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Analysis of atrazine, terbutylazine and their *N*-dealkylated chloro and hydroxy metabolites by solid-phase extraction and gas chromatography–mass spectrometry and capillary electrophoresis–ultraviolet detection

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Abstract

Solid-phase extraction (SPE) with the styrene–divinylbenzene adsorbent LiChrolut EN was investigated for the extraction of the *s*-triazine herbicides atrazine and terbutylazine, their polar *N*-dealkylated degradation products deethylatrazine (DEA), deisopropylatrazine (DIA) and deethylterbutylazine (DET) and for the hydrophilic hydroxytriazine degradation products (HTDPs) hydroxyatrazine (HA), hydroxyterbutylazine (HT), deethylhydroxyatrazine (DEHA), deisopropylhydroxyatrazine (DIHA) and deethyldeisopropylhydroxyatrazine (ameline). The optimum pH value for the extraction of the HTDPs from fortified tap water at 2 μg/l is 3.0. Recovery values with 200 mg LiChrolut EN are >80% for HA, HT, DEHA and 30% for DIHA from 200 ml spiked tap and river water. Atrazine, terbutylazine, DEA, DIA and DET are quantitatively extracted by LiChrolut EN. The chlorotriazines are analyzed by GC–MS and the HTDPs by capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC) with an acetate buffer at pH 4.6 or a sodium borate–sodium dodecyl sulfate buffer at pH 9.3. The combined method of SPE enrichment and CE analysis allows the determination of HTDPs in the low μg/l range. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

s-Triazines are used worldwide as selective pre- and post-emergence herbicides for the control of weeds in many agricultural crops like corn, wheat, maize and barley, as well as in railways, roadside verges and golf courses. Due to their extensive use, high persistence, high water solubility and weak adsorptivity they contaminate the aquatic environment through agricultural run-off and leaching. These compounds are regularly found in stream, lake, ground and well water [1–7]. Because of widespread environmental pollution, the commercial use of atrazine has been forbidden in Germany since 1991, and has been replaced by terbutylazine [8,9]. Nevertheless, atrazine and its degradation products are still detectable.

In water and soil the parent *s*-triazine herbicides atrazine and terbutylazine (or simazine and propazine) are subjected to various biotic and abiotic degradation processes such as photolysis, oxidation,

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hydrolysis and biodegradation leading to dealkylation of the amine groups, dechlorination, deamination and hydroxylation at position 2, and to a minor extent, ring cleavage [3,4,8,10,11]. In addition to these degradation processes, up to 50% of the originally applied amount of atrazine and its metabolites have been determined as bound residues in soils [2,12–15].

The main degradation products in ground and surface waters are dealkylated chloro metabolites, predominantly deethylatrazine (DEA) [16,17]. Hydroxyatrazine degradation products (HADPs) are formed in the environment via chemical, biological and photochemical hydrolysis of atrazine and chlorinated atrazine metabolites. In soils, abiotical chemical degradation of adsorbed atrazine to hydroxyatrazine (HA) is generally the major degradation pathway with lesser amounts of the chloro metabolites DEA and deisopropylatrazine (DIA) formed by microbial *N*-dealkylation. Photochemical hydrolysis of atrazine in water is catalyzed by OH-radical generating photosensitizers, such as humic substances. HADPs are more persistent than atrazine or DEA and DIA in soil and strongly adsorb to soil organic matter. However, it has been demonstrated that HA is transported in solution by surface run-off events via desorption and leaching from soil sediments [18–21]. Although HADPs exhibit low water solubility [22,23], a recently published long term monitoring study in the midwestern states of the USA by Lerch and co-workers [18,21] confirmed their widespread occurence in streams. HA is the most significant HADP with greater concentrations and frequency of detection than the *N*-dealkylated HADPs. HA was even found to be the predominant atrazine degradation product with greater prevalence than DEA or DIA. The median HA concentrations in streams were 0.22 μg/l. However, in ground-water HA only exists at low ppt levels [11].

In the past, the lack of routine methods for determination of OH-triazines has limited research on the fate and behavior of these hydrophilic metabolites in environmental compartments. A sensitive analytical method is needed to investigate the degradation pathways in water. The parent *s*-triazine herbicides and with some restriction also the dealkylated amino metabolites can be analyzed by gas chromatography–mass spectrometry (GC–MS) (or nitrogen–phosphorus detection, NPD) [5,17,24–29], but the OH derivatives cannot be determined directly by GC because of their high polarity and non-volatility. High-performance liquid chromatography (HPLC) is directly applicable both to *s*-triazines and their OH degradation products and is by far the most frequently applied method for their determination [4,5,7,10,18,21,22,29–33]. In addition, enzyme-linked immunosorbent assays (ELISAs) have been applied to the determination of *s*-triazines and their degradation products [34,35]. Literature describing capillary electrophoresis (CE) analyses of *s*-triazine herbicides [1,2,36–40] and their OH metabolites [3,8,41–43] is still rare. Separations have been performed by capillary zone electrophoresis (CZE) [3,8,36,39,41,43], micellar electrokinetic capillary chromatography (MECC) [1,2,37,38] and isotachophoresis [40,42]. *s*-Triazine herbicides have been successfully extracted from water with conventional RP-C_{18} solid-phase extraction (SPE) materials [25,44,45], or nonpolar polystyrene–divinylbenzene sorbents [45,46], but the quantitative extraction of the more hydrophilic *N*-dealkylated degradation products and the OH metabolites from aqueous media with conventional non-polar sorbents is difficult [10,27,46–49]. With C_{18} materials recoveries for OH-triazines usually vary between 21% for deethylhdroxyatrazine (DEHA) and 75% for HA [29,50], although Geerdink et al. [51] claimed to have achieved extraordinary high recoveries with 1 g C_{18} PolarPlus material (Baker) for DEA and DIA (>90%) as well as for DEHA, deisopropylhydroxyatrazine (DIHA) (64%) and ameline (47%). Very good recoveries of >90% have been achieved by graphitized carbon black (GCB) adsorption for atrazine, terbutylazine, DEA, DIA and didealkylatrazine [24,48] as well as for the HADPs HA, DEHA, DIHA and ameline [5,30,47]. By exploiting the higher basicity of OH-triazines, selective extraction and good recoveries also have been obtained by the use of a strong cation-exchange (SCX) propylbenzenesulfonic acid cartridge [22]. Recently, a SPE enrichment procedure for HA, DEHA and DIHA with a mixture of nonpolar styrene–divinylbenzene and polar methacrylate macroporous resins (Amberchrom CG) has been reported. Even with 1200 mg sorbent mixture, the recovery for DIHA was only 25%, ameline was not retained at all [41].

The aim of this work was to investigate LiChrolut

EN for the extraction of chloro- and OH-triazines from water. LiChrolut EN (Merck, Darmstadt, Germany) is a microporous, crosslinked styrene–divinylbenzene adsorbent material. It has a very large accessible specific surface area and shows high adsorption capacity for polar micropollutants [9,10,28,52,53]. Önnerfjord et al. [10] measured breakthrough volumes for *s*-triazines and their *N*-dealkylated chloro metabolites with C_{18}, nonpolar styrene–divinylbenzene and LiChrolut EN. Quantitative recoveries for DEA and DIA only were achieved with LiChrolut EN [28]. Schlegel et al. [9] reported very good recoveries for terbutylazine and its polar chloro and OH metabolites of >90% for the extraction from tap water with 200 mg LiChrolut EN.

2. Experimental

2.1. Chemicals and reagents

The *s*-triazine standard substances were obtained from Riedel-de Haën (Seelze, Germany) and Dr. Ehrenstorfer (Augsburg, Germany). The internal standard *n*-propylatrazine was synthesized in our laboratory by Dr. Michael G. Weller. The structures, acidity constants (pK_a), *n*-octanol–water partition coefficients (log K_{OW}) and the water solubilities of the different *s*-triazines and their degradation products are given in Table 1. Sodium tetraborate ($Na_2B_4O_7$, anhydrous), sodium dodecyl sulfate (SDS), hydrochloric acid (37%), ammonium acetate, glacial acetic acid (100%) and sodium hydroxide all of analytical grade were purchased from Merck (Darmstadt, Germany). Methanol, acetone and toluene (purity for pesticide residue analysis) were supplied by Promochem (Wesel, Germany). Ultrapure water was prepared by ultrafiltration with a Millipore Q_{plus} apparatus (Millipore, Bedford, MA, USA).

s-Triazine standard stock-solutions of 50 mg/l were prepared by dissolving 5 mg of each compound in 100 ml methanol. Acidification of these solutions is necessary for the solubilization of the OH-triazines. Therefore, 1 ml hydrochloric acid (37%) was added to the OH-triazine standards. The working standard solutions were prepared by further dilution of the stock standard solutions with toluene for GC–MS and Milli-Q water for CE analyses. The standard mixtures for calibration and preparation of fortified SPE samples were produced from these single-compound solutions. All solutions were stored at 4°C in the dark.

2.2. Solid-phase extraction

The solid-phase adsorption material LiChrolut EN was obtained from Merck. LiChrolut EN (200 mg) was filled in 3-ml glass cartridges (Merck ordering No. 1.19878.0001) between two PTFE frits (Merck, 1.19891.0001, porosity 10 μm). The adsorbent was activated and conditioned first with 5 ml of a

Table 1
Names, abbreviations, chemical structures, acidity constants (pK_a), *n*-octanol–water partition coefficients (log K_{OW}) and water solubilities of the investigated *s*-triazines [22,31,32,41,43,46,47,54–56]

Compound	Abbreviation	R_1	R_2	R_3	pK_a	Log K_{OW}	Water solubility (mg/l)
Atrazine	A	Cl	NH-C_2H_5	NH-$CH(CH_3)_2$	1.68–1.71	2.2–2.7	33
Terbutylazine	T	Cl	NH-C_2H_5	NH-$C(CH_3)_3$	1.95	3.04	8.5
Deethylterbutylazine	DET	Cl	NH_2	NH-$C(CH_3)_3$	–	–	–
Deethylatrazine	DEA	Cl	NH_2	NH-$CH(CH_3)_2$	1.3–1.65	1.52	3200
Deisopropylatrazine	DIA	Cl	NH-C_2H_5	NH_2	1.3–1.58	1.13	670
n-Propylatrazine	PA	Cl	NH-$(CH_2)_2CH_3$	NH-$CH(CH_3)_2$	–	–	–
Hydroxyatrazine	HA	OH	NH-C_2H_5	NH-$CH(CH_3)_2$	4.9–5.2	1.4	5.9
Hydroxyterbutylazine	HAT	OH	NH-C_2H_5	NH-$C(CH_3)_3$	5.2	–	–
Deethylhydroxyatrazine	DEHA	OH	NH_2	NH-$CH(CH_3)_2$	4.57–4.75	0.2	26.7
Deisopropylhydroxyatrazine	DIHA	OH	NH-C_2H_5	NH_2	4.65	−0.1	22.0
Deethyldeisopropylhydroxyatrazine	Ameline	OH	NH_2	NH_2	4.5 pK_{a2}=9.4	−1.2	21.0

methanol–acetone (3:2, v/v) solvent mixture and then with 5 ml water (without application of vacuum). For recovery studies, 1-l water samples (Milli-Q, tap or river water) were spiked with known volumes of a *s*-triazine standard mixture. In case of the OH-triazine mixture, the samples were pH-adjusted with hydrochloric acid (37%) either to pH 1.8, 3.0 or 5.2 (or not adjusted for pH 7.0 and 7.9). The tap water for the extraction of the chlorotriazines was not pH-adjusted (pH 7.9). The spike level for each OH-triazine for the recovery study experiments was 2 μg/l, and for the chlorotriazines 0.1 μg/l and 10 μg/l. From the 1-l sample, 200 ml water was taken and filled into glass reservoirs connected to the extraction cartridge and was then drawn through with a flow-rate of approximately 5 ml/min. After the extraction, the cartridges were washed with 5 ml water and then dried with a stream of nitrogen to remove any remaining water. The compounds were eluted with 4 ml of the methanol–acetone (3:2, v/v) solvent mixture into glass vials. The solvent was evaporated under a gentle stream of nitrogen and the OH-triazines redissolved in 100 μl water for CE analysis (enrichment factor: 2000) and the chlorotriazines in 20 μl toluene for GC–MS analysis (enrichment factor: 10 000). Absolute recoveries were determined using external calibrations. The mean values for recoveries were calculated from six determinations ($n=6$).

2.3. Samples and sample pretreatment

The tap water used was from the water supply system of Munich and the river water from the river Isar, the major river running through Munich. The samples were collected in August 1998. The *s*-triazine contaminated groundwater was from the drinking water system of a rural village near Munich. No special sample pretreatment was applied.

2.4. Gas chromatography–mass spectrometry

GC–MS analyses of the chlorotriazines were carried out on a Hewlett-Packard HP 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) connected to a VG AutoSpec mass spectrometer with a resolution of 10 000 (Micromass, Manchester, UK), using a HP-5-MS column (Hewlett-Packard) 60 m×0.25 mm I.D., 0.25 μm film thickness, and helium as carrier gas. The temperature of the splitless injector and the GC–MS interface was 270°C. The GC temperature program was: 90°C held for 2.0 min (solvent toluene), then at 10°C/min to 250°C, 250°C held for 5.0 min, and at 40°C/min to 300°C, 300°C held for 5.0 min. The injection volume was 1 μl.

Electron impact (EI) ionization of the sample was performed using an electron energy of 40 eV with a trap current of 800 μA. The two electric fields of the mass spectrometer were operated at a voltage of 8000 V. Mass analyses were performed in the selected ion monitoring (SIM) mode. The components were detected by recording the following molecular-ions m/z: DIA 173.0468, DEA 187.0625, DET 201.0781, atrazine 215.0928, terbutylazine and *n*-propylatrazine 229.1094. The concentrations of the real water samples were determined by internal calibration with the internal standard *n*-propylatrazine.

2.5. Capillary electrophoresis

Separations were performed with a Crystal CE system equipped with a fixed-wavelength UV detector Model PU 4225 (both from Unicam Chromatography, Kassel, Germany). UV detection was performed at 210 nm. Bare fused-silica capillaries of 60 cm (length to detector 50 cm)×75 μm I.D.×375 μm O.D. were used (Unicam). A constant voltage of 20 or 25 kV was applied with the cathode end at the detector. The temperature of the capillary was maintained at 30°C by the instrument thermostatting system. Samples were pressure injected with 50 mbar for 12 s (cathodic injection). The injection volume is approximately 10–60 nl and can be calculated by Hagen–Poiseuille's Law. Data acquisition was performed with 4880 Unicam Chromatography data handling software.

MECC separations of the OH-triazines were routinely performed with a 30 m*M* sodium borate–30 m*M* SDS buffer at pH 9.3. The 100 m*M* acetic acid buffer (pH 4.6) was prepared by mixing sodium acetate and glacial acetic acid (each 100 m*M*) and adding 10% methanol. The buffers were filtered through 0.45 μm cellulose acetate filters (Sartorius, Göttingen, Germany). The capillary was conditioned

every morning before starting a sequence of runs by rinsing in the high-pressure mode for at least 10 min with 0.1 *M* NaOH, 5 min with water and 5 min with running buffer. After every run the capillary was rinsed for 5 min with 0.1 *M* NaOH, 3 min with water and 2 min with running buffer in order to remove adsorbed material from the walls of the capillary. Pre-run rinsing for equilibration was performed with running buffer for 2 min.

3. Results and discussions

3.1. GC–MS analysis

Due to its higher sensitivity and selectivity, routine separations of chlorotriazines were performed by GC–MS in the SIM mode. Fig. 1 shows a standard chromatogram at a concentration range of 100 μg/l for each compound. The most polar *s*-triazine DIA is eluting first (retention time 16 min and 5 s) because of its lowest retention on the nonpolar HP-5 column. The separation of DIA and DEA (16.15) is critical, but with a clean injector inlet it is baseline. Otherwise, peak-tailing occurs. For sensitivity enhancement, a detection window was set after the elution of DET (16.33) at 17.00 min. In function 2, atrazine (17.28), terbutylazine (17.50) and the internal standard *n*-propylatrazine (18.33) are detected.

Calibration for the chlorotriazines was performed in the concentration range between 1 and 2000 μg/l with GC–MS. The calibration graphs are linear in this range. Regression data is not shown. The correlation coefficients r ($n=9$) are between 0.996 and 0.999. The response factors relative to the internal standard *n*-propylatrazine were measured from this calibration.

The limit of detection (LOD) concerning detector sensitivity ($S/N=3$) was determined to be 1 μg/l, which corresponds to absolute 1 pg for a 1 μl injection. The blank value dependent LOD of the combined SPE–GC–MS method for the extraction of tap 200 ml water with 200 mg LiChrolut EN is

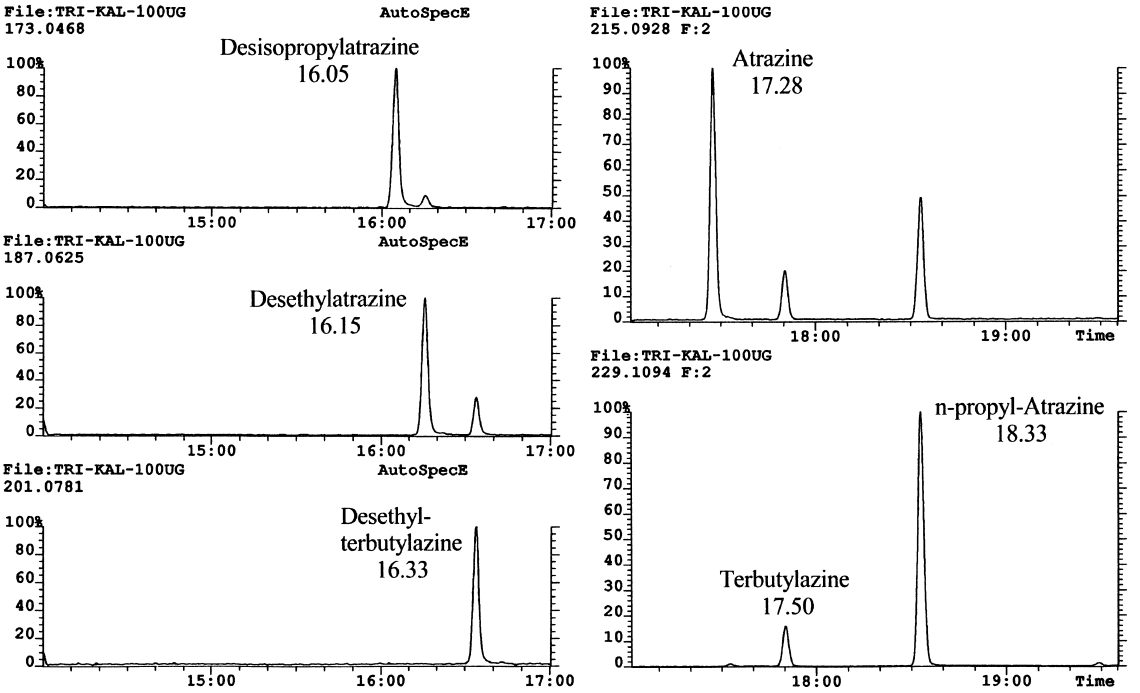

Fig. 1. GC–MS chromatogram of a 100 μg/l chlorotriazine standard. 60 m HP-5-MS column, resolution 10 000, injection of 1 μl. Time in min.

approximately 5 ng/l (LOD=blank value+3×RSD of the blank value).

3.2. CE analysis

s-Triazines are weakly basic compounds which can be protonated in aqueous solution to give the corresponding cations (see Fig. 5) [3,23,31,43]. However, owing to their low pK_a values (<2.0), protonated chlorotriazines are unsuited for CZE separations. In an acidic medium (citric, phosphoric, Tris–trichloroacetic, citrate–HCl or perchloric acid buffers), measurements are unstable due to a weak electroosmotic flow (EOF) and long migration times [1,8,36]. Conversely, OH-triazines with pK_a values of 4.5–5.2 are quite well suited for CZE. Schmitt and co-workers [3,8] reported that the best buffer for OH-triazine separations is acetate at pH 4.6. Therefore, separation of the chosen OH-triazines HA, HT, DEHA, DIHA and ameline was investigated under these conditions. The best separation was obtained at pH 4.6 with a 100 m*M* acetate buffer and the addition of 10% methanol (Fig. 2). The use of 50 m*M* acetate resulted in shorter migration times and decreased separation efficiency (electropherogram not shown). The electropherogram in Fig. 2 shows that DEHA and DIHA are not completely separated with the acetate buffer. Also the addition of more organic modifier methanol to the buffer did not result in better separation. Therefore, MECC which usually provides better separations was applied to the determination of the OH-triazines. Different concentrated sodium borate–SDS buffers were tested for the separation of the five OH-triazines. Separation and peak shapes are very susceptible to the buffer molarity and the applied voltage. The best and reproducible separation results were obtained with 30 m*M* borate–30 m*M* SDS and a voltage of 20 kV (Fig. 3). With a buffer of lower molarity (25 m*M*) separation efficiency of DEHA and DIHA was reduced (due to shorter migration times) and caused peak-splitting for HA and HT. A higher voltage of 25 kV induces Joule heating of the capillary which degrades separation and also results in shorter migration times.

Calibration for the OH-triazines was performed in

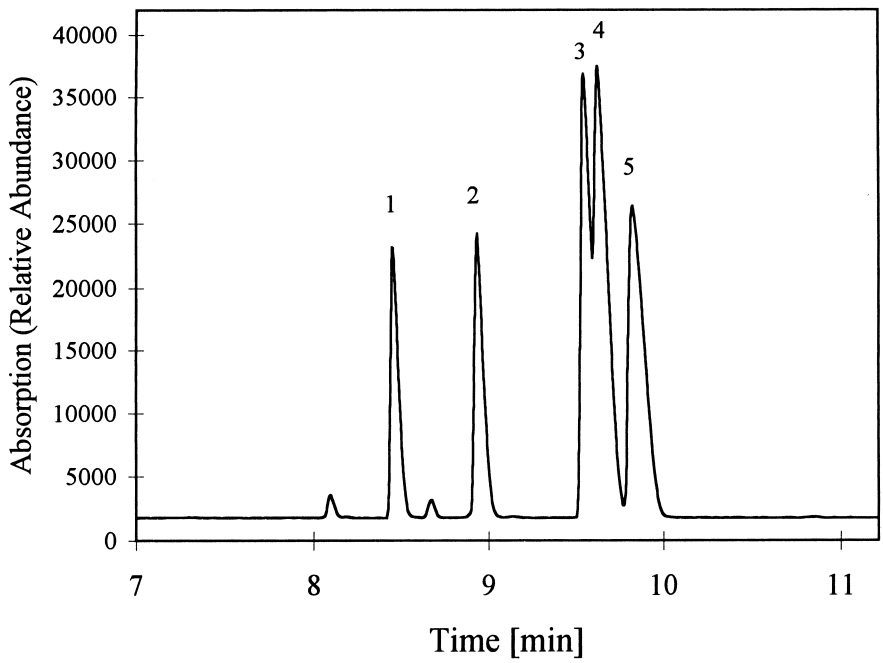

Fig. 2. Electropherogram of a five-compound OH-triazine mixture containing 10 mg/l of each compound. Conditions: running electrolyte 100 m*M* acetate buffer with 10% methanol, pH 4.6, capillary 60 cm (50 cm to detection window)×75 μm I.D., voltage 25 kV, temperature 30°C, pressure injection 50 mbar for 12 s, UV detection at 210 nm. Peaks: 1=HT, 2=HA, 3=DEHA, 4=DIHA, 5=ameline.

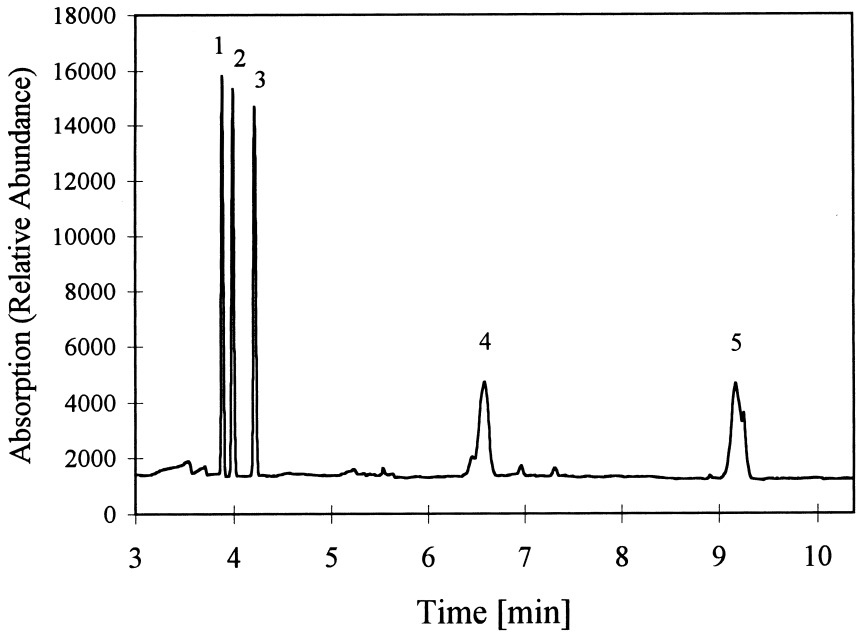

Fig. 3. Electropherogram of a five-compound OH-triazine mixture containing 5 mg/l of each compound. Conditions: running electrolyte 30 m*M* sodium borate–30 m*M* SDS, pH 9.3, capillary 60 cm (50 cm to detection window)×75 μm I.D., voltage 20 kV, temperature 30°C, pressure injection 50 mbar for 12 s, UV detection at 210 nm. Peaks: 1=DIHA, 2=DEHA, 3=ameline, 4=HA, 5=HT.

the concentration range between 0.2 mg/l (for DIHA, DEHA and ameline) or 0.5 mg/l (for HA and HT) and 10 mg/l with MECC (30 m*M* borate–30 m*M* SDS) and UV detection at 210 nm. The calibration graphs are linear in this range. Regression data is not shown. The correlation coefficients *r* (n=5 or 6) are between 0.966 for DEHA and 0.999 for HA.

The LOD is approximately 0.2 mg/l ($2 \cdot 10^{-6}$ *M*) for the early eluting DIHA, DEHA and ameline and 0.5 mg/l for HA and HT (S/N=3). By extracting 200 ml water and redissolving in 100 μl, an enrichment factor of 2000 is achieved. Hence, the LOD of the combined method of SPE enrichment and CE analysis is approximately between 0.1 and 0.25 μg/l for 200 ml water samples.

Separations of chlorotriazines have been achieved as uncharged compounds at neutral pH by MECC [1,2,37,38]. However, we experienced difficulties analyzing chlorotriazines by MECC due to peak splitting or very broad peaks. Fig. 4 shows the obtained peaks for atrazine, DEA and DET with the 30 m*M* borate–30 m*M* SDS buffer. Only DEA gives a single but relatively broad peak, the later eluting atrazine and DET are split dupletts. The reason for this peak-splitting is probably the existence of different *s*-triazine rotational isomers as the rotation of the alkyl-sidechains is hindered in the *s*-triazine molecule. These isomers are separated by CE due to its very high resolution efficiency.

3.3. Solid-phase extraction and recovery studies

s-Triazine concentrations in environmental waters are very low, therefore an enrichment step in combination with CE–UV as well as with GC–MS determination is required. SPE with LiChrolut EN was investigated for chloro- and OH-triazine enrichment from water. Tap water was spiked with 0.1 or 10 μg/l for each of the chosen chlorotriazines (Table 2) and Milli-Q, tap or river water samples with 2 μg/l for the optimization of the OH-triazine extraction (Table 3) with LiChrolut EN. The tap water for the chlorotriazine enrichment was not pH-adjusted, it had a pH value of 7.9. SPE of the OH-triazines was investigated at different pH values (pH 1.8, 3.0, 5.2,

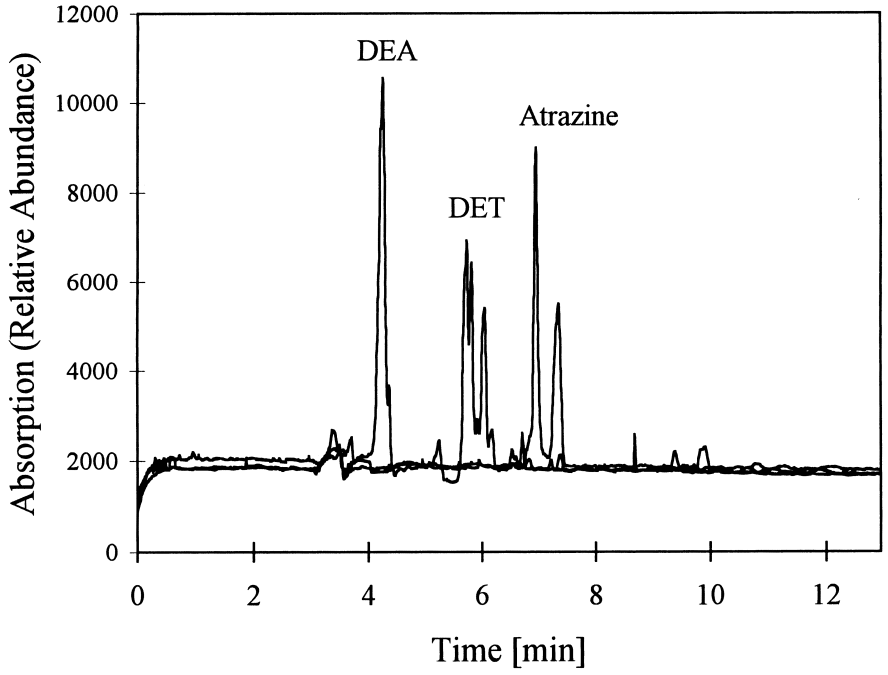

Fig. 4. Electropherograms of chlorotriazines. Conditions as in Fig. 3.

Table 2
Recovery of chlorotriazines from 200 ml fortified tap water with 200 mg LiChrolut EN, pH 7.9 (n=6)

s-Triazine	c_0=0.1 μg/l		c_0=10 μg/l	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
DIA	99.8	11.8	98.4	8.4
DEA	102.5	8.5	93.5	5.4
DET	103.5	6.0	122.5	16.2
Atrazine	124.8	4.6	100.6	5.5
Terbutylazine	103.0	8.5	102.2	9.9
n-Propylatrazine	96.3	6.7	106.9	7.9

Table 3
pH dependent recovery of HTDPs from 200 ml fortified water at 2 μg/l with 200 mg LiChrolut EN (n=6)

	Milli-Q water						Tap water						River water	
	pH 1.8		pH 3.0		pH 7.0		pH 3.0[a]		pH 5.2		pH 7.9		pH 3.0	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
DIHA	12.0	1.5	25.4	3.0	72.6	2.7	28.4	1.5	43.6	4.4	14.1	2.7	43.8	6.2
DEHA	47.1	1.1	67.7	3.2	83.8	3.6	82.3	4.6	67.4	5.1	16.9	4.8	83.5	5.7
HA	86.3	3.2	76.8	5.8	78.6	12.1	89.8	16.3	71.8	8.4	27.6	7.2	92.1	11.3
HT	85.8	3.8	93.7	8.9	79.6	10.7	93.4	8.3	81.3	9.2	44.4	4.7	93.4	14.8

[a] Values with 500 mg LiChrolut EN (n=3): DIHA 72.5% (±10.4), DEHA 105.8% (±13.5), HA 112.6% (±15.3) and HT 97.1% (±11.8).

7.0 and 7.9). Analyses were performed for the chlorotriazines by GC–MS and for the OH-triazines by MECC and recovery studies were carried out. Recovery data for the chlorotriazines is given in Table 2 and for the OH-triazines for Milli-Q, tap and river water at different pH values in Table 3.

Recovery of the chlorotriazines from 200 ml spiked tap water at pH 7.9 with LiChrolut EN is quantitative. Recovery values range from 94% for DEA (c_0=10 μg/l) to 125% for atrazine (c_0=0.1 μg/l). No pH adjustment is necessary for the extraction of the relatively nonpolar herbicides atrazine and terbutylazine and their medium polar monodialkylated degradation products DEA, DIA and DET. The average precision of recovery of the method indicated by the RSDs are between 5 and 16%. The high recovery of 125% for atrazine at the lower spike-level of 0.1 μg/l might be an indication for a blank value problem.

Recovery of the OH-triazines from ultra-pure Milli-Q water was compared at pH 1.8, 3.0 and 7.0, recovery from tap water at pH 3.0, 5.2 and 7.9 (Table 3). What is quite astonishing is the big recovery difference from Milli-Q water compared with tap water at neutral pH (7.0 for the Milli-Q and 7.9 for the tap water). The extraction from Milli-Q water at pH 7.0 yielded very good recovery values even for the most hydrophilic compound DIHA (73%), whereas the recovery rates from tap water at pH 7.9 were <50% for all compounds. However, ameline was not extracted neither from tap nor from Milli-Q water. From Milli-Q water, the dealkylated HADPs DIHA and DEHA are worse retained at lower pH values, but HA and HT are slightly better retained at pH 1.8. Similarly, the highest recoveries for the extraction from tap water were obtained at the lowest pH 3.0 (82% for DEHA, 90% for HA and 93% for HT). However, for DIHA the optimum extraction pH was 5.2 (44%). Consequently, the river water samples were extracted at pH 3.0 only. The RSDs were determined to be <17% in all cases. With 500 mg adsorbent material (LiChrolut EN) the recovery of DIHA was increased to 73% (±10.4, n=3). Higher values for the other compounds were obtained too. Therefore, the lower recovery with 200 mg can be explained by early breakthrough of this polar compound. Breakthrough volumes were not determined.

OH-triazines are weakly basic compounds with dissociation constants between 4.5 and 5.2 (Table 1) [3,22,23,31,43]. According to Fig. 5, they may exist in a variety of forms – uncharged tautomeric keto-enol species, zwitterionic, anionic or protonated-keto isomers dependent upon pH. They are anionic species at pH values above 11.5, predominantly a zwitterionic keto-enol form between pH 11.5 and approximately 4.6, and they are easily protonated at an amine-nitrogen of the both sidechains or at the *N*-heterocycle at acidic pH to form a protonated keto species [31,43]. Our recovery results show that retention of the HTDPs on LiChrolut EN is increased at lower pH values when the compounds are present in their cationic form. Therefore, it must be concluded that LiChrolut EN has anionic adsorption sites on its surface which enables cation-exchange properties. Retention on the polystyrene–divinylbenzene sorbent of the charged OH-triazine species is probably also induced by their hydrophobic properties (this phenomenon is also known as hydrophobic effect [57]).

The advantages of the present SPE method with LiChrolut EN for the extraction of hydrophilic OH-triazines are its simplicity, fastness and the economic consumption of only 200 mg LiChrolut EN adsorbent per extraction. Better recoveries were obtained for DIHA compared with even 1200 mg Amberchrom resin [41]. However, GCB adsorption methods yield higher recoveries [5,30]. One drawback of LiChrolut EN is its inability to retain the very hydrophilic ameline.

3.4. Analysis of real water samples

To investigate the applicability of the present SPE procedure for real environmental analysis of chloro- and hydroxytriazines, tap, ground and river water samples were analyzed by MECC and GC–MS after analyte enrichment with LiChrolut EN. The tap water in Munich is very clean and has drinking water quality as it comes directly from mountain springs or sources in the Austrian and German Alpes. The river Isar running through Munich also origins from the Alpes and flows mainly through cattle farming areas up to Munich. Neither chloro- nor OH-triazines were found in these waters by MECC–UV and GC–MS analysis after analyte enrichment with LiChrolut EN.

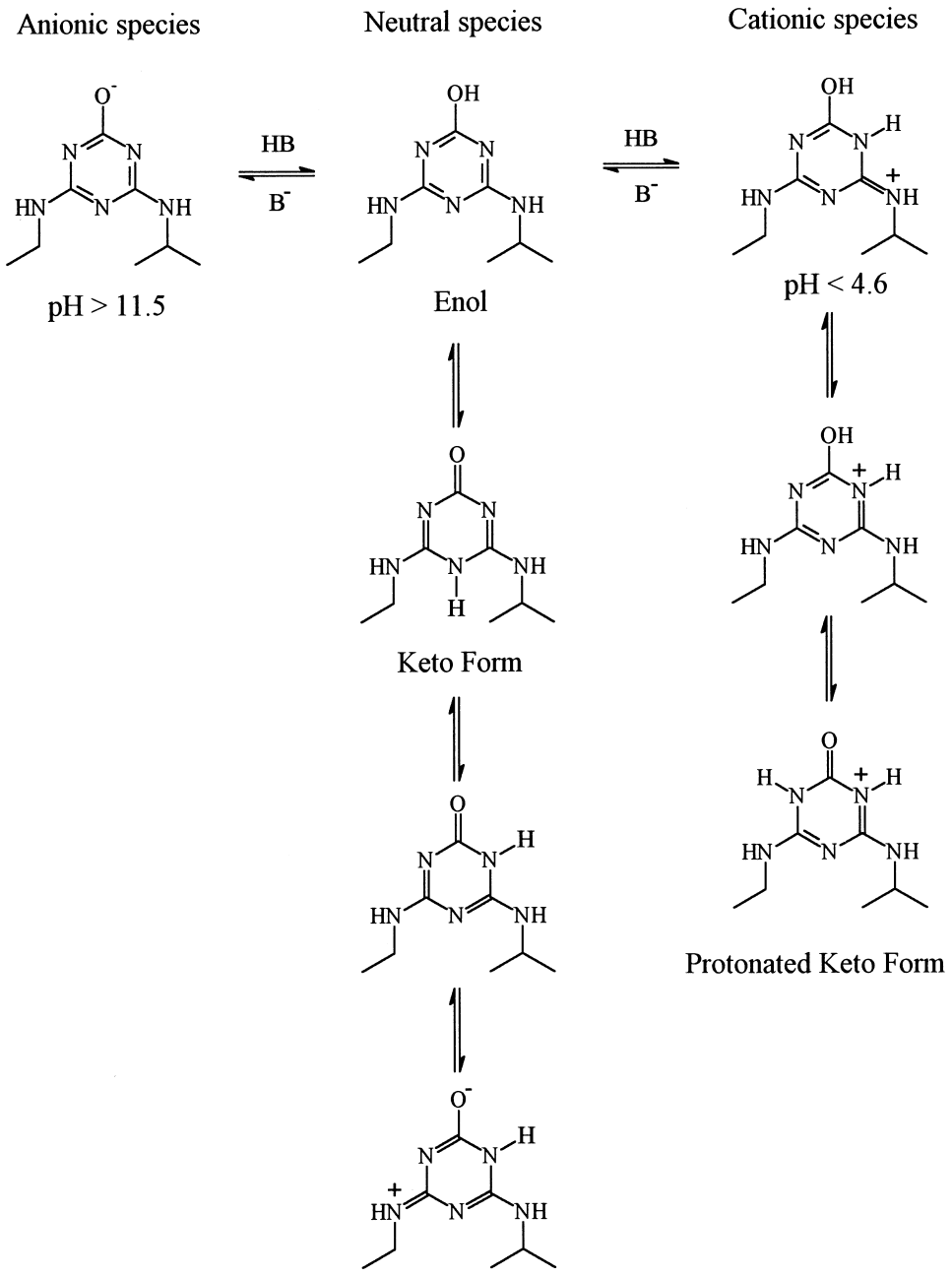

Fig. 5. Tautomeric forms of hydroxyatrazine at different pH values (B^-=base) [31,43].

Therefore, we spiked tap and river Isar water samples with OH-triazines. Fig. 6 shows electropherograms of these spiked water samples at an enrichment factor of 2000 recorded with the 30 m*M* borate–30 m*M* SDS buffer. The spike level for the OH-triazines was 2 μg/l. The lower plot represents for comparison the standard OH-triazine mixture at 2 μg/l. The electropherogram of the river Isar extract shows a much higher background absorbance caused by interfering matrix compounds such as humic

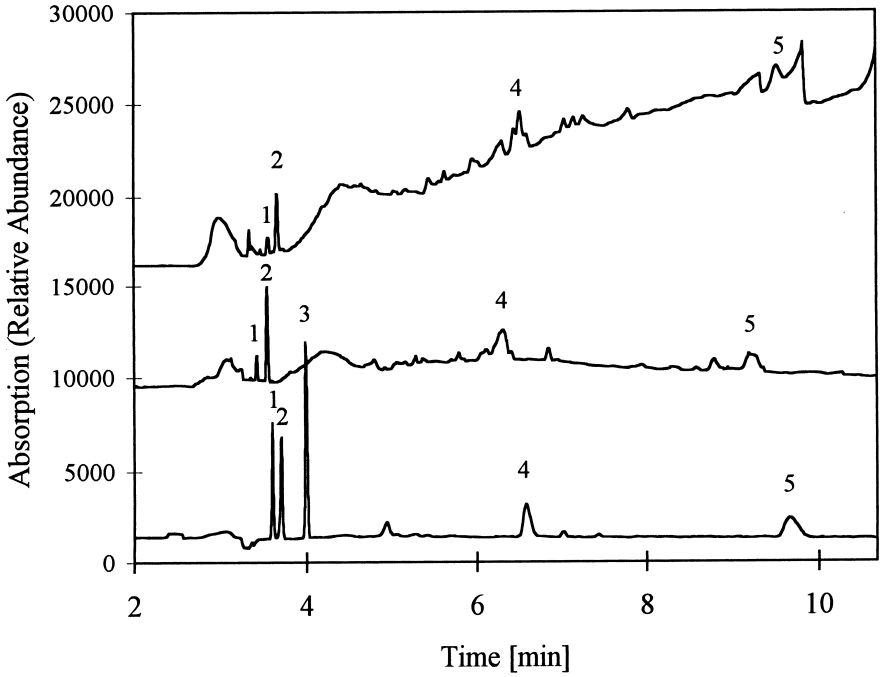

Fig. 6. Electropherograms of OH-triazine spiked (2 μg/l) tap and river Isar water samples after SPE with LiChrolut EN (enrichment factor 2000). Conditions as in Fig. 3. Peaks: 1=DIHA, 2=DEHA, 3=ameline, 4=HA, 5=HT.

substances. Ameline (3) is not detected in the samples after SPE as it is not extracted with LiChrolut EN. The peak for DIHA (1) is quite small due to poor recovery. In the tap and especially in the river water samples some additional interfering signals are observed. Separation of HT (5) from these interferences in the river water is quite difficult. The peak form of HA (4) in the river water sample is not ideal. Nevertheless, the electropherograms show that the present SPE–MEIC method allows real water analysis of OH-triazines in the low μg/l range.

Groundwater from the drinking water system of a rural village near Munich was analyzed routinely for chlorotriazines by SPE with LiChrolut EN and GC–MS determination (chromatograms not shown). Atrazine and DEA were regularly detected in this groundwater. The concentration for DEA was between 70 and 120 ng/l and for atrazine between 20 and 35 ng/l. Terbutylazine and DET were not found in the water. DIA is difficult to detect as its concentration is close to or below the LOD of the method of approximately 5 ng/l. These results confirm the current knowledge about atrazine metabolism. DEA is the major atrazine degradation product in groundwater with much higher prevalence than DIA. DEA shows a greater mobility and better solubility in water than atrazine, and therefore it is present in a higher concentration range in the groundwater. DEA sometimes even exceeds the threshold value of 0.1 μg/l for a single pesticide in Europe.

4. Conclusions

SPE with the styrene–divinylbenzene adsorbent LiChrolut EN was investigated and optimized for the extraction of *s*-triazine herbicides and their hydrophilic hydroxylated degradation products (OH-triazines) from water. Compared to other conventional adsorbent materials like RP-C_{18} or Amberchrom styrene–divinylbenzene resins, good recovery values were obtained with only 200 mg LiChrolut EN. The optimum pH value for SPE of the OH-triazines with LiChrolut EN was 3.0. As the OH-triazines are

present in acidic media in their cationic, protonated form, these results indicate the presence of anionic adsorption sites on LiChrolut EN. Hence, LiChrolut EN exhibits cation-exchange properties and is not a completely nonpolar styrene–divinylbenzene adsorbent material. Separation efficiency for the OH-triazines by MECC was higher than with CZE. The applicability of the combined method of SPE enrichment and CE analysis was checked by the analysis of spiked water samples in the low μg/l range. Chloro- and OH-triazines were not found in the river Isar. Atrazine and deethylatrazine were regularly detected in groundwater of an agricultural area used for drinking water production in the low ng/l range by GC–MS after SPE with LiChrolut EN. The simplicity and fastness of the LiChrolut EN SPE enrichment procedure in combination with CE–UV or GC–MS analysis makes the presented method time efficient, economic and easy to handle.

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