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On-line dual-precolumn-based trace enrichment for the determination of polar and acidic microcontaminants in river water by liquid chromatography with diode-array UV and tandem mass spectrometric detection

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Abstract

Dual-pre-column-based trace enrichment combined on-line with liquid chromatography–diode-array UV and tandem mass spectrometric detection was used to determine a wide polarity range of organic microcontaminants in river water. Various sorbents were studied for their extraction efficiency of (highly) polar and acidic compounds and their ability to selectively remove humic substances, which are normally co-extracted and interfere in the UV detection of polar microcontaminants. An optimised on-line dual-pre-column set-up with PLRP-S in the first pre-column and Hysphere-1 in the second pre-column was used to study the analytical performance of the procedure. Tandem MS was used for confirmation purposes and to quantify the organic microcontaminants in river water at the low-ng/l level. In addition, the influence of the type of sample (drinking and river water) on suppression of analyte responses in electrospray ionization MS was studied.

Keywords: Solid-phase extraction; Water analysis; Environmental analysis; Tandem mass spectrometry; Pesticides

1. Introduction

Today, the monitoring of organic microcontaminants in surface water can be performed in automated systems which use trace enrichment by means of solid-phase extraction (SPE) followed by desorption and on-line separation by column liquid chromatography (LC) and diode-array UV (DAD UV) detection. Quantification in the range of 0.1–5 μg/l has been reported for a variety of medium volatile, and, mostly, neutral compounds such as organophosphorus pesticides, carbamates, triazines and phenylureas [1,2]. For the trace enrichment of these compounds in on-line SPE–LC, styrene–divinylbenzene copolymers such as PLRP-S are frequently used. However, the on-line analysis of highly polar and acidic compounds still creates problems, since they are not always efficiently preconcentrated on PLRP-S and show early breakthrough. Secondly, matrix constituents such as humic substances are co-extracted which results in a huge hump in the early part of the UV chromatogram, which interferes with the compounds of interest [1,2]. Thirdly, with ionic compounds such as phenoxy acid herbicides, which are hardly concentrated under real-life conditions (pH 6–8) due to their charge, acidification of the sample

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is a solution to enhance their retention. Unfortunately, however, this also increases the enrichment of the humic substances. Several groups of workers have studied these problems and come up with various solutions, but none of these has been generally accepted. As regards the determination of (highly) polar and acidic microcontaminants, both polymeric phases, either in an off-line [3] or on-line [4,5] set-up, and graphitized carbon black material, again in an off-line [6,7] or on-line [8,9] set-up, have been recommended as sorbents. Here, one should note that in on-line SPE–LC not only the trapping efficiency of analytes is important, but also the compatibility of the SPE sorbent and the stationary phase in the LC column which should preferably have the same or similar retention capabilities.

Another approach to eliminate the above problems is to use an on-line dual-pre-column set-up [10,11] in which the conventional SPE–LC system is modified by mounting a second pre-column on the second valve. During trace enrichment, the first and, often, the major part of the sample solution of, e.g., 40–50 ml is loaded on the first pre-column. Next, the second pre-column is switched in-line and the final portion of the sample of, say, 5–10 ml, is led through both pre-columns. With this set-up, which ensures removal of the bulk of the humic substances from the most polar analytes, reliable quantitative results were obtained for a number of phenolic compounds up to phenol [10,11] and for several acid herbicides [12]. In the latter study in-line acidification was performed between the first and second pre-column to retain the acidic compounds. However, detection and identification at the very low μg/l level still remained difficult because of the presence of matrix interferences. Consequently, the use of an alternative detection technique such as mass spectrometry (MS) which is frequently used for identification and confirmation, but increasingly also for quantification of compounds of environmental interest [13,14], should be considered. The use of tandem mass spectrometry (MS–MS) in the selected-reaction monitoring (SRM) mode for the quantification of analytes at the low ng/l concentration level in water needs more attention [15–17].

In the present study, the behaviour of various SPE sorbents in terms of their extraction efficiency of (highly) polar and acidic compounds and their capacity to selectively retain humic substances was studied using an on-line dual-SPE–LC–UV set-up. The performance of the overall method was evaluated and the practicality of DAD UV and MS–MS detection for the determination of polar microcontaminants in real-life samples was compared.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade acetonitrile and methanol were purchased from J.T. Baker (Deventer, The Netherlands). HPLC-grade water, generated by distillation of demineralised water in a laboratory-built quartz system, was used to prepare the LC eluent and in the SPE system. The pH of the LC–UV eluent was adjusted by adding the appropriate amount of 1 *M* orthophosphoric acid to a sodium dihydrogenphosphate solution (J.T. Baker) and subsequent dilution to obtain a 10 m*M* solution with a pH of 2.7. In LC–MS experiments, 10 m*M* aqueous ammonium formate (pH 3.0) was used which was prepared in a similar way from formic acid and an ammonium formate solution (J.T. Baker). In addition, 10 m*M* aqueous ammonium acetate (pH 4.0) was prepared with ammonium acetate and acetic acid (Riedel-de Haën, Seelze, Germany). Nitric acid (Riedel-de Haën) was used for acidification of the samples.

The various analytes were purchased from Riedel, Janssen Chimica (Beerse, Belgium), Dr. S. Ehrenstorfer (Augsburg, Germany) and Fluka (Neu-Ulm, Germany). A 1 mg/ml stock solution of each individual compound was prepared in methanol (HPLC-grade; J.T. Baker) and was kept at −20°C. Standard mixtures of 20 μg/ml were made by dilution in methanol. Mixture 1 contained oxamyl, isocarbamide, desethylatrazine, phenol, aldicarb, dicamba, 2-chloroaniline, bentazone, atrazine, diuron, (2-methyl-4-chlorophenoxy)acetic acid (MCPA), 2,4,5-T, alachlor and dinobuton. Mixture 2 contained desethylhydroxy-4-atrazine, oxamyl, isocarbamide, desethylatrazine, phenol, dicamba, bentazone, MCPA and 2,4,5-T.

Nitrogen (99.999% purity; for MS experiments) used for drying and as nebulizing gas, and argon

(99.9995% purity; for MS–MS experiments) were from Praxair (Oevel, Belgium).

2.1.1. Sample treatment

River water samples (Rhine, Lobith, Dutch/German border) were filtered over a 47-mm diameter filter (0.45-μm pores; Millipore, Bedford, MA, USA) mounted in a vacuum filtration unit (Millipore). Tap water samples were used without any pre-treatment. Spiking was carried out just before analysis by adding the appropriate volume of a 20 μg/ml standard mixture to the samples.

2.2. Instrumentation and columns

2.2.1. SPE

A Prospekt (Spark Holland, Emmen, The Netherlands) sample preparation module was used in combination with a LC–DAD UV system. For detailed information on the total set-up and programme conditions, the reader should consult Refs. [11,15]. Briefly, the Prospekt consists of a solvent delivery unit (SDU), a valve-switching unit with three Rheodyne (Berkeley, CA, USA) six-port valves and a clamping system for automated exchange of the pre-columns. For SPE, 10 mm×2 mm I.D. pre-columns packed and kindly provided by Spark Holland were used. The first pre-column is mounted on valve 1 by the clamping system. The second pre-column (mounted on valve 2) was placed in a laboratory-built holder. Some 20 packing materials (particle size in brackets) were tested for their sorption efficiency. These included: two types of C_2 (8 μm), an end-capped C_2 (8 μm), three types of C_8 (two, 8 μm; one, 40–70 μm), three types of end-capped C_8 (two, 8 μm; one, 40–70 μm), three types of C_{18} (two, 8 μm; one, 40–70 μm), two types of end-capped C_{18} (8 μm and 40–70 μm), end-capped CN (8 μm), two types of PLRP-S (15–25 μm and 10–15 μm) from Polymer Labs. (Church Stretton, UK), LiChrolut EN from Merck (Darmstadt, Germany), Isolute ENV+ from International Sorbent Technology (Cambridge, UK), and Hysphere-1 (Spark Holland).

2.2.2. LC–DAD UV

A HP 1090 LC system equipped with a ternary solvent delivery unit and an HP1040 diode-array detector (Hewlett-Packard, Waldbronn, Germany) was used. Separations were carried out on a 250 mm×4.0 mm I.D. Hypersil BDS C_{18} column equipped with a 4 mm×4 mm I.D. LiChrospher 60 RP select B guard column, both packed with 5 μm particles. The system was fully automated and was software-controlled using HPChem software packages.

2.2.3. LC–MS–MS

Tandem mass spectrometry was performed on a Quattro II triple-stage quadrupole equipped with an electrospray ionization (ESI) interface (Micromass, Altrincham, UK). Masslynx software was used for data acquisition and data analysis.

2.3. Analytical procedures and conditions

2.3.1. SPE

The dual-pre-column procedures were similar to those reported in Refs. [11,12]. Briefly, the first pre-column was exchanged after every run. Both pre-columns were conditioned with 5 ml of methanol and, next, with 5 ml of HPLC-grade water at 2.5 ml/min. After flushing of all solvent lines, 40–50 ml of sample was loaded onto the first pre-column at 5 ml/min. In these experiments, the last 5–10 ml of sample was also loaded onto the second pre-column after switching it in-line with the first pre-column. Finally, analytes retained on each pre-column were desorbed in two subsequent gradient runs by switching, first the second and, then, the first pre-column into the LC eluent stream to the analytical column. On both occasions a closure contact signal initiated the data acquisition and the LC gradient programme.

To study the sorption power of various sorbents, analyte breakthrough was studied by loading sample volumes in steps of 5 ml, from 5 to 40 ml, onto the first pre-column. A similar experiment was performed by loading 50 ml of sample on the first pre-column and switching the second pre-column in-line with the first after 45–10 ml and subsequently loading the last 5–40 ml, in steps of 5 ml, onto the second pre-column.

2.3.2. LC

A multi-step gradient programme of 10 m*M* sodium phosphate (pH 2.7) and acetonitrile at 1.0

ml/min was found to be suitable for the separation of all compounds. Between LC runs, the flow-rate was programmed to 0.1 ml/min. In case of MS detection, a linear gradient of 10 m*M* aqueous ammonium formate (pH 3.0) (A) and methanol (B) was applied (10% to 75% B in 14 min). To maintain the flow-rate of the LC eluent entering the ESI interface at 120 μl/min, a post-column splitter was inserted. Details of the various LC–UV gradient programmes are given in the text and the legends to the figures.

2.3.3. DAD UV

For the registration of LC chromatograms, the use of three wavelengths, 215, 254 and 275 nm, was found to be sufficient. The signal recorded at 215 nm was used for quantification. During separation, UV spectra of all peaks were recorded from 200 to 400 nm.

2.3.4. MS–MS

With the selected flow-rate of 120 μl/min, the source temperature was maintained at 120°C. The flow-rates of the drying and nebulizing gas were set at 350 l/h and 20 l/h, respectively. Mass spectra were collected in the full-scan positive-ion (PI) and negative-ion (NI) mode (*m/z* 100–300; scan time, 2 s; interscan time, 0.1 s). The sample cone voltage was optimised for each of the test compounds (for specific values, see text and figures) with a skimmer lens offset of 5 V. The electrospray capillary voltage was set at 4.0 kV for the PI, and at −4.0 kV for the NI mode. MS–MS experiments were carried out with an argon pressure of approx. $2.5 \cdot 10^{-3}$ mbar in the collision cell (Q2). The collision energy was optimised for each of the compounds. Full-scan product-ion spectra were collected with the first quadrupole (Q1) set at a fixed *m/z* value representing $[M+H]^+$ or $[M-H]^-$ in the PI and NI modes, respectively, and with the third quadrupole (Q3) scanning from *m/z* 40 up to 10 u above the molecular mass of the compound studied (scan time, 1 s; interscan time, 0.1 s). For SRM experiments, both Q1 and Q3 were set at fixed *m/z* values, viz. $[M+H]^+$ and $[M-H]^-$ for Q1 in the PI and NI modes, respectively, and two of the most intense product ions for Q3 (dwell time, 0.1 s; interchannel delay, 0.01 s).

2.3.5. Data analysis

Since the band broadening occurring during the on-line elution of the Hysphere-1 pre-column resulted in a less accurate comparison between results obtained in standard and trace-enrichment experiments, loop injections were always performed over both the pre-column and the analytical column. Recoveries and relative standard deviations (RSDs) were calculated relative to the peak areas observed after elution of the first and the second pre-column.

3. Results and discussion

3.1. Selection of SPE sorbents

In order to create a point of reference in our dual-pre-column studies, a set-up with both pre-columns packed with PLRP-S was used for the analysis of the 14-compound mixture 1. Water samples were spiked at the 4-μg/l level and 50 ml were pumped through the first pre-column, and the final 10 ml also through the second pre-column, with subsequent LC–DAD UV analysis. As expected, the medium and non-polar compounds were efficiently retained on the first pre-column, and their quantification created no problems. Breakthrough to, and retention on, the second pre-column occurred for the acidic and (highly) polar compounds. When the river water was acidified prior to analysis, most of the acidic compounds were efficiently retained on the first pre-column; however, the humic acid hump then also increased and reliable quantification was not possible. In-line acidification largely solved this problem. Unfortunately, since the most polar and acidic analytes could be retained on PLRP-S without serious losses due to breakthrough from sample volumes of, at best, 10 ml, detection limits were too high (ca. 1 μg/l). Obviously, other sorbent combinations would be required to reach our goal.

3.1.1. Use of alternative SPE sorbents

Some 20 sorbents were briefly tested with regard to their retention power. None of the alkyl-bonded silicas, whether end-capped or not, and irrespective of their particle size, showed better characteristics than PLRP-S, and no useful selectivity towards

specific analytes was noted either (retention of humic substances, see below). The same was true for the CN-modified silica. The three remaining sorbents, Hysphere-1, LiChrolut EN and Isolute ENV+, however, displayed much stronger sorption towards the polar and acidic compounds – which agrees with literature findings [3–5]. They were, therefore, studied in more detail by means of conventional SPE–LC–DAD UV. In order to obtain results which were not influenced by interfering matrix constituents, experiments were performed with HPLC-grade rather than surface water. Typical results are shown in Table 1. Some interesting observations are that, for oxamyl, the recoveries increased from 40% on PLRP-S to 80–100% for the other three sorbents, and for phenol, from 10% to 60–70% (if pH 7 was used). Such results are in fair agreement with literature data: recoveries of about 80% were found for both oxamyl and phenol with Hysphere-1/50 ml acidified HPLC-grade water [5], and some 65% phenol was recovered from 50 ml of acidified groundwater when using LiChrolut EN or Isolute ENV+ [4]. Because of the very strong sorption of most of the analytes on the selected sorbents, on-line forward-flush gradient elution caused severe band broadening and hampered reliable quantification. Back-flush elution with a gradient starting at 10 m*M* phosphate buffer (pH 2.7)–acetonitrile (90:10, v/v) which caused peak compression on the top of the analytical LC column gave much better results.

As is evident from Table 1, similar recoveries were obtained for all acidic compounds with PLRP-S, Hysphere-1 and LiChrolut EN if the samples were acidified. Hysphere-1 was the only sorbent that gave essentially the same results for neutral and acidified water samples. It is interesting to add that Masqué et al. [18] reported that recoveries of their polar and acidic compounds obtained upon the addition of a 10% solution of Na_2SO_3 to their surface water samples (pH 2.5) were similar to those obtained when Milli-Q standards were analysed. To our opinion, the explanation is that addition of sulphite after sample acidification will cause an increase of the pH. This will cause a decrease of the amount of co-extracted humic substances and, consequently, a decrease of the huge hump in the UV chromatogram. Since the retention power of Hysphere-1 is comparable under acid and neutral condition (cf. above), an overall improved result will be observed.

The present results show that for the on-line SPE–LC analysis of medium and non-polar neutral compounds such as atrazine, diuron and alachlor, PLRP-S performs best in terms of recoveries. This is in line with abundant literature information. For the highly polar and acidic compounds, Hysphere-1 is the preferred choice. In subsequent dual-pre-column

Table 1
Recovery data (%) for analysis in HPLC-grade water (50 ml, 2 μg/l, $n=10$) using four SPE sorbents in on-line SPE–LC–DAD UV[a]

Compound	PLRP-S		Hysphere-1		LiChrolut EN		Isolute ENV+	
	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3
Oxamyl	38	42	97	100	84	100	100	83
Isocarbamide	92	99	97	100	89	100	99	96
Desethylatrazine	80	98	84	83	77	80	75	70
Phenol	10	10	71	56	63	37	62	40
Aldicarb	76	88	80	76	84	74	75	71
Dicamba	–	98	–	99	–	99	–	–
2-Chloroaniline	78	90	74	75	84	73	76	68
Bentazone	17	95	92	96	31	87	–	–
Atrazine	88	100	84	85	74	79	78	78
Diuron	85	100	83	82	75	81	69	75
MCPA	20	98	100	100	64	98	–	–
2,4,5-T	47	96	95	96	51	97	–	–
Alachlor	83	92	83	79	86	77	82	80
Dinobuton	78	93	100	76	76	54	77	66

[a] –, Not detected in UV.

experiments, PLRP-S was therefore used in the first pre-column and Hysphere-1 in the second one, and mixture 2 which contains polar and acidic compounds only, was used for testing.

3.2. Dual-pre-column–LC–UV

Breakthrough of the selected pesticides in the PLRP-S/Hysphere-1 dual-pre-column system was studied by analysing 50-ml water samples; a volume increasing from 5 to 40 ml was led through the second pre-column. At pH 7, no breakthrough on Hysphere-1 was observed for desethylhydroxyatrazine, oxamyl and phenol up to the largest sample volume, 40 ml, whereas breakthrough on PLRP-S was found after about 10 ml. In river water, the corresponding data were 25 ml for Hysphere-1, and 5–10 ml for PLRP-S. Consequently, in all further experiments, 50 ml of river water were loaded onto the PLRP-S pre-column, with the final 25 ml being led to the Hysphere-1 pre-column.

A typical application of the proposed procedure is shown in Fig. 1 for 50 ml of (non-acidified) river Rhine water. Satisfactory results at the 2-μg/l spiking level used in this case, were obtained for the most polar analytes (desethylhydroxyatrazine, oxamyl, phenol; Hysphere-1 pre-column) as well as isocarbamide and desethylatrazine (PLRP-S pre-column) (Fig. 1A). However, the results for the remaining (acidic) analytes were disappointing, with peak broadening seriously affecting the results. If the pH of the surface water samples was adjusted to 3, the

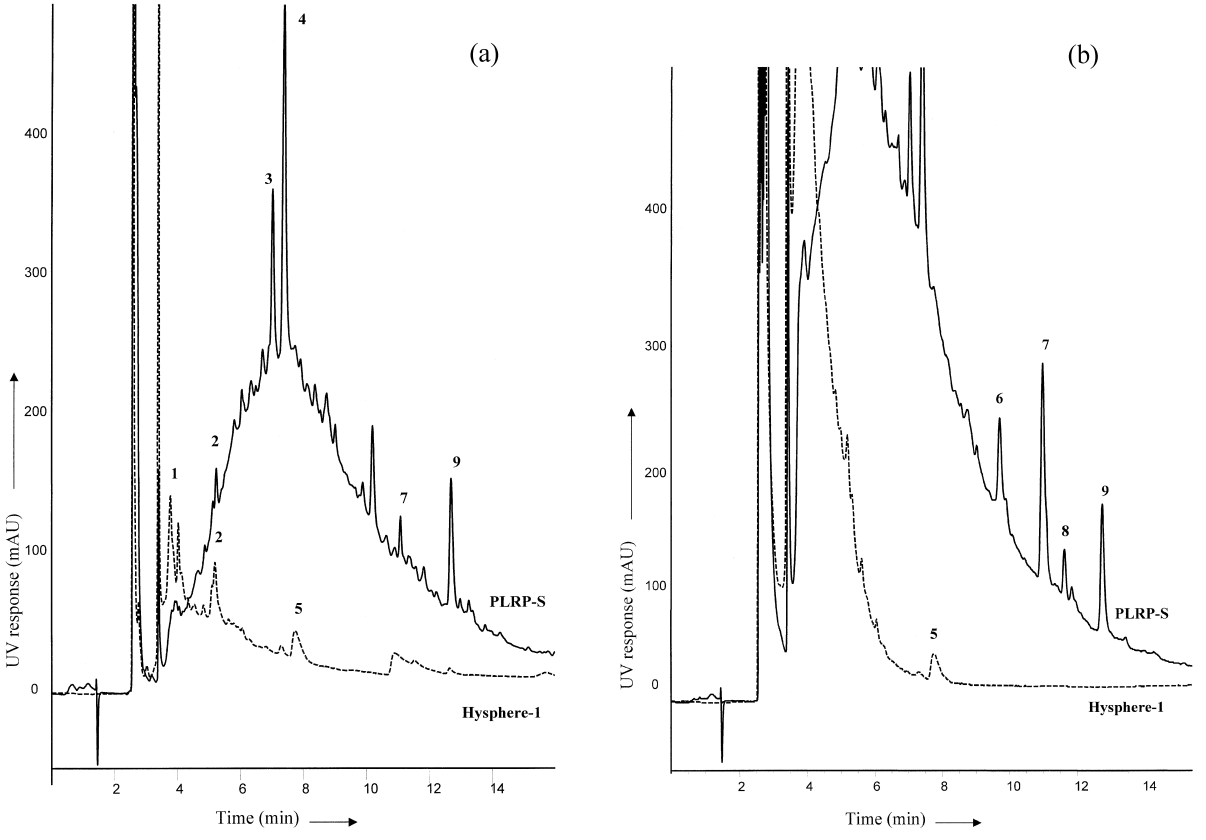

Fig. 1. Dual-SPE–LC–DAD UV chromatograms of river Rhine water spiked at the 2-μg/l level with mixture 2, and trace enrichment of 50 ml of sample on first pre-column (PLRP-S, upper trace) and final 25 ml on second pre-column (Hysphere-1, bottom trace) in (A) non-acidified, and (B) acidified sample (pH 3). LC conditions: gradient elution of first and second pre-column with 10 m*M* phosphate buffer (pH 2.7)–acetonitrile (85:15 to 25:75, v/v) in 14 min. UV detection at 215 nm. Peak assignment: (1) desethylhydroxyatrazine, (2) oxamyl, (3) isocarbamide, (4) desethylatrazine, (5) phenol, (6) dicamba, (7) bentazone, (8) MCPA and (9) 2,4,5-T.

Table 2
SPE–LC–DAD UV of 50 ml river Rhine water using on-line dual-pre-column PLRP-S (50 ml)/Hysphere-1 (25 ml)

Compound	Pre-column	pH	Calibration equation[a]	R^2	Detection limit (μg/l)	Recovery[b] (%)	RSD[b] (%)
Oxamyl	Hysphere-1	7	$y=96.1\ (3.1)x-16.3\ (14.4)$	0.996	0.5	65 (4)	6
Isocarbamide	PLRP-S	3	$y=165.5\ (2.6)x+16.4\ (10.4)$	0.999	0.1	90 (3)	4
Phenol	Hysphere-1	7	$y=112.5\ (2.1)x+0.3\ (8.4)$	0.998	0.5	75 (1)	3
Dicamba	PLRP-S	3	$y=151.4\ (2.5)x+10.7\ (11.7)$	0.999	0.3	75 (1)	2
Bentazone	PLRP-S	3	$y=330.5\ (1.6)x-17.8\ (6.4)$	0.999	0.1	91 (2)	2
MCPA	PLRP-S	3	$y=82.0\ (1.3)x+1.5\ (5.1)$	0.999	0.3	73 (3)	1
2,4,5-T	PLRP-S	3	$y=44.5\ (0.5)x-8.0\ (9.8)$	0.999	0.2	92 (1)	2

[a] y=Area; x=concentration (μg/l); SDs in parentheses. Analyte range, 0.1–10.0 μg/l; $n=7$; data points in duplicate.
[b] Spiked at the 1-μg/l level, $n=10$; RSDs (%) of recovery, RSDs (%) of peak areas are in parentheses.

overall picture (on the PLRP-S pre-column) improved considerably, despite the (expected) dramatic increase of the humic acid hump. However, the trapping role of the Hysphere-1 pre-column now became very limited, with desethylhydroxyatrazine and oxamyl being lost and only phenol showing up properly (Fig. 1B). Data on analytical performance for seven test analytes, collected under proper pre-column/pH conditions, are shown in Table 2. They can be called satisfactory, with analyte recoveries of over 70% in all but one case (oxamyl, 65%), and RSD values of 1–6%. Good linearity was observed for the calibration plots of the 10-μl loop injections as well as 50-ml sample trace-enrichment experiments, with R^2 values of 0.996–0.999, and detection limits of about 0.3–0.5 μg/l, which are acceptable for the present set of difficult compounds.

On the positive side, the above results indicate that even highly polar compounds can be analysed in an on-line system and with modest sample volumes. That is, screening for suspect samples in which alert/alarm threshold levels of 1–3 μg/l are exceeded, can be performed. On the other hand, the data of Table 2 and the chromatographic traces of Fig. 1 also reveal that (i) the present procedure cannot be used at the 0.1–0.5 μg/l level often desired today, and (ii) the approach is insufficiently robust for routine application, as it is obvious that – even with the two runs required according to Table 2 – too much will depend on the behaviour of each new compound of interest and, even more so, the type of water to be analysed (cf. above). In order to meet these more stringent demands, an equally sensitive but much more selective detection technique has to be used. Since target analysis is the major goal in this case, MS(–MS) detection is the obvious choice.

3.3. Dual-pre-column–LC–MS–MS

Infusion experiments of mixture 2 showed that, in ESI-MS, four of the eight compounds could be detected only in the PI mode and four in the NI mode, while phenol could not be detected at all (Table 3). LC–MS was used to optimise inlet conditions (see Experimental) in order to achieve

Table 3
Relative response of test analytes for different eluent compositions in LC–ESI-MS–MS[a]

LC eluent[b]		PI				NI			
A	B	Desethylhydroxyatrazine	Oxamyl	Isocarbamide	Desethylatrazine	Dicamba	Bentazone	MCPA	2,4,5-T
AA	ACN	25	50	5	35	45	60	50	45
AA	MeOH	50	50	40	65	70	90	85	80
AF	ACN	55	90	10	50	80	85	70	70
AF	MeOH	100	100	100	100	100	100	100	100

[a] For SRM transitions used, see Table 4.
[b] AA, Ammonium acetate; AF, ammonium formate; ACN, acetonitrile; MeOH, methanol.

maximum formation of protonated (PI mode) and deprotonated (NI mode) molecules. In addition, different LC eluent compositions (aqueous ammonium acetate, aqueous ammonium formate or formic acid as solvent A and acetonitrile or methanol as solvent B) were studied to obtain the highest analyte response for the eight test compounds in an LC gradient run (Table 3). Combination of 10 m*M* aqueous ammonium formate (pH 2.5) and methanol in a 14-min linear gradient from 10 to 75% (v/v) methanol gave the best results.

For quantitative analysis, both MS in the selected-ion monitoring mode, and MS–MS, in the SRM mode, are extremely useful. Selected-ion monitoring, in principle, yields the best analyte detectability, but it is generally recognised that, with complex samples, SRM – which is distinctly more selective – is to be preferred for both identification and quantification purposes [16,19]. The latter alternative was therefore used in our work. The SRM transitions used are included in Table 4.

The response linearity of LC–ESI-MS–MS which was studied by injecting standard solutions of the eight compounds (seven concentrations from 0.1 to 2.0 μg/ml; 10-μl loop injections, and SRM detection in the PI and NI modes) gave satisfactory linearity (R^2>0.9995) for all compounds. Detection limits were 1–5 ng/ml in the PI mode and 10–50 ng/ml in the NI mode. Next, the complete procedure was evaluated with dual-pre-column–LC–MS–MS of 50 ml river water spiked with the eight compounds. Table 4 shows some relevant results. The linearity of the calibration plots, now constructed over the range of 0.01–2 μg/l was as good as for the loop injections. Detection limits were in the range of 1–20 ng/l and 3–50 ng/l in the PI and NI modes, respectively. Fig. 2 shows typical SRM chromatograms of dual-pre-column–LC–MS–MS of 50 ml river water spiked at the 50-ng/l level for detection in the PI mode and the 100-ng/l level for detection in the NI mode.

The highly satisfactory results for all test analytes, and the absence of a background in all recorded traces demonstrate the potential of SRM for the identification and quantification of the polar and acidic analytes at or below the 100-ng/l level. This implies that the intended goal has been achieved. Actually, the results allow one to speculate that other compounds eluting in the same retention-time window can be subjected to the same analytical procedure, provided they are amenable to ESI-MS–MS detection.

3.3.1. Signal suppression by matrix

Even if the analytical performance of the above procedure is fully satisfactory, there is one further point that briefly merits attention. It is rapidly becoming recognised that analyte response in LC–

Table 4
Dual-pre-column–LC–MS–MS of river Rhine water using on-line PLRP-S (50 ml)/Hysphere-1 (25 ml) and SRM PI (pH 7) and NI (pH 3) detection

Compound	Pre-column	Calibration equation[a]	R^2	Detection limit (ng/l)	SRM transitions
PI mode (pH 7)					
Desethylhydroxyatrazine	Hysphere-1	y=129 594 (3486)x+242 (1504)	0.996	5	170→128, 86
	PLRP-S	y=5190 (93)x−12 (40)	0.998	20	
Oxamyl	Hysphere-1	y=7913 (111)x+54 (48)	0.999	20	220→90, 72
	PLRP-S	y=6072 (75)x+30 (33)	0.999	20	
Isocarbamide	PLRP-S	y=212 102 (2403)x+172 (1037)	0.999	1	186→87
Desethylatrazine	PLRP-S	y=152 199 (1713)x+4718 (739)	0.999	1	188→146
NI mode (pH 3)					
Dicamba	PLRP-S	y=2057 (42)x+108 (37)	0.998	50	219→175
Bentazone	PLRP-S	y=74 988 (3290)x+4875 (2864)	0.995	3	239→197, 132
MCPA	PLRP-S	y=32 366 (622)x−49 (541)	0.998	3	199→141
2,4,5-T	PLRP-S	y=12 792 (244)x−108 (213)	0.999	10	253→195

[a] y=Area; x=concentration (μg/l); SDs in parentheses. Analyte range 0.01–2.0 μg/l; n=7, data points in duplicate.

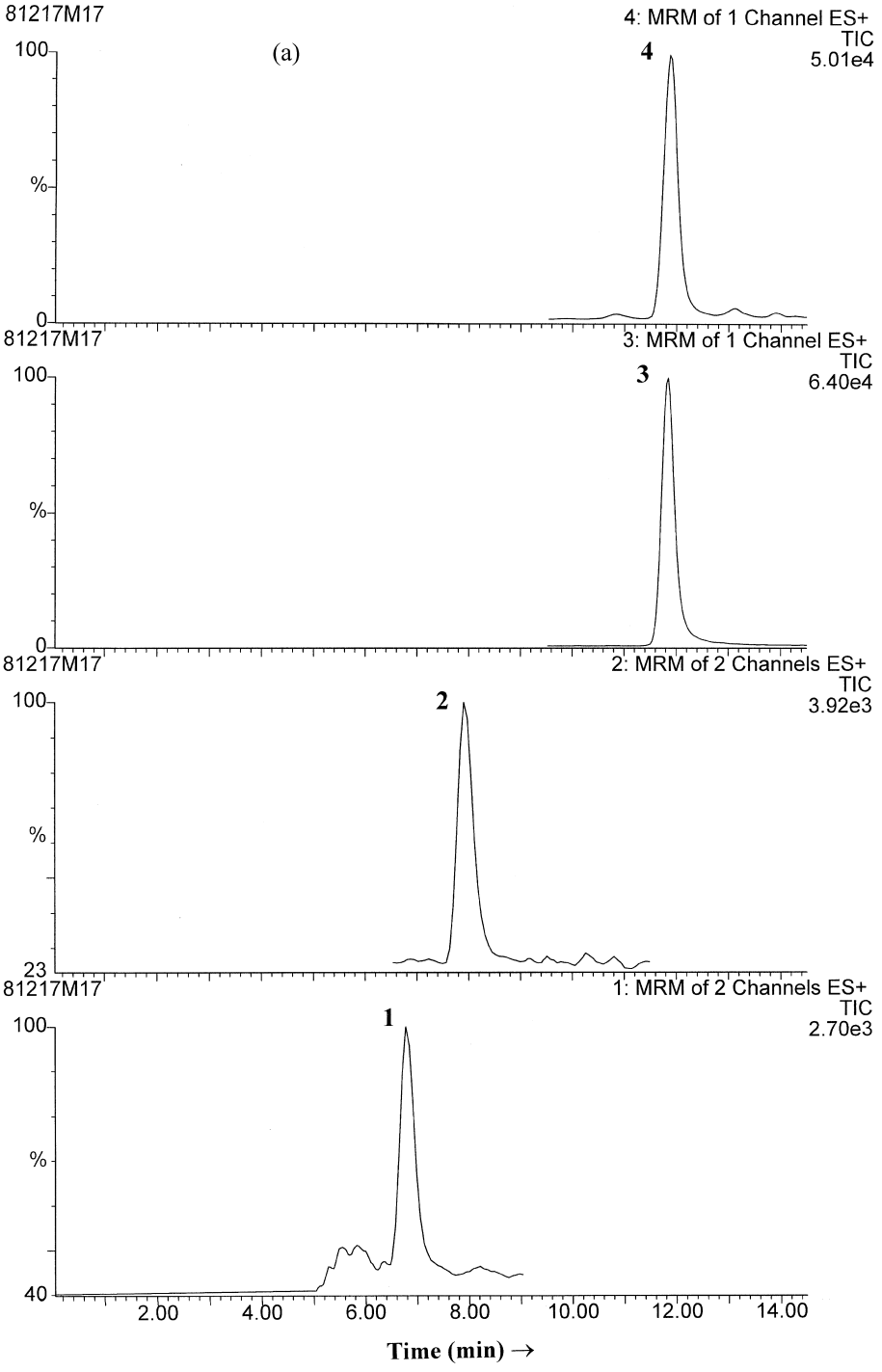

Fig. 2. Dual-SPE–LC–MS–MS SRM chromatogram of 50 ml of river Rhine water spiked with a mixture of pesticides at the 0.05-μg/l level and detected in the PI mode (A) and spiked at the 0.1-μg/l level after acidification to pH 3 and detected in the NI mode (B). LC conditions: gradient elution of first and second pre-column with 10 m*M* aqueous ammonium formate (pH 3.0)–methanol (90:10 to 25:75, v/v) in 14 min. SRM detection: for precursor-ion/product-ion transitions, see Experimental and Table 4. Peak assignment: see Fig. 1.

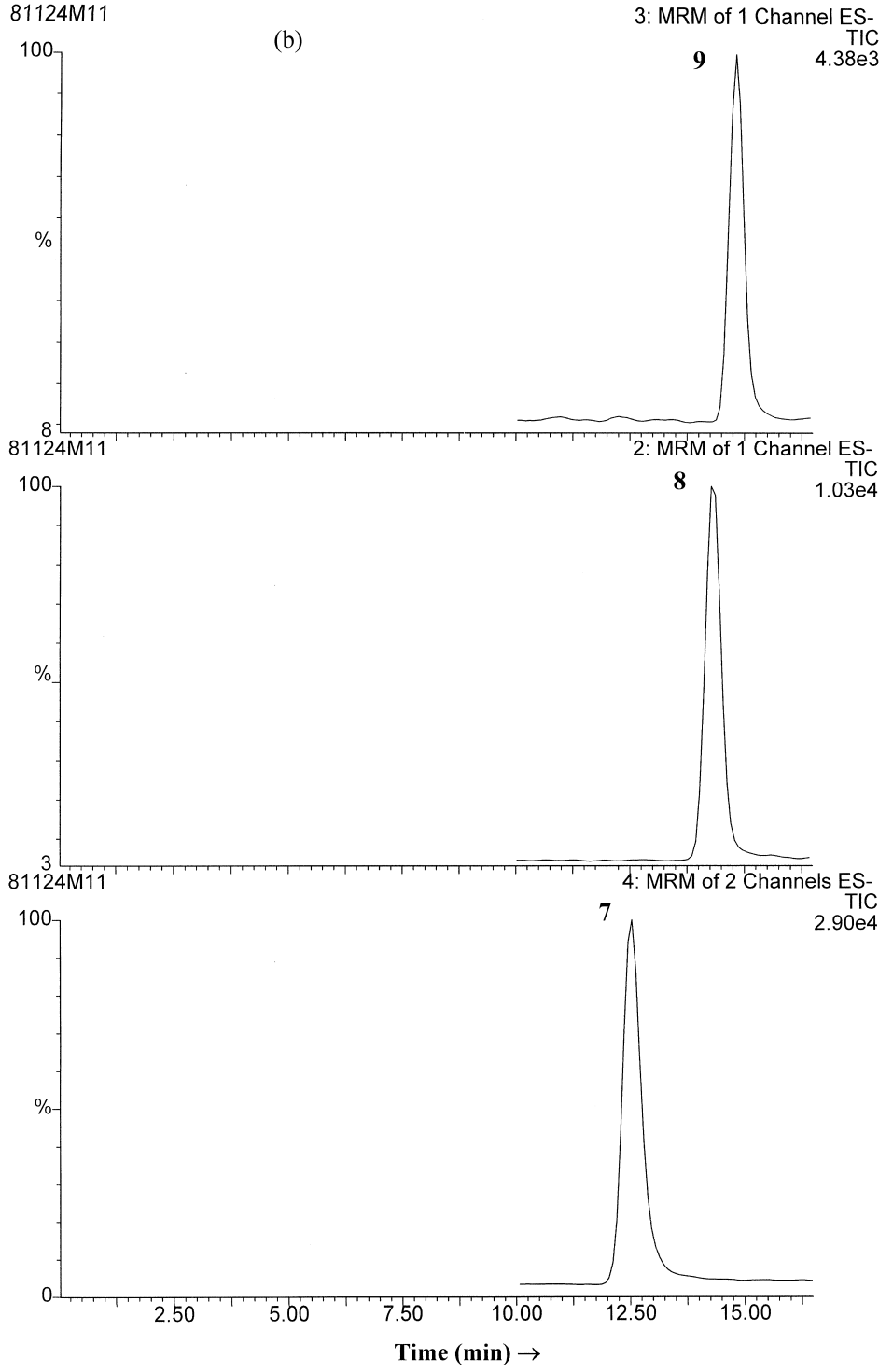

Fig. 2. (*continued*).

MS procedures can be strongly affected by, specifically, the presence of humic substances. Next to quenching, the continual introduction of humic-containing samples can cause contamination of the ion source and gradually decrease the efficiency of the procedure [20,21].

For a brief evaluation of this matrix effect in the current application, the overall recoveries – which reflect the extraction recoveries plus the signal suppression – of the test analytes studied under PI conditions, were compared for HPLC-grade water (data set at 100%), tap and surface water. Table 5 compares data obtained with the preferred PLRP-S/Hysphere-1 set-up, a set-up using C_2/Hysphere-1 (where the first pre-column can be expected to trap humic substances, but essentially no analytes) and a conventional single-SPE PLRP-S system. Two conclusions are obvious, viz. (i) the expected overall decrease from HPLC-grade to tap to surface water is indeed observed, and (ii) the presence of a protective extra pre-column clearly reduces the differences between tap and surface water (i.e., from ca. 50% with a single pre-column to 30–35% in dual-SPE). Another series of experiments with the NI-sensitive analytes – which, for obvious reasons, had to be limited to the single PLRP-S and the dual C2/Hysphere-1 set-up – showed similar trends with regard to the suppression effect. The data, which are included in Table 5, help to demonstrate that the dual-SPE set-up serves a useful purpose even if MS–MS detection is used.

4. Conclusions

Dual-SPE–LC with UV detection allows the determination of acidic as well as highly polar analytes from aqueous samples. PLRP-S/Hysphere-1 is the recommended pre-column combinations. Even analytes such as desethylhydroxyatrazine and oxamyl can be included in the test set. However, the results show that, with the 25–50 ml samples that can be handled, analyte detectability (0.3–0.5 μg/l) leaves something to be desired. In addition, the inclusion of other analytes and/or the study of other types of water sources will regularly require adaptation of the procedure.

The above problems completely disappear when ESI-MS–MS detection is introduced. Detection limits now are in the 1–50 ng/l range and analytical performance is fully satisfactory. Even for this system, the use of a dual-SPE set-up has some advantages because signal suppression and/or ion-source contamination due to the presence of, specifically, humic substances can be reduced.

Table 5
Recoveries of test analytes from spiked tap and river Rhine water compared with HPLC-grade water using on-line single- and dual-SPE–LC–MS–MS in the SRM mode[a]

Compound	Recovery (%)					
	Single-PLRP-S		(PLRP-S)–Hysphere-1		(C_2)–Hysphere-1	
	Tap	River	Tap	River	Tap	River
PI mode (pH 7)						
Desethylhydroxyatrazine	60 (4)	32 (3)	52 (2)	37 (2)	51 (2)	32 (1)
Oxamyl	94 (4)	50 (3)	96 (5)	65 (5)	80 (1)	53 (3)
Isocarbamide	94 (2)	34 (5)	–[b]	–	89 (2)	39 (3)
Desethylatrazine	94 (2)	53 (2)	–	–	91 (2)	56 (4)
NI mode (pH 3)						
Dicamba	77 (4)	22 (3)	–	–	100 (10)	49 (3)
Bentazone	100 (4)	54 (1)	–	–	100 (10)	98 (5)
MCPA	98 (2)	79 (2)	–	–	100 (5)	90 (4)
2,4,5-T	96 (2)	71 (3)	–	–	100 (2)	94 (5)

[a] Recoveries on second (Hysphere-1) or single (PLRP-S) pre-column using 50 (25) ml samples spiked at 0.1 μg/l; RSD values in parentheses; $n=5$.

[b] –, Not determined, because of (intended) retention on first pre-column.

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