0
4.02	2.5	100	0	0
8.02	2.5	100	0	0
8.03	2.5	0	0	100
14.03	2.5	0	0	100
14.04	1.5	100 (l.g.)	0	0
29.04	1.5	0	100 (l.g.)	0
32	1.5	0	100	0
33	1.5	100	0	0

The conditions apply to a 20 mm column, and include conditioning (0.01–4.01 and 4.02–8.02 min), sample loading (8.03–14.03 min) and separation [14.04–29.04 min, linear gradient (l.g.)]. With the 60 mm column, all flow-rates were set to 1.5 ml/min, while the total volumes used were kept the same as before.

approach will be studied, the following aspects merit some attention.

(1) By carrying out trace enrichment and separation on one (short) column instead of using a precolumn plus a (long) analytical column, band broadening will be significantly reduced. This is especially true because the conventional SAMOS approach for practical reasons uses a copolymer sorbent in the precolumn which displays much higher retention towards most analytes of interest than does the C_{18}-bonded silica packing of the analytical column. On the other hand, breakthrough volumes will be considerably smaller in the simplified system.

(2) With the single short-column approach, the total time of analysis can be rather short. However, since the high-pressure packed short column (as yet) is not really inexpensive, repeated re-use is a necessity. Sample loading should therefore preferably be carried out at moderate flow-rates or, in other words, sample size should be limited.

It will be rather obvious that the above considerations indicate a preference for a very short rather than a moderate-size column (20 mm versus 60 mm in the present study), and that duration of run, limits of detection versus sample size, and column re-use are the main aspects of interest.

3.1. Duration of gradient run

In order to find out to which extent the time of run can be reduced without compromising the separation power of the system too much, a series of injections was performed of a standard mixture of 16 organophosphorus pesticides (OPPs) on the 20 mm column. Norberg et al. [9] —in their study on the optimisation of the 55-min linear gradient of the usual SAMOS approach— recommended a linear gradient from 5 to 95% acetonitrile (in 30 min) for the class-selective separation of a mixture of 20 OPPs on a 250 mm × 4.6 mm I.D. column. Using a 30-min gradient in our system, i.e. with the 20 mm column, baseline separation was observed for all but two of the test compounds; some peak overlap was found for fenchlorphos and bromophos-methyl only (Fig. 2). The last eluting OPP, bromophos-ethyl, had a retention time of 18.7 min as against 32.5 min on the 250 mm column.

In view of the rather unexpectedly good re-

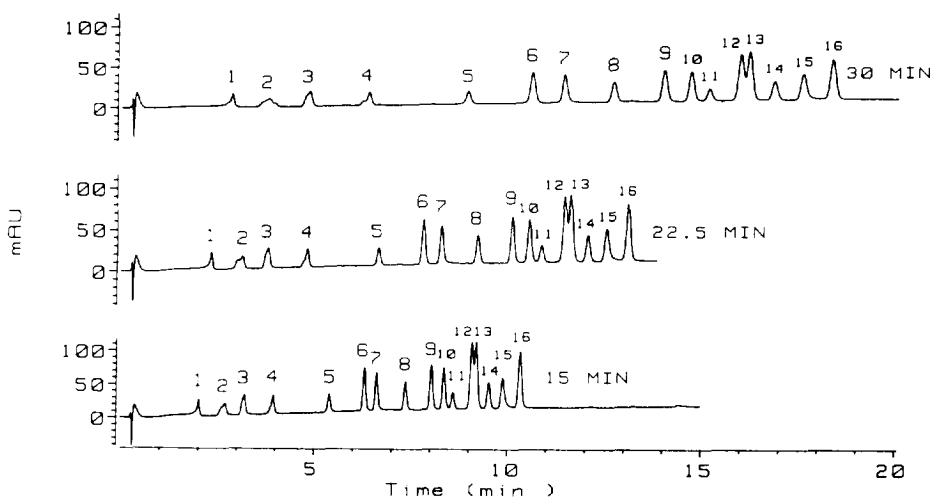

Fig. 2. Influence of gradient run time on resolution for a mixture of 16 OPPs. Column: 20 mm × 4 mm I.D. Hypersil ODS; linear gradient: 10 m*M* phosphate buffer (pH 3) to acetonitrile in 15, 22.5 and 30 min. Injection volume, 25 μl containing 5 μg/ml of each OPP. Peaks: 1 = monocrotophos; 2 = dimethoate; 3 = mevinphos; 4 = phosphamidon; 5 = paraoxon; 6 = azinphos-methyl; 7 = fenamiphos; 8 = fenitrothion; 9 = fenthion; 10 = coumaphos; 11 = phoxim; 12 = fenchlorphos; 13 = bromophos-methyl; 14 = chlorpyryphos; 15 = carbophenothion; 16 = bromophos-ethyl. UV detection at 210 nm.

sults, it was decided to omit —at least for the time being— studies dealing with the 60 mm long column, especially because preliminary results showed that this alternative primarily increased back-pressure and loading time, while the additional resolution which was obtained had no real priority anymore. Instead, restricting the study to the 20 mm long column, in a next step gradient run times of 22.5 and 15 min were used. As is evident from the pertinent chromatographic traces included in Fig. 2, even with the 15-min run (with bromophos-ethyl being eluted after less than 11 min), there still is baseline resolution for essentially all peaks. Since the main goal of our study is to achieve adequate resolution only in an early-warning situation, whilst having low limits of detection and a high sample throughput, the 15-min gradient run obviously is the best choice.

3.2. Trace enrichment

In a next series of experiments, trace enrichment was included in the operation. To this end, surface water from the river Meuse was spiked with the mixture of sixteen OPPs, and analysed on the 20 mm column according to the procedure of Table 1. In order to keep the time of analysis rather short, the sample size was only 15 ml. Subsequent analysis of five samples spiked at the 4 μg/l level, on the same column, gave relative standard deviations (peak areas) of less than 10% for all but two OPPs (dimethoate and paraoxon, 20–25%). Satisfactory recoveries of 75–95% were observed for all analytes eluting between 5 and 9 min. As was to be expected, much lower recoveries of typically 45–50% were found both for the early eluting peaks (due to early breakthrough) and the late eluting OPPs (adsorption to inner walls of the tubing, etc. because of high hydrophobicity). Whereas the former problem can only be solved —for the same sorbent material— by using lower sample volumes or a longer column, thereby decreasing the overall analyte detectability or sample throughput, the latter problem can in principle be solved by adding a few per cent of modifier (e.g. methanol or acetonitrile) to the sample solution [4,9]. A typical chromatogram of a river Meuse water sample, without and with the OPP spike (recorded after the column had been used for several such samples), is shown in Fig. 3.

The limits of detection of the OPPs typically

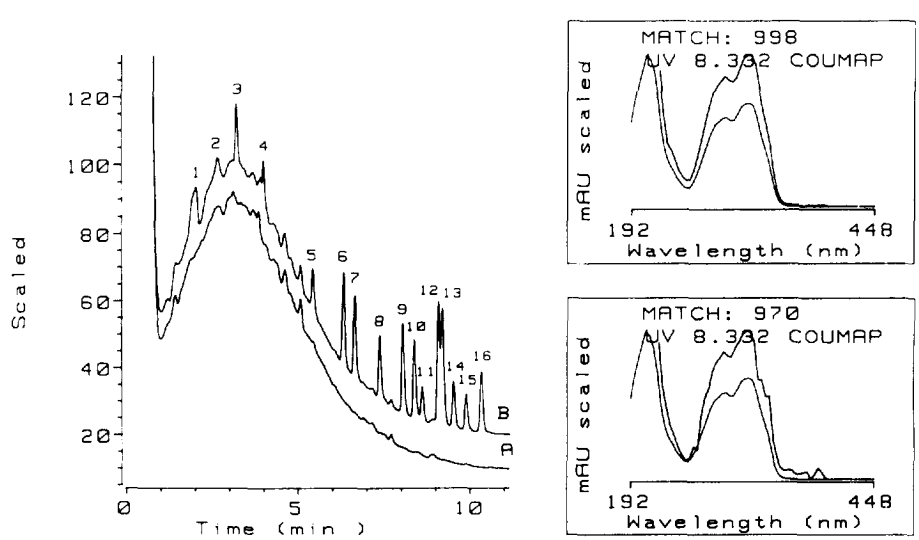

Fig. 3. Short-column LC–DAD of 15-ml samples from the river Meuse (A) without, and (B) with a 4 μg/l spike of sixteen OPPs. Insets on the right-hand side: UV spectra of coumaphos (peak 10) at 4 μg/l (top) and 1 μg/l (bottom) compared with library spectra. System characterization: 20 mm column; 15-min gradient; UV detection at 210 nm. Peak Nos. as in Fig. 2. For all other details, see text.

were about 1 μg/l in surface as well as in tap water. Actually, at the said concentration level most analytes gave good UV spectra with library matches of at least 905 for all but the first three eluting compounds. A relevant example is included in Fig. 3. Linearity was tested over the short, but relevant, concentration range of 1–8 μg/l. The results were satisfactory, with R^2 values of at least 0.992 for all but two compounds (dimethoate and paraoxon).

Column performance was remarkably good. Retention times were constant within 0.02 min, and peak widths typically were 0.1 min. Column lifetime has not been studied extensively, but on at least one 20 mm column, some 40 real samples (30 surface water and 10 tap water samples) were analysed without any noticeable column deterioration showing up. For the rest, on the basis of our (admittedly limited) experience it seems safe to state that a 20 mm column can be used for at least some 20–30 15-ml sample runs.

Other analytes

Preliminary runs were carried out with pesticides other than OPPs. Good results, i.e. detection and/or identification at the 0.5–1 μg/l level, were obtained for atrazine and for a series of phenylureas which included metoxuron, monuron, chlortoluron, diuron, chlorbromuron and neburon. Fig. 4 shows chromatograms obtained for a surface water sample spiked at the 1 μg/l level, and for a 12-μl syringe injection of a 2.5 mg/l standard solution. Recoveries were 80–100% for all but the early eluting desmethyl-metoxuron, which was not detected at this concentration as a result of early breakthrough and poor peak shape.

It is interesting to add that atrazine and diuron were identified in several of the samples tested, viz. at concentration levels of 0.2–0.5 μg/l.

3.3. Short-column LC–MS

In order to extend the scope of the present study, we briefly examined the direct on-line combination of short-column (20 mm) LC and MS. A PB interface was used, because this would enable the recording of electron impact mass spectra. The analytical conditions were as described in the Experimental section, with the exception that the aqueous part of the LC eluent now contained 0.1 *M* ammonium acetate buf-

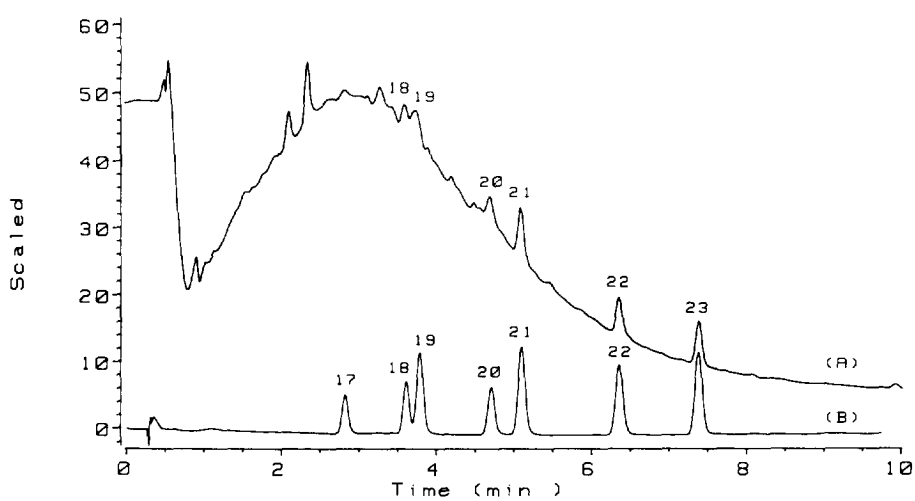

Fig. 4. Short-column LC–DAD of (A) 15-ml Meuse sample spiked with seven phenylurea herbicides at 1 μg/l and (B) a 12-μl syringe injection of a 2.5 mg/l mixture of the phenylureas. Peaks: 17 = desmethyl-metoxuron; 18 = metoxuron; 19 = monuron; 20 = chlortoluron; 21 = diuron; 22 = chlorbromuron; 23 = neburon. System characterization: 20 mm column; 15-min gradient; UV detection at 254 nm. For other details see text.

fered to pH 4 and that the flow-rate was reduced to 0.4 ml/min, which is the highest flow the PB interface can handle. Since the gradient time was kept at 15 min, the gradient itself became steeper; as a consequence peak widths were larger in time units, but smaller in units of volume.

With a series of six phenylureas (cf. [1]) as test compounds, short-column LC–PB-MS was performed with Meuse water spiked at the 0.1–10 μg/l level. Four of the test compounds could be detected over the whole concentration range in the selected ion mode (SIM), and at the 1 μg/l level with full-scan acquisition. Chlorbromuron (m/z 207) could only be detected at the 10 μg/l level (full-scan acquisition). Desmethyl-metoxuron appeared as a small broad peak in the early part of the chromatogram (cf. above), but could be detected (m/z 142) at 1 μg/l using ion extraction. Fig. 5 shows an extracted ion chromatogram of river Meuse water spiked with the six phenylureas at the 1 μg/l level. In the non-spiked sample, a peak (m/z 72) was observed with the same retention time as diuron. SIM and extracted-ion chromatograms (see Fig. 5) obtained after standard addition indicated a diuron concentration of 0.1 μg/l. Over 20 real-life samples were analysed by means of automated short-column LC–PB-MS without any deterioration of the system performance.

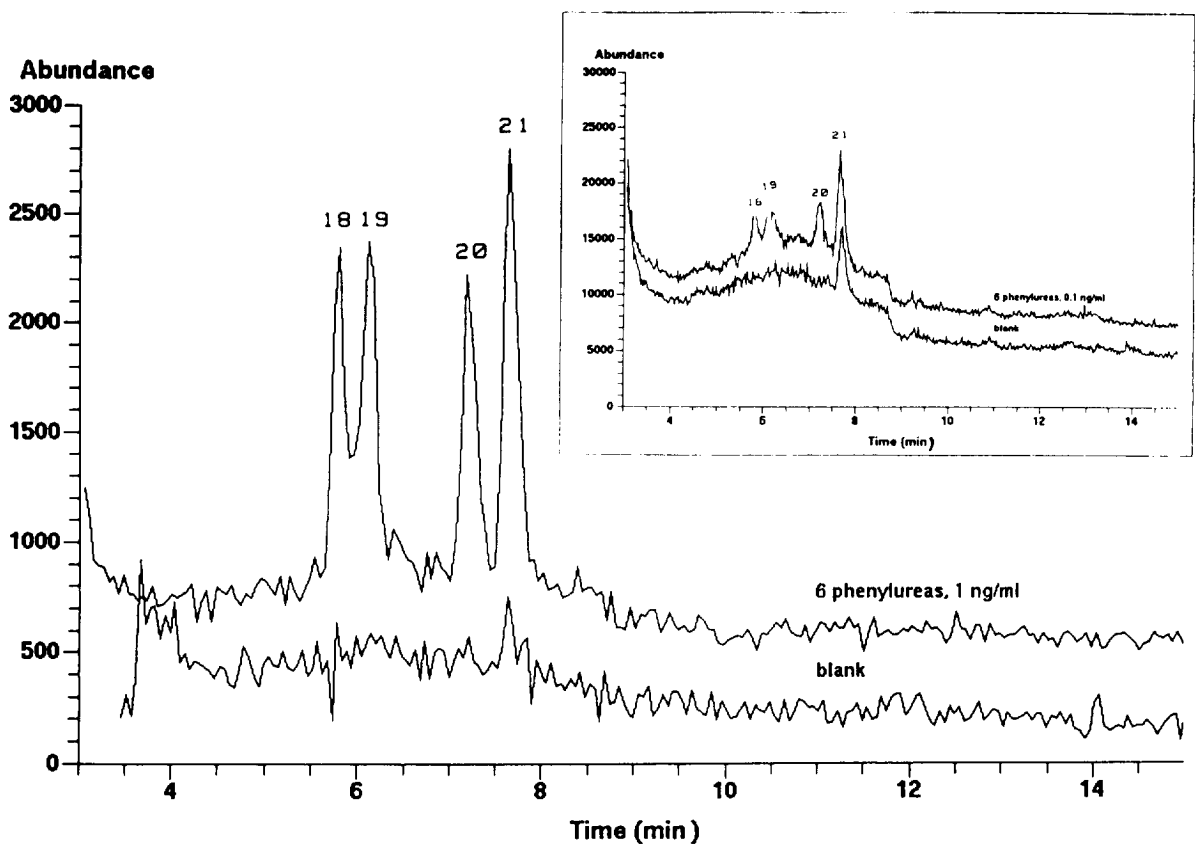

Fig. 5. Extracted ion (m/z 72) chromatograms of 15 ml of river Meuse water without (blank) and after spiking with six phenylureas at the 1 μg/l level. Inset: SIM chromatogram (m/z 72) of 15 ml of the same sample and after spiking with the six phenylureas at the 0.1 μg/l level. For analytical conditions, see text. Peaks: 18 = metoxuron; 19 = monuron; 20 = chlortoluron; 21 = diuron.

4. Conclusions

The practicality of single short-column LC for the trace enrichment and separation of polar pesticides in aqueous samples has been demonstrated. For many surface-water monitoring studies in which the number of microcontaminants actually showing up in each sample is very low, analyte detectability is much more important than high resolution. With the short-column LC–DAD system, the majority of the test analytes could be detected and identified down to concentrations of typically, 0.5–1 μg/l (8–15 ng injected). The total analysis time was 25 min, and a single 20 mm × 4 mm I.D. short LC column could be re-used some twenty times.

The results of this preliminary study and those reported in Ref. [8], and especially those on the use of MS detection instead of DAD are encouraging, and indicate that further optimisation may well lead to the development of a straightforward and relatively inexpensive procedure for the analysis of a large number of water samples in, especially, early-warning situations. Here, one should point out the distinct parallels existing between the present work and earlier studies on coupled-column LC in which two high-pressure packed short columns were used in series for the trace-level determination (large-volume injection, clean-up and separation) of highly polar analytes [10,11].

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