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Coupled-column reversed-phase liquid chromatography–UV analyser for the determination of polar pesticides in water

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ABSTRACT

Coupled-column RPLC with UV detection using direct large volume injections of up to 4 ml can be used for the rapid and sensitive determination of single polar pesticides in environmental water samples. The limits of determination for pesticides such as bentazone and isoproturon are 0.1 μg/l in real-life samples and the sample throughput is 5–7/h. Linearity is observed over at least three decades and the repeatability is satisfactory (relative standard deviations 3–7% at spiking levels of 0.1–0.5 μg/l). The set-up is fully automated and shows good robustness. The coupled-column RPLC–UV analyser has successfully been used in several monitoring programmes.

INTRODUCTION

Today there is a growing concern over the contamination of drinking-water sources by pesticides. The European Community (EC) Directive on the Quality of Water Intended for Human Consumption states (in paragraph OJI 229 30.80) that the concentration of pesticides and related products should not exceed the level of 0.1 μg/l for individual compounds and 0.5 μg/l for total pesticides. Consequently, there is a need for fast, sensitive and reliable techniques for the determination of pesticides in aqueous environmental samples. Ideally, it should be possible to meet the above requirements by injecting an aqueous sample of interest into an analyser without any sample pretreatment.

In many laboratories reversed-phase column liquid chromatography (RPLC) is routinely used to determine polar pesticides in environmental water samples. In almost all instances, however, time consuming sample pretreatment is required and the total analytical procedure cannot be carried out on-line. Still, in recent years an important step forward has been made by the

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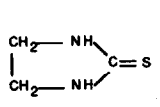
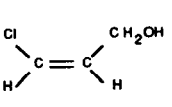
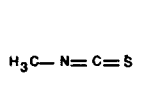
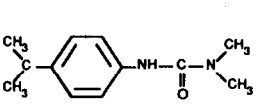
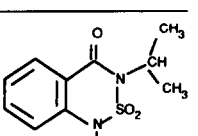
further development of so-called coupled-column or column-switching procedures which involve preseparation of a sample on the first —often low-efficiency— column, heart-cutting of the analyte-containing fraction to the second column and final analysis of this fraction on the latter —invariably high-efficiency— column. If such procedures are based on RPLC-type separations and are used for the analysis of aqueous samples, it is an additional advantage that relatively large volumes can be injected without causing extensive band broadening. In other words, a certain degree of on-line trace enrichment can be effected. Such approaches are well documented [1–8] and do not require further discussion. However, problems are encountered when highly polar analytes have to determined. Retention now is low even on highly hydrophobic C_{18}-bonded-silica phases and, consequently, trace enrichment becomes difficult because the analyte starts to elute during injection. Moreover, clean-up becomes less efficient, because the possibility of separating the analytes of interest from the invariably present early-eluting interferences will be rather limited.

In recent papers, we have used the above approach for the trace-level determination of three very polar analytes, *viz.* chloroallyl alcohol (CAAL) [9], ethylenethiourea (ETU) [10] and methylisothiocyanate (MITC) [11]. When two LC columns of essentially the same high efficiency were used in a coupled-column LC–UV set-up, the solutes of interest could be determined in water samples down to a level of 1 μg/l by means of direct large-volume (200–800 μl) injections. The total time of analysis was a mere 5–7 min. Unfortunately, however, each of the quoted analytes displayed one distinctly unfavourable characteristic, *viz.* very low retention on C_{18}-bonded silica (ETU), an extremely non-selective detection wavelength (CAAL), or a small molecular extinction coefficient (MITC) (see Table I). This prevented a further improvement of the on-line system performance to meet the EC requirement of a 0.1 μg/l detection limit. (To achieve that, RPLC-UV had to be preceded by off-line liquid–liquid extraction.)

Still, the simplicity of coupled-column LC and its high sample throughput of 7–10 h make it rather attractive for screening purposes if (single) analytes that are somewhat less polar than ETU, CAAL and MITC, and have similar or slightly

TABLE I

DETAILS OF POLAR COMPOUNDS ANALYSED BY MEANS OF LARGE-VOLUME-INJECTION COLUMN-SWITCHING RPLC WITH UV DETECTION

Parameter	Ethylenethiourea (ETU)	Chloroallyl alcohol (CAAL)	Methylisothiocyanate (MITC)	Isoproturon	Bentazone
Formula					
Water sol. (g/l)	20	Infinite	8	0.07	0.5
k' [a]	1.6	7.0	20	>100	>100
λ (nm)	233	205	237	244	220
ε (l/mol cm)	18 000	10 000	3000	17 000	25 000
Sample vol. (ml)	0.20	0.20	0.77	4.00	2.00
LOD (μg/l)[b]	1	1	1	0.1	0.1
Time of analysis (min)	5	7	7	10	10

[a] On 5-μm Hypersil ODS; mobile phase, pure water for ETU/CAAL/MITC/isoproturon and aqueous 0.1% phosphoric acid for bentazone.

[b] Detection limit (signal-to-noise ratio = 3) of analyte in ground, surface and rain water.

better UV detection characteristics, have to be determined. In other words, coupled-column RPLC-UV using direct large-volume injections may well prove to be an elegant monitoring method for a large majority of the many medium-polarity pesticides in use today. In the present paper this is demonstrated using isoproturon and bentazone, two typical representatives of this class of compounds, as test solutes (Table I).

EXPERIMENTAL

Materials

Isoproturon and bentazone (content > 99.5%) were obtained from Dr. S. Ehrenstorfer (Promochem, Wesel, Germany). Acetonitrile (HPLC-grade S), methanol (HPLC grade) and phosphoric acid (analytical-reagent grade, 89% pure) were from Rathburn (Walkburn, UK), Promochem and Merck (Darmstadt, Germany), respectively. HPLC-grade water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Bedford, MA, USA). A 1000 μg/ml stock solution of each pesticide was prepared in acetonitrile. For LC analysis the stock solution of isoproturon was diluted in HPLC-grade water and the stock solution of bentazone in a 0.02 *M* phosphate buffer, pH 2.3. The diluted solutions were kept in a refrigerator at 4°C.

RPLC analyser

The schematic of the coupled-column RPLC analyser is shown in Fig. 1. The system consists of two isocratic Model 305 LC pumps (P-1 and P-2) from Gilson (Villiers-le-Bel, France), a Model 232 autosampler (AS) from Gilson equipped with a Type 7010 high-pressure column-switching valve (HP) from Rheodyne (Berkeley, CA, USA), a Model 116 variable-wavelength UV–Vis detector (D) from Gilson and two analytical separation columns, both packed with 3-μm C_{18} Microspher from Chrompack (Bergen op Zoom, Netherlands) with dimensions of 50 × 4.6 mm I.D. (C-1) and 100 × 4.6 mm I.D. (C-2), respectively.

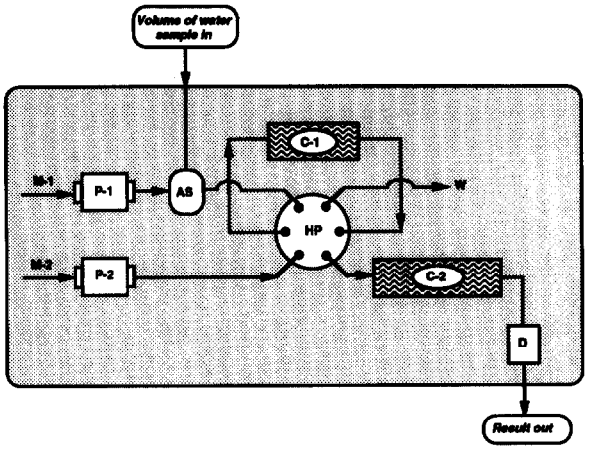


Fig. 1. Schematic of the coupled-column RPLC analyser. M-1, M-2 = first and second mobile phases; C-1, C-2 = first and second C_{18} separation columns; P-1, P-2 = LC pumps; AS = autosampler; HV = high-pressure switching valve; D = UV detector; W = waste.

Procedures

Isoproturon. Approximately 20 ml of sample were passed through a 0.45-μm Millex filter (Millipore) and collected in an autosampler vial of 20 ml. A volume of 4.00 ml was taken from this vial and injected onto C-1. After injection, column-switching RPLC was carried out using a clean-up volume of 5.85 ml (injection volume included) of M-1 [acetonitrile–water (47.5:52.5, v/v) at 1 ml/min], and, after switching C-1 on-line with C-2, a transfer volume of 0.40 ml of M-1 (duration, 24 s). In this instance, separation on C-2 was performed with a mobile phase, M-2, having the same composition and flow-rate as M-1. UV detection was at 244 nm.

Bentazone. A 20 ml water sample was acidified by adding 20 μl of phosphoric acid, passed through a 0.45-μm filter and collected in an autosampler vial. A volume of 2.00 ml was taken from this vial and injected onto C-1. After injection, a clean-up volume of 4.65 ml (injection volume included) of M-1 [methanol–0.02 *M* phosphate buffer, pH 2.3 (50:50, v/v)] at 1 ml/min was used. Analyte transfer was carried out for 27 s (0.45 ml of M-1) after switching C-1 on-line with C-2. Separation on C-2 was carried out with methanol–0.02 *M* phosphate buffer, pH 2.7 (50:50, v/v), at 1 ml/min as mobile phase M-2; UV detection was at 220 nm.

Both of the above procedures were fully automated. Quantification was done by peak height comparison after RPLC–UV of equal volumes of aqueous standard and sample solutions.

RESULTS AND DISCUSSION

In several earlier studies, the main parameters governing analyte detectability and sensitivity in coupled-column RPLC have been discussed, and a general scheme for method development has been represented [11]. Briefly, there are two basic requirements. Firstly, one needs sufficient preseparation between the analyte and UV-absorbing ionic species, such as anions and humic acids, in order to enhance the selectivity of the chromatographic process. Secondly, relatively large sample volumes have to be introduced into the RPLC system without undue band broadening in order to obtain the desired low limits of detection. Table I shows the relevant properties of the five compounds discussed in this paper. As outlined in the Introduction, when only one out of the three key characteristics (k', λ_{max}, ε_{max}) is unfavourable, direct 200–800 μl sample analysis still yields limits of detection of 1 μg/l.

Obviously, sample analysis using the coupled-column RPLC analyser of Fig. 1 will permit one to reach the EC drinking-water limit of detection of 0.1 μg/l for all analytes having marginally better characteristics than the three compounds referred to above, *i.e.* for essentially all (polar) pesticides and many related individual compounds of current interest. In order to verify this assumption, isoproturon and bentazone —which can be considered to possess typically "average" characteristics within the quoted class of compounds— were selected as test solutes. As can be calculated from their molecular extinction coefficients included in Table I, injection volumes of about 4.00 ml (isoproturon) or 2.00 ml (bentazone) should be sufficient to reach a 0.1 μg/l detection limit. Because of the high k' values in purely aqueous solutions, such volumes can be injected without causing serious additional band broadening. However, one should bear in mind that the total volume of mobile phase used in this type of analysis typically is less than 10 ml. In other words, a sample injection of more than 1 ml represents a significant part of the total chromatographic process, *i.e.* the most polar interferences will start to elute during injection. To out it differently, in the proposed procedures sample injection is the first step of a (multi)step-gradient elution with the sample as the first mobile phase. In practice, no additional band broadening was observed by us for the present large-volume injections of isoproturon and bentazone. That is, the requirement concerning analyte detectability can obviously be met.

As regards selectivity, it lies at hand to use an earlier simulation programme for the development of methods using step-gradient elution [12]. Unfortunately, this approach cannot easily be used for the determination of polar compounds in aqueous samples, because the interfering peak(s) originating from the matrix cannot be defined properly by plots of ln k' *vs.* mobile phase composition, as it required for this simulation programme. Especially in the case of acidic compounds such as bentazone, interferences show up as a broad (tailing) hump, eluting in the same region as the analytes [13]. Trial and error seems to be the only way to obtain an acceptable solution. Still, on the basis of earlier experiences, we can define two major boundary conditions, *viz.* (i) the clean-up volume, which is the volume of mobile phase M-1 used on column C-1, should at least be twice the dead volume of that column, and (ii) the capacity factor of the analyte in the mobile phases M-1 and M-2 should be between 2 and 5, in order to achieve short times of analysis and good sensitivity. On the basis of the above considerations, coupled-column RPLC–UV procedures were elaborated for isoproturon and bentazone. Since, in such procedures, the somewhat contradictory demands of large-volume injections and high sensitivity/selectivity have to be met, large-diameter (4.6 mm I.D) analytical columns packed with high-efficiency material (3-μm Microspher C_{18}) were invariably used.

Determination of isoproturon

Initial experiments showed acetonitrile to be preferable to methanol because of slightly better peak shapes and a lower operational pressure.

Acetonitrile–water (50:50, v/v) —which gave $k' = 3$ for isoproturon— was the first choice as mobile phase M-1. Under these conditions sample volumes of up to at least 4.00 ml could be injected without any noticeable band broadening compared with 100-μl injections. Using the same mobile phase compositions for M-1 and M-2, and a suitably adjusted small transfer volume, isoproturon could now be determined down to a level of 0.1 μg/l in aqueous standard solutions. However, for spiked surface water samples the separation between analyte and matrix interferences in the first part of the chromatogram was not sufficient. Improved results were obtained by lowering the eluotropic strength of both M-1 and M-2 (47.5% instead of 50% of acetonitrile). The application of gradient elution, *i.e.* the use of different percentages of acetonitrile in M-1 and M-2, did not improve selectivity and/or sensitivity substantially. Using a clean-up volume of 5.75 ml on column C-1—that is 4.00 ml of injected water sample plus 1.75 ml of M-1 (!)— and a transfer volume of 0.40 ml of M-1, isoproturon could be determined in surface water samples down to a level of 0.1 μg/l. A typical example of a real-life analysis is shown in Fig. 2.

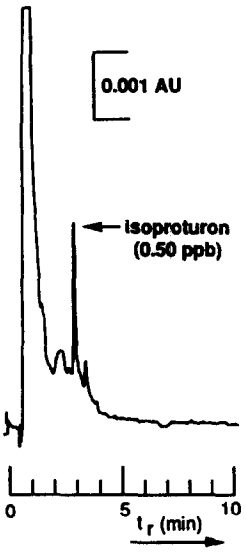


Fig. 2. Column-switching RPLC–UV (244 nm) using direct 4.0-ml sample injection of (A) a surface water sample spiked with 0.5 μg/l (ppb) isoproturon and (B) a blank surface water. Displayed chromatograms start after clean-up on C-1. For LC conditions, see Experimental section.

The repeatability of the procedure was tested with surface water. Samples spiked with 0.2–0.5 μg/l isoproturon showed a mean recovery of 98% and a relative standard deviation (R.S.D.) of 2.8% ($n = 8$). Calibration curves were linear ($r = 0.9998$) over the range 0.1–200 μg/l (Five data points in duplicate.)

Determination of bentazone

When analysing water samples for an acidic compound such as bentazone, two additional boundary conditions are important during method development. The pH of mobile phase M-1 should be as low as possible for the processing of large sample volumes, pH 2.3 being about the best one can achieve when working with alkyl-modified silicas. In addition, modifier-based gradients should be avoided in order to prevent interferences due to the continuous release of humic and/or fulvic acids from the column during such a gradient.

The analytical columns, C-1 and C-2, were the same as in the previous example. In this instance, however, mobile phases based on methanol–phosphate buffer were preferred (cf. Ref. 14). With pH 2.3–2.7, the capacity factor of bentazone was about 3 when using methanol–phosphate buffer (50:50, v/v) as mobile phase. Under these conditions, a sample of up to at least 2.00 ml could be injected on C-1 without additional band broadening of the bentazone peak.

Fig. 3 gives a nice impression of the problems encountered in the development of the coupled-column procedure for bentazone. As can be seen, small changes in the mobile phase composition have a dramatic impact on the final result. As in earlier studies, a conventional step gradient was used. Fig. 3A shows the chromatogram obtained with a step gradient from 50 to 60% methanol, which, apparently, releases quite a lot of interferences. However, omitting the step gradient does not provide enough selectivity, as is demonstrated in Fig. 3B. Fig. 3C shows that the combined use of a modifier and a pH gradient has a distinctly beneficial influence, but quantification of bentazone will still be rather

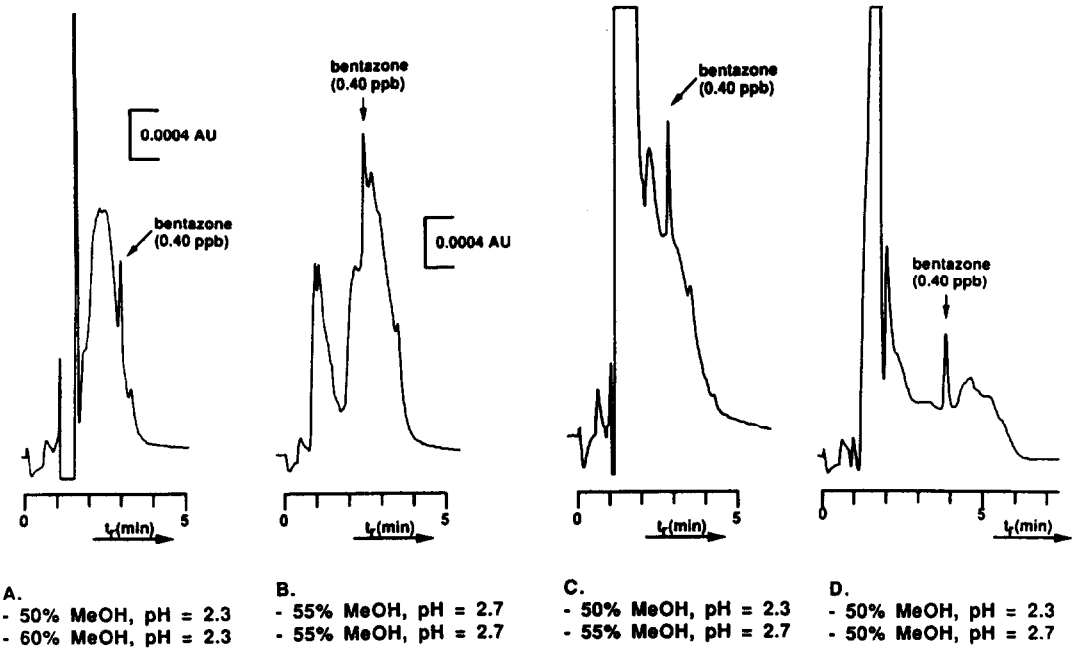


Fig. 3. Selectivity effected with the different step gradients given above using coupled-column RPLC of a surface water containing 0.40 μg/l bentazone, using direct sample injection (2.00 ml). Clean-up volumes: A, C and D, 4.65 ml of M-1 and B, 3.75 ml of M-1; transfer volumes: A, C and D, 0.50 ml of M-1 and B, 0.40 ml of M-1; MeOH = methanol. For LC conditions, see text. Displayed chromatograms start after clean-up on C-1.

difficult. In the end, restricting ourselves to a pH step gradient only gave the best approach, as can be seen from Fig. 3D. This figure also shows that the final goal of a coupled-column procedure enabling detection at the 0.1 μg/l level has been reached. As an illustration of the potential of the procedure, the results of RPLC–UV analyses of a drinking and a surface water sample spiked with bentazone at the 0.1 μg/l level are shown in Fig. 4. Compared with our earlier procedure, which involved a manual liquid–liquid extraction [14], the gain in sample throughput is considerable (total time of analysis, 8–10 min; seven samples per hour).

It is an interesting feature of the present heart-cutting procedures that only a small fraction of the interfering material reaches the second column, C-2, and subsequently, of course, the detector. This is nicely illustrated in Fig. 5 for a surface water sample; in this instance, the clean-up time, t_c, is included in the RPLC–UV traces shown. When processing a series of samples, the next analysis can be started shortly (*ca.* 0.5 min)

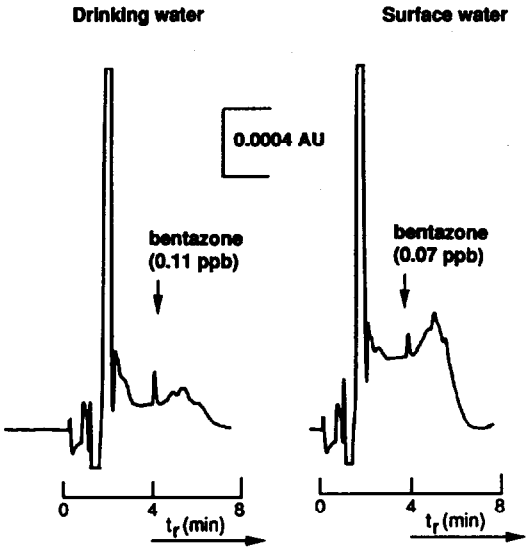


Fig. 4. Column-switching RPLC–UV (220 nm) with direct sample injection (2.00 ml) of a drinking water and a surface water sample spiked with *ca.* 0.1 μg/l. For LC conditions, see Experimental section. Displayed chromatograms start after clean-up on C-1.

TABLE II

DETERMINATION OF BENTAZONE IN SURFACE WATER BY RPLC–UV USING LIQUID–LIQUID EXTRACTION (LLE) OR DIRECT LARGE-VOLUME INJECTION

Sample No.	Bentazone content (μg/l) found using	
	LLE	Direct sampling
1	0.06	0.08
2	0.37	0.40
3	<0.02	<0.05
4	0.66	0.71
5	0.35	0.33

after analyte transfer to column C-2. The real time of analysis therefore is only about 8 min.

Calibration curves were linear ($r = 0.9999$) over the range 0.11–110 μg/l (five data points in duplicate). The recovery of bentazone form drinking water spiked at the 1.1 and 0.11 μg/l level was 98% (R.S.D., 0.5%; $n = 5$) and 86% (R.S.D., 5.7%; $n = 6$), respectively. Similar results were obtained for rain water. Two real-life surface water samples containing bentazone were analysed on five consecutive days. The reproducibilities were 6.0% and 6.5% at the 0.40 μg/l and 0.08 μg/l level, respectively. Five surface water samples were analysed both by the

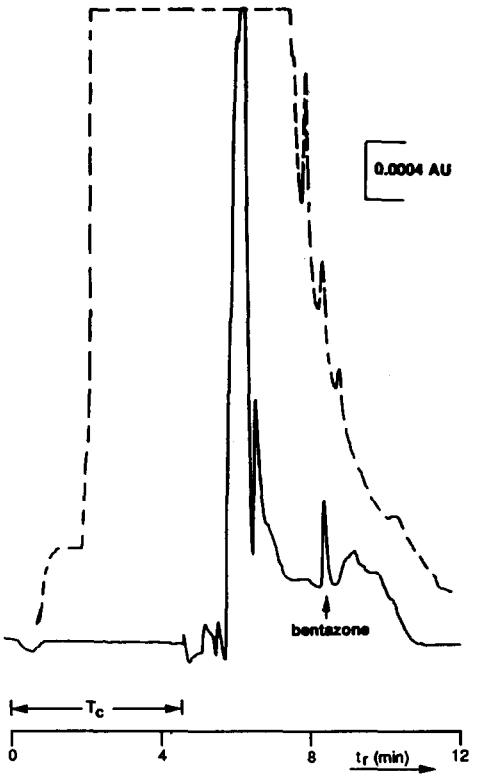


Fig. 5. RPLC–UV (220 nm) of a surface water sample containing 0.40 μg/l bentazone; injection volume, 2.00 ml. Solid line, chromatogram obtained with the coupled-column procedure (see Experimental section); dashed line, chromatogram obtained with the same two columns coupled on-line without column switching using a mobile phase of methanol–0.02 *M* phosphate buffer, pH 2.7 (50:50, v/v) at 1 ml/min; t_c, clean-up time on C-1 with coupled-column procedure.

TABLE III

TYPICAL RESULTS OF REAL-LIFE WATER SAMPLE ANALYSES USING THE COUPLED-COLUMN RPLC-UV ANALYSER

Date	Pesticide	Type of sample	No. of samples investigated	No. of positive samples	Pesticide content (μg/l)
11/10/91	Bentazone	Surface water	5	5	0.1–0.7
07/09/92	Bentazone	Ground water	8	3	0.3–0.5
07/09/92	Isoproturon	Surface water	5	0	<0.1
10/09/92	Methabenzthiazuron	Surface water	7	0	<0.1
19/10/92	Linuron	Surface water	12	12	2–8
30/01/93	Bentazone	Ground water	12	4	0.4–90
09/02/93	Bentazone	Ground water	10	8	2–10

present method and by the earlier method involving liquid–liquid extraction. As listed in Table II, the agreement between the two sets of results was fully satisfactory.

CONCLUSIONS

Coupled-column RPLC with UV detection using direct large-volume injections is a highly useful technique for the rapid trace analysis of single polar pesticides in aqueous environmental samples such as drinking, ground and surface water. For compounds with LC retention and UV detectability characteristics typical for this class of compounds, detection limits are of the order of 0.1 μg/l. The results presented in this paper demonstrate the viability of this approach, which features a throughput of 5–7 samples per hour. The coupled-column RPLC–UV analyser used in our studies is fully automated and has shown good robustness over a period of more than 16 months. Typical results of monitoring programmes carried out during this period of time for bentazone and isoproturon as well as two other pesticides, metribuzine and linuron, are summarized in Table III. The development of the various dedicated procedures was relatively rapid in all instances.

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