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Semi-permeable surface analytical reversed-phase column for the improved trace analysis of acidic pesticides in water with coupled-column reversed-phase liquid chromatography with UV detection Determination of bromoxynil and bentazone in surface water

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Abstract

The coupled-column (LC–LC) configuration consisting of a 3 μm C_{18} column (50×4.6 mm I.D.) as the first column and a 5 μm C_{18} semi-permeable-surface (SPS) column (150×4.6 mm I.D.) as the second column appeared to be successful for the screening of acidic pesticides in surface water samples. In comparison to LC–LC employing two C_{18} columns, the combination of C_{18}/SPS-C_{18} significantly decreased the baseline deviation caused by the hump of the co-extracted humic substances when using UV detection (217 nm). The developed LC–LC procedure allowed the simultaneous determination of the target analytes bentazone and bromoxynil in uncleaned extracts of surface water samples to a level of 0.05 μg/l in less than 15 min. In combination with a simple solid-phase extraction step (200 ml of water on a 500 mg C_{18}-bonded silica) the analytical procedure provides a high sample throughput. During a period of about five months more than 200 ditch-water samples originating from agricultural locations were analyzed with the developed procedure. Validation of the method was performed by randomly analyzing recoveries of water samples spiked at levels of 0.1 μg/l (n=10), 0.5 μg/l (n=7) and 2.5 μg/l (n=4). Weighted regression of the recovery data showed that the method provides overall recoveries of 95 and 100% for bentazone and bromoxynil, respectively, with corresponding intra-laboratory reproducibilities of 10 and 11%, respectively. Confirmation of the analytes in part of the samples extracts was carried out with GC–negative ion chemical ionization MS involving a derivatization step with bis(trifluoromethyl)benzyl bromide. No false negatives or positives were observed. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Coupled columns; Water analysis; Environmental analysis; Pesticides; Bentazone; Bromoxynil

1. Introduction

The trace analysis of acidic pesticides in environmental samples employing reversed-phase liquid chromatography with UV detection (RPLC–UV) is usually severely hampered by co-extracted humic

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and fulvic acids [1–8]. These types of interferences show up in the chromatogram as a broad hump. Most analytes co-elute on the steep slope or, even worse, on top of the hump making reliable quantification difficult or impossible. In case of water samples improved separation has been obtained by using selective solid-phase extraction (SPE) sorbents for off-line [1–6] or on-line [1,7,8] preconcentration.

Because of the large differences in molecular masses of pesticides (small molecules) and humic compounds (large molecules) a (pre)separation on the basis of molecular size seems attractive. For example, for the trace analysis of several types of pesticides in soils gel permeation chromatography (GPC) has been applied as an effective off-line technique for the clean-up of soil extracts prior to instrumental analysis [9–12]. However, the relatively large elution volumes of the analyte-containing fractions and the low compatibility between the mutual mobile phases severely limits the on-line coupling of GPC and RPLC in this type of analysis.

Analytical columns packed with reversed-phase restricted access media (RAM) materials seem attractive in this field of analysis because they provide efficient separation of analytes and exclusion of retention of large molecules. Initially, these RAM columns have been developed and used for the analysis of target compounds in serum by direct injection. The various types of RAM materials and applications in the biomedical field of analysis have been reviewed recently [13,14].

The advantageous use of an analytical RAM column in coupled-column RPLC (LC–LC) was clearly demonstrated by us for the direct trace analysis of the drugs salbutamol and clenbuterol in serum [15]. Analytical columns packed with semi-permeable surface (SPS) and internal-surface reversed-phase (ISRP) material were tested. An ISRP column (50×4.6 mm) appeared most favorable as a first column (C-1) in LC–LC as regards large volume serum injection and fast elution of serum proteins [15].

These results initiated us to investigate the feasibility of analytical RAM columns in LC–LC for the improved pre-separation between humic substances and acidic pesticides. Our first study on this topic involved the trace analysis of the chlorophenoxy acid herbicide mecoprop in soils [16]. In this study the ISRP column (50×4.6 mm I.D.) was selected as the first column (C-1) and a short 3 μm C_{18} column (50×4.6 mm I.D.) as the second column (C-2). It was clearly shown that the ISRP column provided an efficient (pre)separation between analyte and humic substances allowing the LC–LC–UV (220 nm) analysis of mecoprop in uncleaned soil extracts down to a level of 20 μg/kg [16].

This study demonstrates the advantageous use of an analytical SPS column (150×4.6 mm I.D.) as the second column (C-2) in coupled-column RPLC for the trace analysis of bentazone and bromoxynil in environmental water samples.

This work describes the development and validation of the analytical procedure as part of our contribution to a comprehensive study involving the monitoring of the two acidic herbicides in hundreds of ditch-water samples originating from agricultural-use locations.

2. Experimental

2.1. Chemicals

Bentazone and bromoxynil (content>99%) were from Dr. S. Ehrenstorfer (Promochem, Wesel, Germany). Acetone, acetonitrile and methanol, all of HPLC-grade, were from J.T. Baker (Deventer, The Netherlands). Analytical-grade trifluoroacetic acid (TFA) and phosphoric acid (89% pure) were from Merck (Darmstadt, Germany). HPLC-grade water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Bedford, MA, USA).

[$^2H_7$]Bentazone (bentazone-d_7, 95 atom% deuterium) used as internal standard for GC–MS analysis was synthesized as described by Jacquemijns et al. [17]. Potassium carbonate (K_2CO_3) and 3,5-bis(trifluoromethyl)benzyl bromide (3,5-BTFMB-Br) were from Merck and Aldrich (Bornem, Belgium), respectively.

A stock standard solution (ca. 500 μg/ml) of each pesticide was prepared in acetonitrile. For spiking or LC analysis the stock solutions were diluted in acetonitrile–0.1% phosphoric acid in water (20:80, v/v), respectively. The diluted solutions were kept in the refrigerator at 4°C.

The mobile phase (M-1) applied on the first

column (C-1) consisted of acetonitrile–0.03 *M* phosphate buffer, pH 2.4 (35:65, v/v). On the second column (C-2) a mobile phase (M-2) of acetonitrile–0.03 *M* phosphate buffer, pH 2.4 (40:60, v/v) was used.

Disposable 3-ml SPE cartridges containing 500 mg of C_{18} bonded phase (40 μm) were obtained from J.T. Baker. The cartridges were preconditioned with 3 ml of methanol, 3 ml of acetone, 3 ml of methanol and 6 ml of 0.1% phosphoric acid in water, respectively.

2.2. Equipment

A Baker-10 system of J.T. Baker was used to perform SPE. The HPLC system consisted of a Model 231 XL autosampler from Gilson (Villiers-le Bel, France) equipped with an additional six-way programmable high-pressure valve (type 7010, Rheodyne, Cotati, CA, USA) for column switching, a Model 305 and a Model 306 isocratic LC pump from Gilson, and a Model 785A UV detector from Applied Biosystems (Foster City, CA, USA).

In the coupled-column LC analysis a 50×4.6 mm I.D. column packed with 3 μm C_{18} Microspher (Chrompack, Middelburg, The Netherlands) and a 150×4.6 mm I.D. column packed with 5 μm SPS-5PM-S5-100-ODS (Regis, Morton Grove, IL, USA) were used as a first column (C-1) and second column (C-2), respectively. A pre-column (10×3 mm I.D.) packed with the same material was installed before the first analytical column (C-1).

A semi-preparative guard column (10×10 mm I.D.) packed with 5 μm SPS 5/100 octyl was tested as C-1 in LC–LC.

The LC columns were kept at 30°C with a laboratory-made column oven connected to a Model 1441 circulating water system from Braun (Melsungen, Germany). Quantitative measurements of peak heights were made with the PC-1000 integrator system of TSP employing a Model 800 DP integrator from Fisons.

The gas chromatography–mass spectrometry (GC–MS) system consisted of a Model HP 5890 gas chromatograph from Hewlett-Packard hyphenated to a Finnigan MAT SSQ710 system, with a Digital 5000/25 workstation and ICIS software. A Rtx-1 capillary column of 50 m×0.25 mm I.D. (film thickness 0.1 μm) was from J&W Scientific (Folsom, CA, USA).

2.3. Sample pretreatment

2.3.1. Extraction

A 200-ml water sample was brought to pH 2.1 (±0.1) with 200 μl of TFA, and percolated through a preconditioned 500 mg C_{18} cartridge at a flow of approximately 4 ml/min. After sample loading the cartridge was dried by passing air for 20 min. The cartridge was transferred to the top of a calibrated tube, and by means of slight over-pressure, 2 ml of acetone was passed through the cartridge and collected in the tube. After the volume was adjusted to 4 ml of acetone, the solution was split into two portions of 2 ml for the processing of LC analysis (always) and GC–MS confirmatory analysis (optional), respectively.

Prior to the LC analysis the acetone extract was evaporated to dryness using a warm water bath and a gentle stream of nitrogen. The residue was dissolved by first adding 400 μl of acetonitrile, followed by 1600 μl of 0.1% phosphoric acid in water.

2.3.2. Derivatization (optional)

A 1-ml volume of the acetone extract is transferred into a centrifuge tube and, if necessary (not fully transparent), centrifuged for 5 min (5000 rpm). After transferring the clear organic solvent into a reagent tube, 100 μl of the internal standard solution bentazone-d_7 (about 100 ng/ml) were added. The extract is evaporated with a gentle stream of nitrogen at a temperature of about 60°C. The residue is redissolved in 1 ml of acetonitrile, and about 50 mg K_2CO_3 and 20 μl of BTFMB-Br reagent were added. Derivatization of analytes was performed by heating the solution for 1 h at 90°C. After cooling to ambient temperature, this solution was further processed with GC–MS.

2.4. LC–LC analysis

The mobile phases were adjusted to a flow-rate of 1 ml/min. A volume of 200 μl obtained after the SPE clean-up procedure was injected on C-1. After clean-up with 2.9 ml of M-1 (injection volume included), C-1 was switched on-line with C-2 for

0.90 min for the transfer of the analyte containing fraction to C-2.

Quantification of bentazone and bromoxynil was done by external calibration with standard solutions of the analytes in acetonitrile–0.1% phosphoric acid in water (20:80; v/v).

2.5. Confirmation with GC–MS (optional)

In case of required confirmation an aliquot of 1 to 2 μl of the extract obtained after derivatisation was injected in the splitless mode. The injector and interface temperatures both were 250°C. The column-oven temperature is initially held on 70°C for 1 min and programmed with a rate of 15°C/min to 220°C and subsequently with a rate of 5°C/min to a final temperature of 260°C.

The mass spectrometer was operated in the negative ion chemical ionization mode at 70 eV with methane (or ammonia) as moderator gas. The filament emission current is 50 mA.

In most cases the mass spectrometer was operated in the full scan mode from m/z 200 to m/z 450 in 0.3 s. Operation in selected ion recording (SIR) for monitoring of the m/z 274, 276, 278 and 420 to 423 for bromoxynil, m/z 239 for bentazone and m/z 246 for bentazone-d_7 (I.S.) is optional when a lower detection limit is demanded.

Semi-quantification and confirmation is carried out by calculating the sample peak areas of the m/z 274, 276 and 278 for bromoxynil, m/z 239 for bentazone and m/z 246 for bentazone-d_7 (I.S.). The ratios 276/246 for bromoxynil and 239/246 for bentazone are compared with the same ratios in a reference sample, viz. a processed blank tap water sample spiked with the analytes at the required lowest concentration level of 0.1 μg/l. The first criterion for a positive confirmation of bentazone and bromoxynil is that the mentioned ratios in the sample extract are higher than the corresponding ratio in that of a reference sample.

A second criterion for a positive confirmation of bentazone is that the relative retention time has to be within a window of 1 second compared to the retention time of deuterated bentazone, that is added as an internal standard.

A second criterion for a positive confirmation of bromoxynil is that the relative retention time has to be within a window of 1 second compared to the retention time of the corresponding analyte in a standard. A third criterion for bromoxynil is that the measured areas of m/z 274 and m/z 278 have to be within a range of 15% of the theoretical ratio related to m/z 276 (bromine cluster 274/276/278 is 49:100:51). A fourth criterion for bromoxynil is the presence of the ion clusters m/z 420/422 (1:1) and 421/423 (1:1).

3. Results and discussion

3.1. General aspects

Our laboratory participated in a monitoring study on the occurrence of the acidic herbicides bromoxynil and bentazone to a level of at least 0.1 μg/l in surface water samples originating from agricultural-use locations. Compassing the time before, during and after agricultural application of the herbicides, the sampling of approx. 200 samples involved a period of about five months.

As explained in more detail before [18], our analytical methodology in monitoring programs usually consists of three steps: (i) concentration/extraction of samples shortly after arrival, (ii) screening of batches of collected series of sample extracts, and, if required, (iii) confirmation of positive/negative samples with GC–MS involving a derivatisation procedure prior to the instrumental analysis [17].

As regards the first step we applied SPE on a C_{18} cartridge of a volume of water after adjustment to a pH of about 2 (see Experimental). Method development concerning the other steps will be discussed in the next sections.

3.2. RPLC–UV method development

Following the guidelines of our general applicable strategy [19], firstly selected LC–LC conditions for the simultaneous analysis of bentazone and bromoxynil consisted of two 3 μm C_{18} columns (C-1, 50×4.6 mm I.D.; C-2, 100×4.6 mm I.D.) and mixtures of acetonitrile–phosphate buffer, pH 2.4 of (35:65, v/v) and (40:60, v/v) for the first (M-1) and second (M-2) mobile phase, respectively.

Unfortunately, the selected LC–LC conditions

employing accurate adjusted clean-up and transfer volumes did not provide sufficient selectivity for the analysis of the type of ditch-water samples involved in this study. This is illustrated in Fig. 1A showing the LC–LC–UV analysis of a ditch-water sample spiked with the analytes at a level of 0.5 μg/l. It clearly can be seen that the background signal of the amount of transferred interferences of such a sample will not allow reliable quantification of bentazone at the required level of 0.1 μg/l. The use of pH-based gradients as successfully applied earlier for the determination of bentazone in river water (river Rhine) and ground water [20] did not substantially improve the analyses of these type of samples.

3.3. The use of SPS columns in LC–LC

In further method development we started to investigate the feasibility of RAM columns packed with SPS material, originally developed by Desilets and co-workers [21,22] and schematically displayed in Fig. 2. Table 1 informs on relevant characteristics, viz. material, retention and efficiency, of the selected columns; concerning SPS columns, a preparative guard pre-column (PC) and an analytical column were tested.

As regards the preferable type of LC–LC column configuration to be applied environmental samples some aspects must be considered. In the analysis of serum and plasma, RAM pre-columns have been successfully applied as a first column in LC–LC [23–25]. In comparison to an analytical column on-line clean-up will performed at higher speed and lower costs. Because of lower efficiency (N) of pre-columns (see Table 1 and Ref. [15]) the back-flush mode is preferably used [23–25] in order to avoid undesirable additional band broadening of analytes. It is to be noted that top-column-focusing of analytes during injection and clean-up is mandatory.

However, we have experienced that in case of environmental samples the eluotropic strength of the first mobile phase (M-1) should be maximized, viz. retention of analytes $1<k<5$, in order to enhance (pre)separation between analytes and interferences. Obviously, top column focusing during clean-up is not possible and in order to beneficially utilize the obtained separation on the first analytical column, forward flush is more adequate in environmental analysis.

Unfortunately, the characteristics of the SPS pre-column (see Table 1) make this column not very attractive for use in the forward flush mode. The high retention capacity makes that the somewhat lower efficiency cannot be repaired by a peak compression on the second column using a mobile phase of higher eluotropic strength. This unfavorable effect of band broadening is demonstrated in Fig. 3 for the LC–LC analysis of a standard of bentazone and bromoxynil. It also shows the presence of a system peak (S) originating from the SPS pre-column as a result of column switching.

Next, the performance of the SPS analytical column without column-switching was investigated. As displayed in Fig. 1B, the obtained elution profile of co-extracted humic interferences of a spiked ditch-water sample makes quantification of bentazone and bromoxynil impossible.

The next step was to investigate the feasibility of column-switching. As regards clean-up, the use of the analytical SPS column as C-1 in LC–LC seems most effective. However, the length of this column makes this configuration not suitable. Time for clean-up and transfer will be unnecessary long and the exposure of such an expensive column to the injection of uncleaned extracts is not attractive. As demonstrated recently in our comprehensive study on the use of RAM columns [26], a short analytical SPS column (50×4.6 mm I.D.) is more adequate for use as C-1. However, such a dimension was not available at the time of this study. Therefore, the analytical SPS column was used as C-2 in combination with a short 3 μm C_{18} column (50×4.6 mm I.D.). The performance of the SPS column in this LC–LC configuration is illustrated in Fig. 1C. One clearly can see that in comparison to the use of two C_{18} columns in LC–LC the C_{18}/SPS combination improves significantly the elution profile of humic acid interferences allowing quantification of both herbicides to the required level in this type of samples.

3.4. Method performance and results

Method validation was performed by mixing each measurement sequence of real samples with a few calibration samples consisting of tap water samples

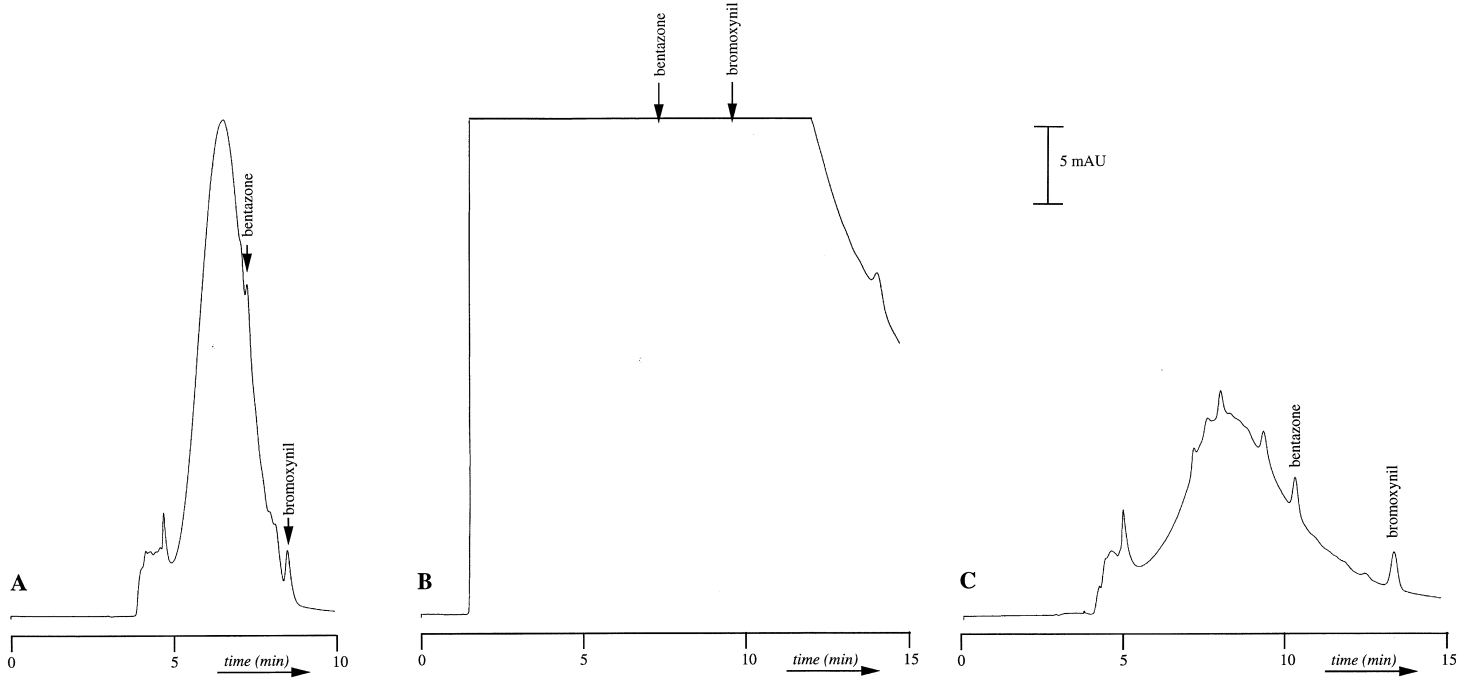

Fig. 1. RPLC–UV (217 nm) of an extract of ditch-water sample spiked with bromoxynil and bentazone at a level of 0.5 μg/l employing different LC column configurations. (A) LC–LC on two 3 μm C_{18} columns (50×4.6 mm I.D.)/(100×4.6 mm I.D.); M-1, acetonitrile–0.03 *M* phosphate buffer, pH 2.4 (35:65); M-2, acetonitrile–0.03 *M* phosphate buffer, pH 2.4 (40:60); clean-up volume, 2.9 ml; transfer volume, 0.9 ml. (B) LC on a 5 μm SPS C_{18} column (150×4.6 mm), M, acetonitrile–0.03 *M* phosphate buffer, pH 2.4 (40:60). (C) LC–LC on 3 μm C_{18} (50×4.6 mm I.D.)/5 μm SPS (150×4.6 mm I.D) columns; further LC conditions as in (A). (A, B and C): Injection, 200 μl of SPE extract corresponding to 10 ml of water sample; flow-rate, 1 ml/min.

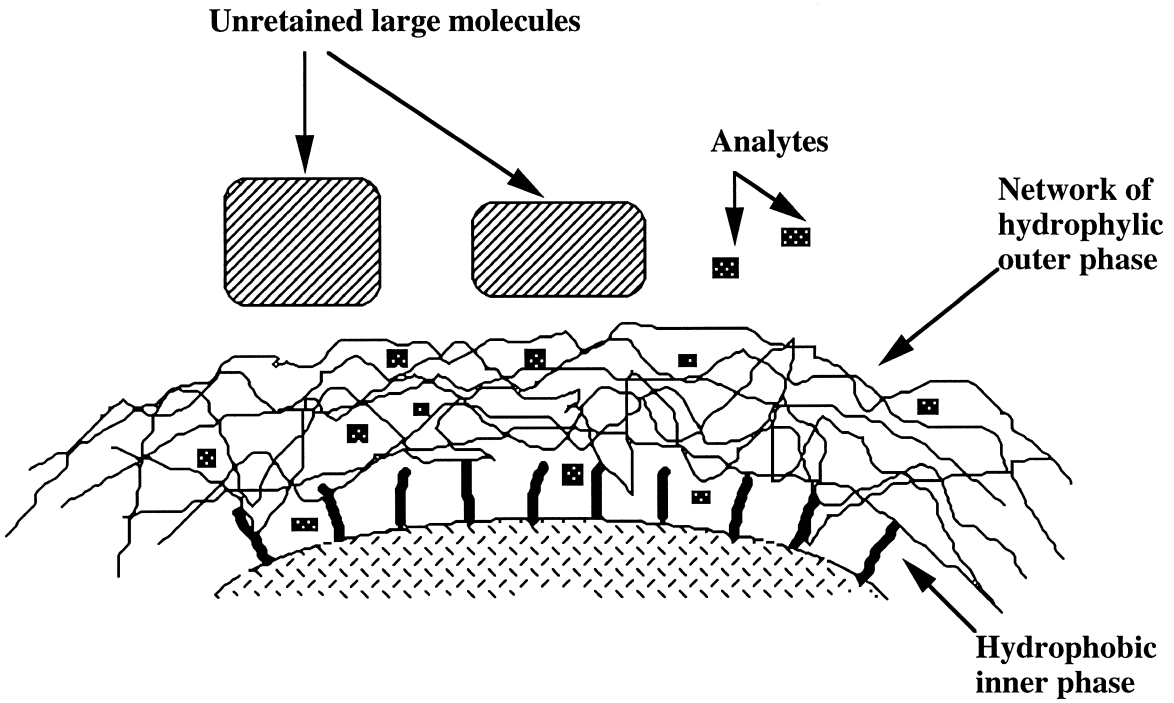

Fig. 2. Schematic representation of the nature and working of the semi-permeable-surface LC packing material (SPS-5PM-5S-100 of Regis). Inner phase, C_8, C_{18}, CN or phenyl chemically bonded to silica surface; outer phase, polyoxyethylene polymer covalent bonded to silica surface.

spiked with bentazone/bromoxynil at different levels. In each series at least one sample spiked at the level of 0.1 μg/l was included in order to guarantee the required lowest level of determination. In total 21 recoveries were analyzed at 11 different days in a five-month period at levels of 0.1 μg/l (n=10), 0.5 μg/l (n=7) and 2.5 μg/l (n=4), respectively. The average recoveries of the whole recovery data set were 95% and 100% for bentazone and bromoxynil, respectively.

The quality of the results of the method was evaluated by processing the obtained recovery data using Calwer 2.2, a computer spreadsheet program for calibration using weighted linear least squares

Table 1
Elution performance of bentazone and bromoxynil on the tested columns

Packing	Column (length×I.D., mm)	Mobile phase composition[a]	V_0 (ml)	Compound	k	N[b] (plates/m)	h[c]
3 μm C_{18}	50×4.6	35:65	0.50	Bentazone	4.6	82 000	4.1
				Bromoxynil	5.7	90 000	3.7
5 μm SPS C_8	10×10	35:65	0.30	Bentazone	3.3	40 000	5.1
				Bromoxynil	5.7	33 000	6.1
5 μm SPS C_{18}	150×4.6	40:60	1.4	Bentazone	3.3	56 800	3.5
				Bromoxynil	5.7	57 000	3.5

[a] Mobile phase constituents (v/v): acetonitrile–0.03 M phosphate buffer, pH 2.4.
[b] Number of column plates per meter, $N=(t_R/\sigma)^2$.
[c] Reduced plate height, $h=H/d_p$ [H, height of one theoretical plate (μm); d_p, average size of one packing particle (μm)].

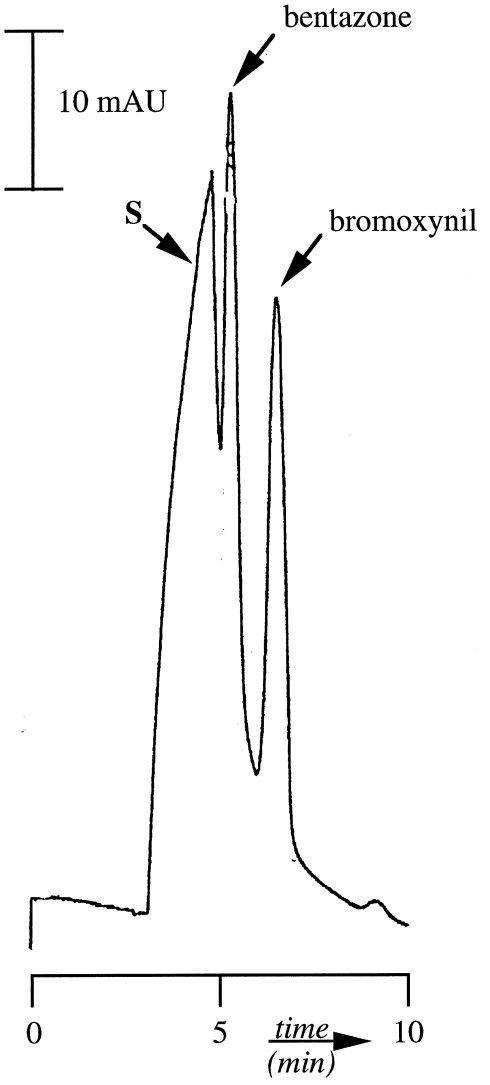

Fig. 3. Performance of the semi-preparative guard SPS column in LC–LC–UV (217 nm) on the elution of bentazone/bromoxynil. C-1, 5 μm SPS C_8 column (10×10 mm I.D.); C-2, 3 μm C_{18} column (50×4.6 mm I.D.); M-1 and M-2, acetonitrile–0.03 *M* phosphate buffer, pH 2.4 (35:65); clean-up volume, 2.5 ml; transfer volume, 2.0 ml. Injection, 200 μl of standard solution (0.12 μg/ml each analyte).

regression analysis [27]. For both compounds various types of calibration models, linear/non-linear, with/without intercept were tested. The contribution from both intercept and non-linearity appeared to be non-significant. Therefore, the most simple model, viz. a straight line through the origin, was used.

Applying weighted regression the variance functions with Calwer 2.2 [27] were estimated as: $s^2_{\text{bentazone}}=(0.016)^2+(0.10\cdot\text{conc.})^2$ and $s^2_{\text{bromoxynil}}=(0.018)^2+(0.11\cdot\text{conc.})^2$.

This implies that the standard deviation of reproducibility within the series of experiments is about 0.015 μg/l for low concentrations (approx. 0.1 μg/l) and about 10% for the ''high'' concentrations (≥0.5 μg/l). The intra-laboratory reproducibilities of 10% (bentazone) and 11% (bromoxynil) emphasizes clearly the robustness of the procedure.

As shown in the variance functions above, the standard deviations (s_0) of the lowest calibration point of the analytical procedure were 0.016 and 0.018 μg/l for bentazone and bromoxynil, respectively, rendering a detection limit ($3s_0$) of 0.05 μg/l for both analytes.

The performance of the screening method is displayed in Fig. 4 showing the LC–LC–UV (217 nm) analysis of a ditch-water sample containing both bentazone (0.37 μg/l) and bromoxynil (2.3 μg/l).

From a total of 221 ditch surfaced water samples screened with the LC–LC–UV method, 31- and 19-times concentrations above the level of 0.1 μ/l were found for bentazone and bromoxynil, respectively.

Despite selective on-line sample clean-up by means of LC–LC, the detection mode is not very selective, hence confirmation of analytes in samples such as displayed in Fig. 4 will be necessary. In agreement with our usual approach [18] confirmation of both bentazone and bromoxynil was carried out by a derivatization with an electron-captive group at the molecule followed by selective/sensitive determination of the reaction products with GC–negative ion chemical ionization MS. The applied semi-quantitative procedure employing BTFMB-Br as reagent for derivatization is described in detail in the Experimental, including the criteria used for positive confirmation.

For GC–MS confirmatory analysis 17 ditch-water samples were selected. This selection included samples with and without residues of bentazone and/or bromoxynil. No false positives or negatives were found in these samples indicating the usefulness of the RPLC–UV screening method.

An important aspect to emphasize is that the LC–LC approach was successful as regards the life time of the expensive RAM column. Under the

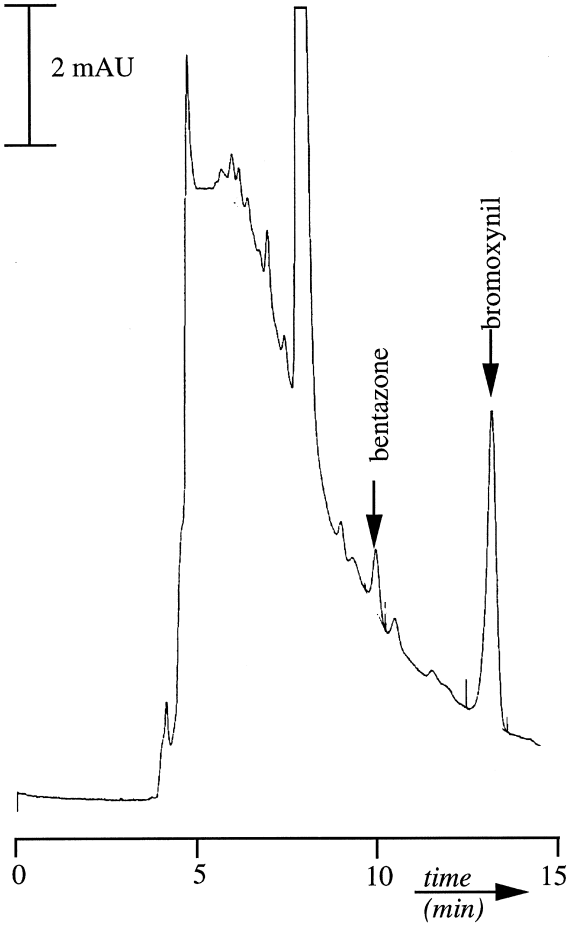

Fig. 4. LC–LC–UV (217 nm) with the C_{18}/SPS column combination of an extract of a ditch surface water sample containing 0.37 μg/l of bentazone and 2.3 μg/l of bromoxynil. Further LC conditions as in Fig. 1C.

rather wearing conditions for bonded silicas, viz. a mobile phase at pH 2.3, the performance of the analytical SPS column remained very well during the five months period of intensive use.

4. Conclusion

The use of an analytical 5 μm SPS column (150× 4.6 mm I.D.) as a second column in coupled column RPLC–UV is favorable for the trace analysis of acidic pesticides in ditch-water samples originating from Dutch agricultural locations. In combination with an efficient 3 μm C_{18} column, the SPS column provides a favorable elution of the co-extracted humic/fulvic acid interferences allowing the determination of bentazone and bromoxynil down to a level of 0.05 μg/l in the ditch-water samples investigated.

An instrumental time analysis of less than 15 min in combination with a rapid SPE provides an efficient procedure with a high sample throughput. The assay of hundreds of samples, the validation data obtained during a five-month period and GC–MS confirmation clearly indicated the good performance and robustness of the coupled-column RPLC–UV (217 nm) screening method.

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