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On-line solid-phase extraction in liquid chromatography using restricted access pre-columns for the analysis of *s*-triazines in humic-containing waters

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Abstract

A coupled-column liquid chromatographic system using solid-phase extraction pre-columns for the seven chloro-triazines, atrazine, desethylatrazine, desisopropylatrazine (DIA), propazine, simazine, desethylterbuthylazine and terbuthylazine, using photodiode-array detection (PDAD) was developed. Restricted access (RA), was investigated as a pre-column material, making use of non-adsorptive size exclusion of macromolecules and simultaneous dynamic portioning of the analytes, for the pre-concentration in environmental analysis. The RA phase was characterised and shown to solve problems with difficult matrix effects in humic-containing surface water samples. Other pre-column materials, e.g. reversed-phase C_{18}, mono-functional C_{18}, extraction disk mixture of C_{18} and styrene–divinylbenzene (PS–DVB) and LiChrolut EN, a polymeric sorbent specially designed for environmental analysis, were investigated and compared for their selectivity and stability, respectively. Comparisons were made with respect to selectivity and efficiency for the SPE columns separately and in combination with analytical columns. Recovery data and matrix dependencies were studied at concentration levels ranging between 0.1–10 μg/l. The RA pre-columns showed superior stability in complex environmental matrices, such as reference humic water and river water, due to the restriction of macromolecules. Retention properties with the RA alkyl–diol–silica were not sufficient for the most polar metabolites. However, efficient sorption/desorption kinetics were found with medium-polar and non-polar compounds. LiChrolut EN was found to strongly retain these polar metabolites as well as medium and non-polar solutes, and was selected for a deeper investigation (e.g. salinity dependence and pH). For a 10×2 mm I.D. pre-column, the breakthrough volume for the polar metabolite DIA was at least 150 ml, while for the RA pre-columns it was less than 10 ml. Unfortunately, this polymeric support was strongly effected, in terms of stability, in complex matrices which lead to repeated and expensive exchanging of pre-columns. However, in order to detect even the polar metabolites these pre-columns were finally used on-line together with liquid chromatography PDAD and thermospray mass spectrometry for the determination of triazines in water samples from the Ebro river during different seasons of the year.

Keywords: Coupled columns; Pre-columns; Environmental analysis; Sample preparation; Extraction methods; Water analysis; Sorbents; Restricted access packings; Triazines; Pesticides

1. Introduction

Atrazine and related triazine herbicides are used throughout the world for the protection of crops from

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broadleaf weeds (pre- and post-emergence weed control in corn, wheat and barley) on golf courses and for non-agricultural purposes such as soil sterilisation and road maintenance. After application, three different types of herbicide degradation occur: chemical (hydrolysis), biological (microbial) [1,2] and physical (temperature and photolysis) [3–5]. Several of the obtained degradation products were included in this study, see also Table 1. The widespread use of pesticides in agriculture leads to an increasing presence of these compounds in surface and drinking water. *s*-Triazines and their degradation products are highly resistant, survive many years in soil and thus the analysis of them is important. To reach concentration levels at 0.1 μg/l, which is set by the European Commission (EC) regulations [6] as the maximum allowed concentration of an individual pesticide in drinking water (no limits for surface water exists), preconcentration of the sample is needed. The analytes are commonly analysed by on- or off-line solid phase extraction (SPE). Pre-columns are being used for simultaneous trace enrichment and sample clean-up in combination with an analytical separation. Various solid supports are utilised with differing selectivities in order to reach optimal chemico-physical interactions. Several sorbent types have been described for the analysis of triazines. Off-line studies were made by Corcia et al. [7], who used graphitized carbon black (GCB) cartridges with very good recoveries for the polar metabolites of atrazine. Berg et al. [8] made a more thorough investigation to determine triazine metabolites using a modification of this technique. Another study using GCB was made by Cai et al. [9] for the analysis of the very polar metabolite, didealkylatrazine. Comparisons of several sorbents, including a cation-exchanger SCX (propylbenzenesulphonic acid), cyclohexyl, phenyl, end-capped cyano-propyl, ethyl, octyl and octadecyl for the analysis of hydroxylated atrazine derivates have been reported [10]. Another off-line study involving Bond Elut C_{18} and ENVI-Carb cartridges as well as Empore C_{18} and polystyrene–divinylbenzene (PS–DVB) disks was recently reported [11]. Investigations using XAD-2 columns [12], cyclohexyl cartridges [13] and mixed-mode resins [14] are also reported in the literature. All of these studies were made off-line, which has its drawbacks, e.g. risks of contamination, extensive and time-consuming manual steps, and injection of an aliquot instead of the whole sample extract. On-line systems are often to be preferred since automation can be complete. On-line studies using Tenax TA [15] and Empore disks [16–18] have been reported.

Until now, restricted access (RA) materials have been designed and used especially in the biomedical area. In this work, the RA stationary phase is characterised and optimised for use in environmental analysis. The alkyl–diol–silica is a new family of chemically and enzymatically modified pre-column packing materials that have a physical diffusion barrier [19]. They are specially developed for the direct and repetitive injection of untreated biological fluids, the quantitative elimination of macromolecules such as proteins, as well as for the dynamic extraction and on-column enrichment of small solutes by classical reversed-phase partitioning [20–22]. These pre-columns have been proven to be highly stable and usable in on-line CLC systems. This paper

Table 1
Trivial names in order of retention on a reversed-phase column, chemical structures, UV_{max} absorbance wavelength and MS ion-fragments of the *s*-triazines investigated in this work (for structure, see Fig. 1)

No.	Compound name, abbreviation	R1	R2	R3	λ_{max}	Ion fragments
0	Didealkylatrazine, DDA	NH_2	NH_2	Cl	–	146, 187
1	Desisopropylatrazine, DIA	NH_2	NHEt	Cl	214, 260	174, 215
2	Hydroxysimazine, HS	NHEt	NHEt	OH	215	184, 225
3	Desethylatrazine, DEA	NHiPr	NH_2	Cl	215, 260	188, 229
4	Hydroxyatrazine, HA	NHiPr	NHEt	OH	214	198, 239
5	Simazine, SIM	NHEt	NHEt	Cl	222, 264	202, 243
6	Desethyl-terbuthylazine, DTBA	NHterb	NH_2	Cl	215, 260	202, 243
7	Atrazine, ATR	NHiPr	NHEt	Cl	222, 263	216, 257
8	Propazine, PROP	NHiPr	NHiPr	Cl	221, 264	230, 271
9	Terbuthylazine, TBA	NHterb	NHEt	Cl	222, 265	230, 271

describes a method for optimising the selectivity and compatibility of RA C_{18}-diol pre-columns in environmental applications. Other commercially available pre-columns were included in this study.

2. Experimental

2.1. Instrumental

The pre-column is placed in a six-port switching valve (MUST, Spark Holland, Emmen, Netherlands) and the analytes are transported to the liquid chromatography system, which consisted of a gradient HPLC pump Model LC-250 (Perkin-Elmer, Norwalk, OH, USA), coupled to a C_8 separation column, Novapak (150×3.6 mm I.D.), 5 μm pore size, (Waters Millipore, Bedford, MA, USA). After the analytical column, a diode array detection (PDAD) system Model LC-480 (Perkin-Elmer) is used for the detection and then data is collected using a PC-computer and the software TurboChrom 4.0 (Perkin-Elmer). For the direct injections, a Model 7125 injection valve furnished with a 20-μl loop (Rheodyne, Cotati, CA, USA) was used. Pre-conditioning of the pre-column and loading of the sample were carried out using an SSI Model 300 HPLC pump (Scientific Systems, State College, PA, USA). The pre-columns used were the following: RA, alkyl–diol–silica (ADS), with a particle size of 25 μm which were packed (approximately 150 mg) in stainless steel pre-columns of (25×4 mm I.D.). These columns were a gift from Prof. K.-S. Boos, Ludwig-Maximilians-Universität (Munich, Germany). LiChrolut EN, irregular particles (40–120 μm) of a highly porous non-ionogenic PS–DVB polymer was kindly provided by Merck (Darmstadt, Germany). Reversed-phase C_{18}, a pre-column (15× 3.2 mm I.D.) packed with reversed-phase C_{18} particles of 10 μm particle size, was purchased from Brownlee Columns, Applied Biosystems (San Jose, CA, USA). Mono-functional (MF) C_{18}, mean particle size 40–70 μm, was kindly provided by Isolute, IST (Cambridge, UK) and finally C_{18} and PS–DVB Empore extraction disks, 47 mm diameter, were purchased from J.T. Baker (Deventer, Netherlands). The analytical columns used were Novapak RP-C_8, 5 μm (150×3.9 mm I.D.), (Waters) and LiChrospher RP-C_{18}, 5 μm (125×4 mm I.D.) (Merck).

2.2. Chemicals

Humic acid, M_r ~600–1000, Fluka (Buchs, Switzerland); humic acid H1675-2, M_r ~5000–10 000, Aldrich (Steinheim, Germany); acetonitrile, LiChrosolv (Merck); 2-hydroxysimazine (HS), C-169550 Lot 40322; desisopropylatrazine (DIA), E-103320 Lot 30128; desethylatrazine (DEA), E-103310 Lot 30505; 2-hydroxyatrazine (HA), E-103330 CH 21123; atrazine (ATR), IPO 005 and simazine (SIM), IPO 692; terbuthylazine (TBA), E-173000; desethyl-terbuthylazine (DTBA), E-173030 were all purchased from Dr. Ehrenstorfer (Augsburg, Germany). Propazine (PROP) 139-40-2 was purchased from Polyscience (Niles, IL, USA). Sea salts (Lot 61H083225) were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade. Stock solutions were prepared in acetonitrile and from these solutions, sample solutions of the pesticides were made daily. The tap water in the laboratory was used as a ground water source. Acetonitrile and HPLC-grade water were filtered through a 47 mm diameter and 0.45 μm pore size nylon filter and degassed under vacuum for 15 min before use. Surface water samples were collected in 2.5 l glass bottles from the bridge in Amposta on different occasions. Samples were immediately stored (as collected) at +4°C, in the refrigerator until use.

2.3. Preconcentration procedure

1. Conditioning of the sorbent with 5 ml of methanol (1 ml/min)
2. Removal of methanol by 3 ml of Milli-Q water (1 ml/min)
3. Loading of the sample X ml (3 ml/min) X_{max}= 150
4. Washing step with Milli-Q water, 9 ml (3 ml/min)
5. Switching of the valve and elution by the mobile phase onto the analytical column.

2.4. Chromatograpic analysis

Separation of the *s*-triazines was accomplished using a gradient program with a mobile phase of acetonitrile (A) and water at pH 7.0 (B) at a flow-rate of 0.9 ml/min. The separation conditions using Novapak C_8 analytical column were: From 0–4 min, 12% A; at 4–25 min a linear gradient from 12–55% A, and finally at 25–30 min 55–100% A.

2.5. Mass spectrometric analysis

A Hewlett-Packard (Palo Alto, CA, USA) Model 5988A thermospray LC–MS quadrupole mass spectrometer and a Hewlett-Packard Model 35741B instrument for data acquisition and processing were employed. The thermospray temperatures for the stem and the tip varied between 125–115°C and 240–245°C, respectively. The ion source temperature was set to 240°C. The filament-on mode was used in all experiments, with conventional positive chemical ionisation (PI) using 0.01 *M* ammonium acetate buffer.

3. Results and discussion

The parent compounds atrazine, simazine and propazine are transformed in nature to form hydroxy- and dealkylated metabolites. Their structural differences are illustrated in Fig. 1 and Table 1. The length of the alkyl chains R1 and R2 will affect the polarity, so that longer chain-containing compounds result in increased hydrophobicity. The hydroxy metabolites are more polar than their parent triazine. The spectral differences were investigated by UV spectra and the corresponding UV_{max} absorbance wavelength are also presented in the table. Spectral analysis was developed for confirmational purposes in complex samples. These differences can be utilised in differentiating between metabolites and parent compounds e.g., the second peak maximum at 260 nm for SIM and ATR is shifted to 235 nm for the hydroxy metabolites, as shown for SIM in Fig. 2a–b.

Successful separations for some of the analytes were achieved by using reversed phase separations with C_8 and C_{18} analytical column materials [5,23]. It is essential to follow a more complete mapping of these pesticides in environmental waters in order to get a complete environmental toxicity figure. However, in this paper we wanted to focus on the simultaneous determination of nine different *s*-triazines. Several silica-based reversed-phase (C_8 and C_{18}) analytical columns were investigated for opti-

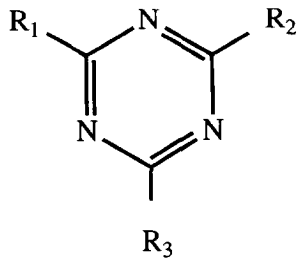

Fig. 1. Chemical structure of *s*-triazines.

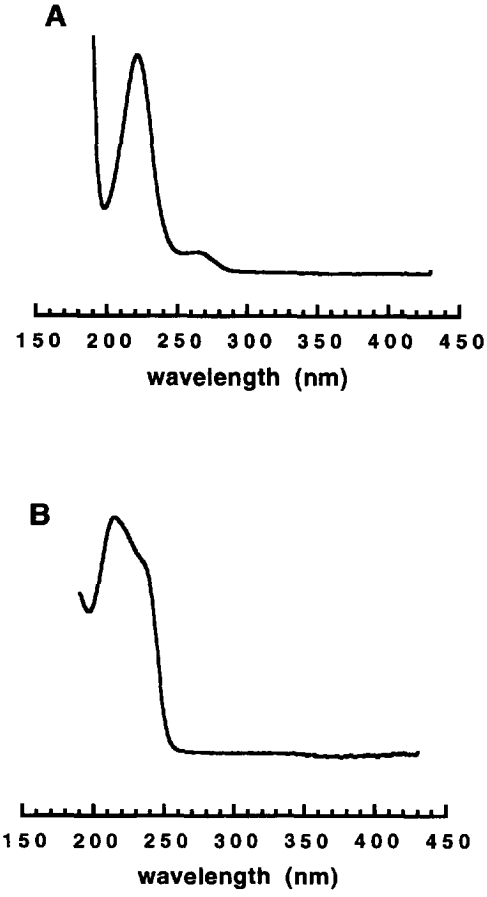

Fig. 2. The UV spectra of simazine (A) and hydroxysimazine (B).

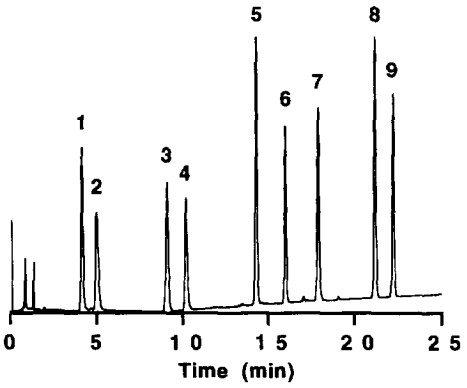

Fig. 3. UV chromatogram obtained with a directly injected standard of nine triazines according to Table 1, using a Novapak C_8 analytical column and a gradient elution programme with a flow-rate of 0.9 ml/min: 12% A (acetonitrile) and 88% B (water) in 4 min, up to 55% A in 25 min and up to 100% A in 30 min.

mal chromatographic resolution and efficiency. Acetonitrile was preferred to methanol as the organic modifier due to its lower UV cut-off and lower viscosity, resulting in improved sensitivity and plate numbers. The use of C_{18} columns allowed usage of stronger mobile phases, which is important for the desorption kinetics. Unfortunately, these columns were not able to separate the polar hydroxytriazines from DEA and DIA, respectively. The best separation efficiency was obtained using the Novapak C_8 column (Fig. 3), which resulted in baseline separation of all nine analytes in one run within 25 min. This column, together with LiChrospher C_{18}, was used throughout the experiments as the analytical column.

In order to analyse trace levels of triazines, trace enrichment of larger volumes of environmental waters is necessary. This was made by positioning a pre-column on-line prior to the separation column. The pre-columns containing RA and C_{18} (Applied Biosystems) were pre-packed and ready to use, while LiChrolut EN and MF C_{18} were packed in the laboratory by manually pressing a methanol slurry of the solid support through a stainless-steel column (10×2 mm I.D.) with a plastic syringe. The final amount of sorbent in these two pre-columns was approximately 15 mg and 50 mg, respectively. The extraction disks were cut and put in a small PTFE holder, according to the method of Brouwer et al. [16], using a combination of six discs of C_{18}-bonded silica and four discs of PS–DVB, achieving similar capacities and selectivities as reported by Chiron et al. [17]. The analytes were adsorbed and enriched on the pre-columns and characterisation of these was made by breakthrough experiments. The breakthrough volume (V_b) is measured as the volume where the analyte is no longer adsorbed on the pre-column due to saturation. V_b can be measured in several ways [24], but, in this case, different volumes of a 0.5–1 μg/l sample solution were percolated through each pre-column and the V_b were estimated as the point where the response in the experimental plot differs $\geq$5% from the extrapolated calibration plot [25]. By percolating different volumes, the V_b is easily seen as the point where linearity levels off. The results presented in Table 2 show the V_b for all the triazines investigated using five different sorbents. The breakthrough of the hydroxy metabolites HS and HA were in the same range as DIA and DEA, respectively. The restricted access column shows sufficient retention for the moderately polar and non-polar analytes, while having poor retention for the polar breakdown products. The cross-linked polymer, LiChrolut EN, has multiple binding sites with mixed mechanistic adsorption properties and shows a strong retention even for very polar degra-

Table 2
Breakthrough values (ml) for different *s*-triazines in ground water spiked at concentrations of 1 μg/l, using various pre-columns

Compound	RA	LiChrolut	RP C_{18}	C_{18}+PS–DVB	M–F C_{18}
DIA	3	>100	<20	<20	<20
DEA	6	>100	<20	40	<20
SIM	30	>100	40	80	50
DTBA	35	>100	40	>100	60
ATR	>50	>100	80	>100	>100
PROP	>50	>100	>100	>100	>100
TBA	>50	>100	>100	>100	>100

dation products. No breakthrough occurs at sample volumes of up to 150 ml (groundwater spiked at 1 μg/l) using this polymeric support. Since larger sample volumes would require an extended sampling time, no further efforts were made to investigate the exact V_b. Among the other supports, extraction discs (C_{18} and PS–DVB) were found to give the best V_b values while the breakthrough using the other C_{18} phases were in the same range as the RA material. Minimal analyte loss is accomplished when working with sample volumes below the V_b. Since the enrichment factor is sufficient to reach a detection limit of around 0.05 μg/l for a 50-ml sample, most experiments were carried out with volumes ranging between 25 and 60 ml. When coupling the pre-column with the analytical separation column, the hydroxy-metabolites HS and HA co-eluted with DIA and DEA. These two analytes were therefore disregarded in further investigations.

High V_b-values need to be combined with fast desorption kinetics in order to be compatible with the analytical separation. Band broadening, which needs to be addressed, is always a problem when coupling chromatographic systems. The pre-column containing extraction disks gives the least band broadening, while the most was found using the polymeric support. Comparisons of chromatograms, obtained after interfacing the RA and LiChrolut EN pre-columns in the coupled column system, using separation with gradient elution, are illustrated in Fig. 4a–b, clearly showing the selectivity difference of the two sorbents. The polar degradation products are retained on the polymeric support, while on the RA they are not. The more strongly retaining LiChrolut EN pre-column, causes band broadening for all the analytes which is probably due to strong interaction with the sorbent and thereby less effective desorption into the mobile phase. Increased sample volumes will, in most cases, also increase band broadening for the peak from the analyte breaking through, as is seen for peak 5–6 in Fig. 4b. Efforts to decrease band broadening, were made by increasing the strength of the mobile phase, but this resulted in an unacceptable loss of resolution of the analytical separation. However, by eluting in the backflush mode, the sample band in the pre-column is compressed to a narrow band before entering the analytical column and thereby reducing the band broadening effect.

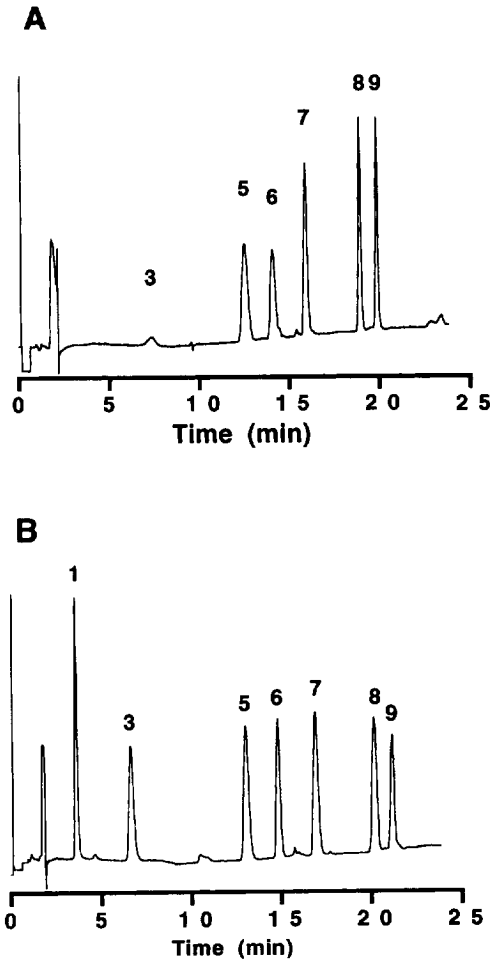

Fig. 4. The selectivity is shown by on-line pre-concentration of seven triazines in a 25 ml groundwater sample using (A) RA and (B) LiChrolut EN precolumns. The chromatographic conditions used were the same as oulined in Fig. 3.

To have a satisfying pre-concentration followed by quantitative LC-analysis, the recovery of the analytes should be close to 100%. Recovery studies were made with five replicates and sample volumes of 25 ml spiked with 1.6 μg/l of triazines in groundwater. The resulting data are shown in Table 3. The same amount of sample (40 ng) was directly injected with a 20-μl loop to calculate the recovery value R_v, which is defined as the peak area after pre-concentration (25 ml) divided by the peak area after a

Table 3
Recovery values (%) using various pre-columns and a pre-concentration of 25 ml groundwater sample compared to a direct injection of 20 μl (mean values, $n=5$)

Compound	RA	LiChrolut	RP C_{18}	C_{18}+PS–DVB	M–F C_{18}
DIA	–	93± 4	–	21±2	2±2
DEA	10±2	94± 2	5± 2	91±2	12±2
SIM	84±2	94± 2	78±12	91±2	81±4
DTBA	86±2	91± 4	73±10	91±2	68±5
ATR	90±4	92± 2	90± 2	90±2	89±2
PROP	91±2	89± 4	89± 2	90±2	91±3
TBA	87±2	73±19	87± 2	86±3	88±3

direct injection (20 μl). The RA column showed good recoveries for the moderately polar and apolar analytes, but insufficient values (<10%) for the determination of polar metabolites. Using C_8 or C_4 as functional groups in RA columns and using the same synthesis procedure as for the alkyl–diol support would favour the chemico-physical binding properties for polar transformation products and the retention of these metabolites would be enhanced [26]. It is obvious that the polymeric sorbent LiChrolut EN gives the highest recoveries, especially for polar analytes (93–94%). Extraction disks show good recoveries for all analytes except DEA and the repeatability or R.S.D. is very good, while the other C_{18} materials had recoveries similar to those of the RA column.

To study the sorbent selectivity regarding matrix interferences, such as humic acids and other humic substances, experiments were carried out by spiking different water types with three analytes representing a polar, a medium polar and a non-polar solute (DIA, SIM, ATR) at a concentration level of 1 μg/l. Three different matrices were investigated: humic containing water from the Ebro river and two different reference humic acids, from Fluka and Aldrich, that were dissolved in Milli-Q water. Normal concentration levels of humic acids in ground, surface and estuarine water are in the range of 0–10 mg/l [27], so similar reference standards were prepared. The resulting chromatograms show a large matrix difference between samples from the Ebro river and the spiked reference humic acid samples, probably due to the fact that the Ebro river samples contained a large number of both humic and fulvic substances. The frontal effect and the characteristic humic hump shown in the chromatograms in Fig. 5 was found to be smaller using the RA-column (Fig. 5a), in comparison to other pre-column materials such as LiChrolut EN (Fig. 5b). It should be pointed out that the dimensions of the RA column are much larger (25×4 mm I.D.) than those of the LiChrolut EN column (10×2 mm I.D.). With the same dimensions, the front effect difference would be more pronounced. The RA pre-columns also showed superior stability, they could be used for more than 100 injections of samples with difficult matrices, while the other pre-columns had to be replaced after every fifth injection. This proves that the restriction of macromolecules also works for environmental applications. It is only the poor retention of the most polar analytes that causes problems. On the other hand, when using LiChrolut EN, the matrix interferences are significantly increased, causing problems with identification and determination of the most polar metabolites by use of PDAD.

The sample pH might affect the preconcentration step in several ways, (i) the charge of the analytes and thereby the retention properties for the sorbent, (ii) the solubility of both analytes and matrix interferences. Less sample handling (e.g., changing the pH) is preferable in order to minimise errors in the analysis. A separate study was made by spiking a solution containing 8 mg/l of reference humic acid (Fluka) with ATR and SIM at 2 μg/l, and loading the sample (25 ml) onto RA at pHs 3 and 7. There was a large increase of the front when the pH was lowered. Obviously, the use of a higher pH lowers the complexity of the sample matrix. Similar results were observed in a study by Liska et al. [28].

Since the polar metabolites are included in this work, some additional optimisations were made with the LiChrolut EN pre-column before running Ebro

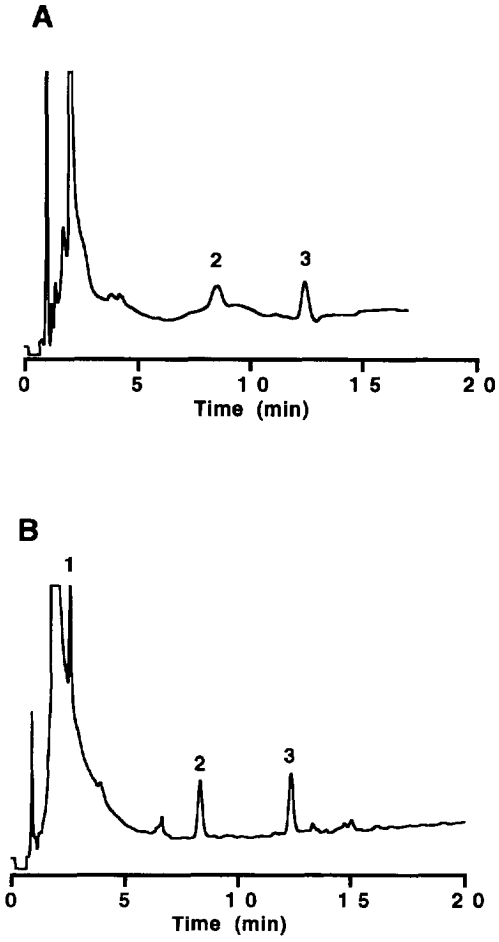

Fig. 5. The prescence of matrix components in chromatograms obtained using on-line pre-concentration of 25 ml Ebro water samples using restricted access (A) and LiChrolut EN (B) precolumns. The river samples were spiked at 1 μg/l of DEA (1), SIM (2) and ATR (3). An analytical column LiChrospher C_{18} was used with a gradient programme of 20% acetonitrile (A) and 80% water (B) from 0–2 min, from 2–25 min 20–60% A, and up to 100% A within 30 min.

water samples. We investigated the pre-concentration efficiency by spiking ground water at 0.5 μg/l at pH 2.5, 7.4 and 10.0 and using a mobile phase at pH 7. We found that the V_b for DIA, the most polar analyte, was not reached by pumping 150 ml of sample volume (see also Fig. 6). No significant recovery difference was found for any of the triazines. Since the breakthrough is not affected by pH, the natural choice was to work at pH values around 7 in all experiments, thereby reducing the matrix effects. Lower detection limits make it possible to detect pesticides even at the low concentration levels in the oceans [29]. Since sampling and analysis of triazines are made in brackish water of the Ebro delta area, it is of great importance to make additional studies with surface waters containing various levels of salt. Salinity aspects were investigated in an earlier study, by spiking ground water with different amounts of sea salts to simulate water from the natural environment of oceans [30]. It is possible to analyse samples with salinity values up to 35‰ using a LiChrolut EN pre-column. However, high salt concentrations were found to affect the behaviour of the pre-column and shorten its lifetime considerably. The pre-column had to be replaced every five injections. It would therefore be beneficial to change it after each injection as is done in an automated on-line system like the Prospekt system [31–35]. The packing procedure of the pre-columns used in this study is time-consuming and quite troublesome (e.g. leaking), so a commercial product similar to the Prospekt cartridges would be greatly appreciated.

4. Application

An integrated LC system was optimised using the LiChrolut EN pre-column, and comprising the C_8 analytical column, for applications to environmental water samples using PDAD and MS detection. Although the RA pre-columns functioned the best, the restriction to medium and non-polar solutes made us choose the LiChrolut column, which enabled the analysis of polar triazines as well. Optimisation of the MS conditions were made by flow injections of stock solutions, in the PI scan mode, in order to obtain the major ion fragments (see Table 1) after ionisation. These molecular ions correspond well with results from Chiron et al. [18] and Barceló et al. [36]. We determined the additional ions found for DTBA (202, 243) and DDA (146, 187) which correspond with the ions $[M+H]^+$ and $[M+H+CH_3CN]^+$. By operating in the time-scheduled selected ion monitoring (SIM) mode, the sensitivity could be increased 10^2–10^3 times. Optimisation with time windows was made by on-line analysis of pre-concentrated samples, resulting in the windows: 3–14 min (compounds 0–4), 14–23 min (5–7), and

Table 4
Approximate concentrations found in Ebro river water using on-line SPE–LC–TSP-MS

Compound	950119	950302	950602
DEA	0.07 μg/l	0.03 μg/l	0.06 μg/l
SIM	0.03 μg/l	0.02 μg/l	0.03 μg/l
ATR	0.07 μg/l	0.03 μg/l	0.07 μg/l

23–30 min (8–9). The final investigation in this work was to determine the levels of triazines in real environmental samples originating from the delta of the Ebro river. Sampling from the Ebro river was made at three different times of the year to get a variation of the sample composition. Since the Ebro delta is an agricultural area, it is well known that the sample complexity will vary with the time of the year. Humic substance composition, and thereby detectability due to complex formation with e.g. humic and fulvic acids will vary. The samples (51 ml each), were processed using the LC–TSP-MS setup described above. The analytes DDA, HS and HA, were included in this target analysis, since the selectivity of the mass detector makes their determination possible, even when co-elution takes place. Fig. 7b, illustrates the separation of an unspiked sample from the Ebro, detecting DEA, SIM and ATR close to the LOD (≈0.02 μg/l). The triazines in the presented chromatogram were detected in all the samples at concentration levels of 0.02–0.07 μg/l, as shown in Table 4. We are working very close to the detection limit, so the quantification is approximate. The metabolite (DEA) concentration corresponds very well to the parent compound (ATR) while the other triazine (SIM) seems to be present in a somewhat lower concentration. It can be noticed that the concentrations are rather low (off-season) and are actually far below the EC limit for drinking water. The UV-chromatogram (220 nm) of the same sample is shown in Fig. 7a. The typical chromatographic hump, originating from humic substances present in the sample, makes trace level determination with PDAD difficult using the LiChrolut EN pre-column. The RA is preferable in this case for the determination of SIM and ATR. Using RA, the effect from humic substances will also minimise the fouling of the MS ion source, improving stability and robustness of the thermospray interface.

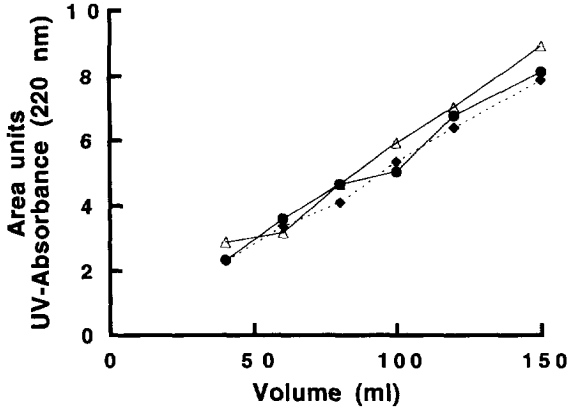

Fig. 6. Breakthrough investigation at different pHs, (●) pH 2.5; (◆) pH 7.4 and (▲) pH 10.0, on a LiChrolut EN precolumn by percolating different volumes of sample. The graph is from the polar metabolite DIA. Chromatographic conditions are the same as in Fig. 3.

5. Conclusions

A number of new and different SPE materials were investigated for triazine monitoring in environmental water samples. Restricted access C_{18}-diol, polymer and silica types were characterised. Several important parameters for their use as pre-columns were investigated.

The RA pre-columns were found to show high reproducibility and stability, superior to the other pre-columns investigated. The RA pre-columns were unfortunately not suitable for the retention of the most polar analytes that were investigated. The breakthrough volumes were too small for a satisfying pre-concentration. However, more than 100 injections of highly complex samples of humic-containing waters could be run with RA, while the LiChrolut EN column couly only be used for a maximum of five injections. This proves that the restriction of macromolecules for environmental applications works as well as it does for biomedical applications. RA pre-columns would be suitable for brackish and salt water analysis or other complex samples, since it can withstand the more difficult matrices without clogging and deterioration. For more apolar compounds, like PCBs, in an on-site alarm system, where

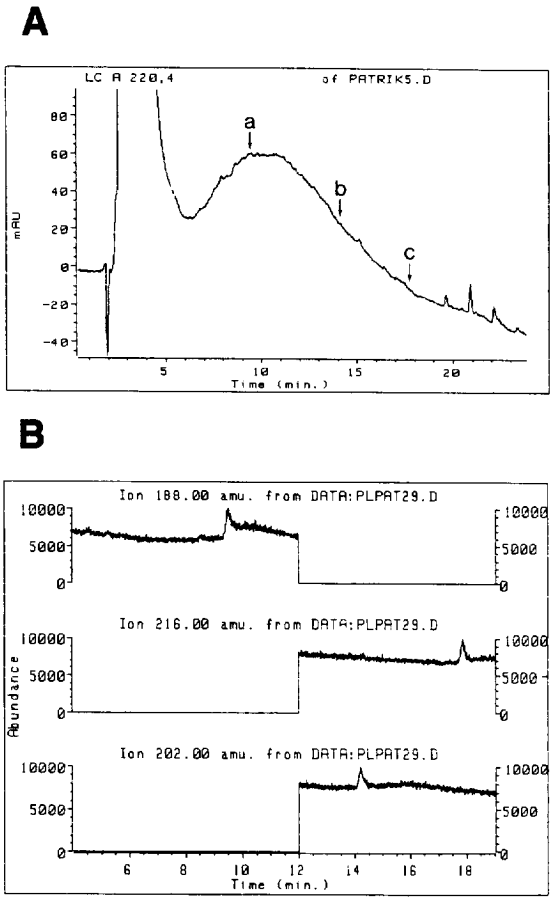

Fig. 7. (A) On-line SPE using a LiChrolut EN pre-column followed by LC–PDAD of a 51-ml unspiked Ebro sample. The abbreviations a–c, corresponds to the retention times where DEA, SIM and ATR should appear. Chromatographic conditions are the same as in Fig. 3, but with an addition of 0.01 *M* ammonium acetate to solvent B. (B) The corresponding chromatogram of the same sample, using LC–MS and thermospray in the time-scheduled SIM-mode. The triazines DEA, SIM and ATR were detected at 0.03, 0.03 and 0.02 μg/l, respectively.

minimal attention (e.g. changing of pre-columns) is desired, this could be a very good application.

LiChrolut EN shows the best retention properties of all SPE materials investigated. The final on-line system using the polymeric sorbent LiChrolut EN was able to pre-concentrate ground water, sea water and river water at pH 2.5–10 without breakthrough ($V<150$ ml), with satisfying recoveries and detection limits of 0.02 μg/l (50 ml). However, still some problems are present e.g. the packing and lifetime of these pre-columns. The largest problem though, is the presence of large quantities of matrix components. The band broadening in the elution step is another problem, which might be solved by using a different packing material (polymeric) in the analytical column and thereby allowing more organic modifier in the mobile phase. Another possibility for decreasing band broadening is to use smaller particles and this is under development (LiChrolut will be available with 7 μm pore size in the near future). Off-line automated pre-concentration should be considered as an alternative to solve elution problems as well as packing and leaking difficulties.

Using extraction disks for the trace enrichment is described in the literature and gives some advantages compared to reversed-phase C_{18} materials. They give higher breakthrough values, little band broadening and good recoveries. They are fast and easy to make and the amount of disks is adjustable depending on the application. Reversed-phase C_{18} was used as a reference standard. The band broadening effect is not so severe, but the retention of the analytes studied was poor. Monofunctional C_{18} has been used, since, according to the manufacturer, it can have different selectivities than trifunctional C_{18}. The difference was unfortunately not significant enough for any conclusions to be made in these analyses. However, there was little band broadening and the packing of this sorbent was not so troublesome as for LiChrolut EN.

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