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Simultaneous Determination of Terbuthylazine and Its Major Hydroxy and Dealkylated Metabolites in Wetland Water Samples Using Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode-Array Detection

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A method based on high-performance liquid chromatography with diode-array detection was developed and validated aiming at the simultaneous determination of terbuthylazine (TER) and its five major metabolites, desisopropyl-hydroxy-atrazine, desethyl-hydroxy-terbuthylazine, desisopropyl-atrazine, hydroxy-terbuthylazine, and desethyl-terbuthylazine. Although s-triazines are used worldwide as herbicides for agricultural and nonagricultural purposes, there is limited information on the environmental impact of TER degradation products. The proposed method includes a solid-phase extraction procedure (using MCX cartridges) with adequate recovery efficiency (70−80%). The statistical evaluation of the method reveals good linearity, accuracy, and precision for the compounds determined, with RSD values less than 14.6%, while the detection limit was found to be 0.05 μg L^{-1} for DIHA and 0.01 μg L^{-1} for the other substances. This method can be employed in biodegradation studies of TER and its metabolites in water samples from constructed wetlands, thus assisting the evaluation of their environmental impact.

1. INTRODUCTION

s-Triazines are used worldwide as selective pre- and post-emergence herbicides for the control of both grasses and broadleaf weeds in many agricultural crops like corn, wheat, maize, barley, sorghum, grape, peaches, apple, and asparagus as well as for nonagricultural purposes such as soil sterilization and road maintenance (*1*). During and after the herbicide application to the farming land, triazines may be transported to both ground and surface water and also into the atmosphere (*2*−*4*). Atrazine (AT) is the most commonly used and the main representative of s-triazines (*5*). Due to environmental pollution, the commercial use of atrazine has been forbidden in the European Union (*6*) and has been gradually replaced by terbuthylazine (TER) (*7*, *8*). TER has lower water solubility (8.5 vs 33.0 mg L^{-1} (pH 7, 20 °C)) than atrazine and stronger soil sorption (*9*). However no methodology exists for routine analysis of TER together with its degradation products. As a result there has been limited information on the environmental impacts of TER degradation products.

In water and soil the parent TER molecule is subjected to various biotic and abiotic degradation processes such as photolysis, oxidation, hydrolysis, and biodegradation, leading to dealkylation of the amine groups, dechlorination, and subsequent hydroxylation (*10*−*15*). The main degradation products in ground and surface waters via biotic mechanism are the dealkylated chloro metabolites, such as deethyl-terbuthylazine (DET) and deisopropyl-atrazine (DIA) (*16*, *17*). Hydroxy-terbuthylazine (HT) is the major abiotic degradation product in water and soil. Other major metabolites of TER are deethyl-hydroxy-terbuthylazine (DEHT) and deisopropyl-hydroxyatrazine (DIHA) (*10*) as shown in Figure 1.

To date, many analytical methods have been developed aiming at the determination of TER. As AT shares common metabolites with TER, several analytical methods have also been proposed for their determination. A method for the simultaneous determination of AT and its dealkylated products in agricultural soil and in associated surface and groundwater has been reported employing a high-performance liquid chromatography-ultraviolet (HPLC-UV) system and a solid-phase extraction (SPE) pretreatment step using cyclohexyl Bond elut cartridges (*18*). In another method, the determination of the dechlorinated products has also been realized using an LC-MS system (*19*).

Nonpolar SPE sorbents are generally selected for extracting triazines from water samples such as the parent molecule of

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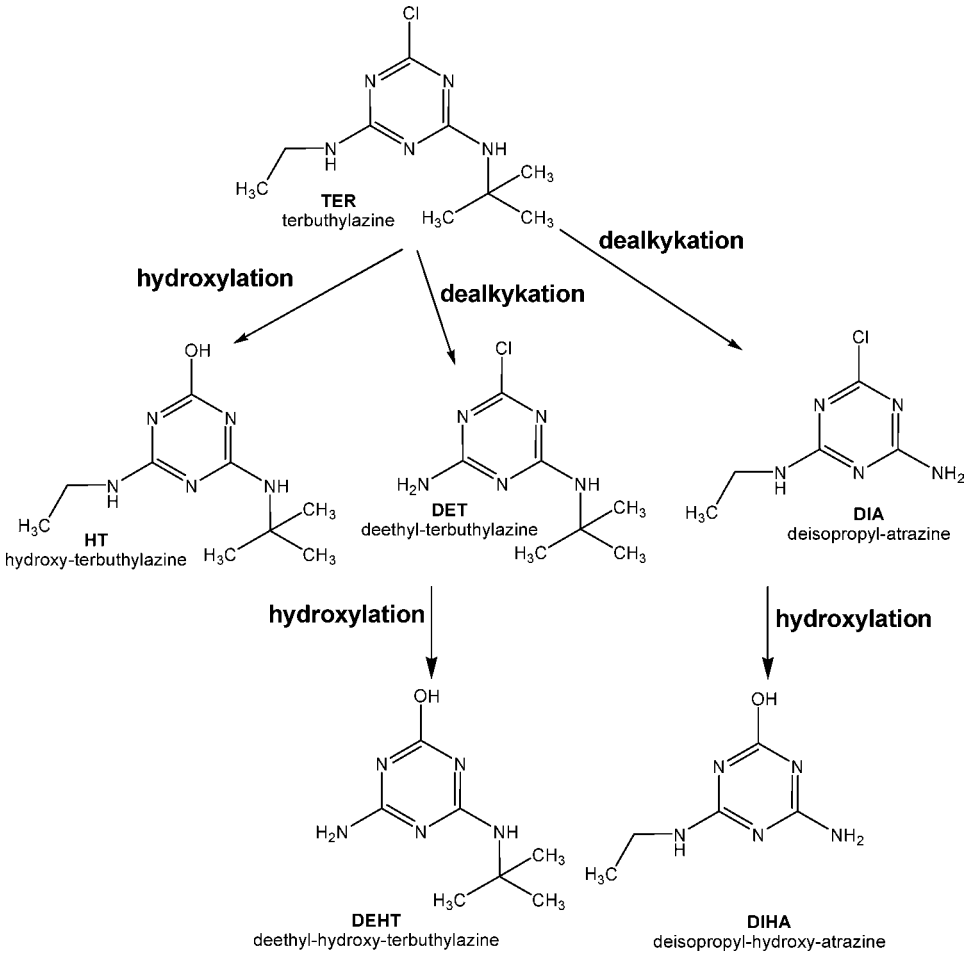

Figure 1. Degradation pathways and respective products of terbuthylazine (TER).

AT and TER. However the degradation products which contain polar functional groups such as the hydroxyl group moiety can be more efficiently extracted using polar sorbents (*14*). While C_{18} bonded silica has been used successfully for extracting AT, breakthrough (*20*) and recovery (*21*) studies for DEA and DIA indicate that these polar analytes can be prematurely lost in aqueous elution from the C_{18} cartridge (*22*). Another analytical method has been reported for the analysis of atrazine, its dealkylated degradation products, and hydroxyatrazine using thermospray HPLC-MS/MS (*12*). AT and its hydroxy degradation products have also been determined by HPLC-UV, following SPE treatment with propylbenzenesulfonic acid (SCX) cation-exchange SPE cartridges (*5*). Similarly, sample pretreatment using graphitized carbon black (GCB) cartridges has been employed in the analysis of the polar substances DEA and DIA (*23*, *24*). AT, DIA, deethyl-atrazine (DEA), and HT have been analyzed in water samples with large volume injection by two coupled LC columns using an HPLC-UV system. Due to polarity differences between the triazine herbicides and their metabolites, the two aforementioned approaches (C_{18} and SCX cartridges) have been used in a single analysis in order to achieve the maximum selectivity and sensitivity (*25*). Also, AT, DIA, DEA, and HT have been analyzed in water samples by GC-MS preceded by a C_{18} SPE pretreatment step (*26*) or by solid-phase microextraction (SPME) GC-MS (*27*). SPE with the styrene-divinylbenzene adsorbent LiChrolut EN has also been investigated for the extraction of s-triazines and dealkylated and hydroxylated degradation products by GC-MS and capillary electrophoresis-UV detection (*10*). On the other hand, parent s-triazine herbicides and with some restriction the dealkylated amino metabolites can be analyzed by GC-MS, but the hydroxylated derivatives cannot be determined directly by GC due to their high polarity and nonvolatility (*10*, *11*) thus making their derivatization necessary (*28*). Other methods have also been developed in conjunction with liquid chromatography−mass spectrometry (LC-MS) using molecularly imprinted polymers (*29*, *30*).

The goal of this work is the development of a sensitive method for analyzing TER, together with the associated N-dealkylated degradation products DET and DIA and the hydroxy products, HT, DEHA, and DIHA in water samples from constructed wetlands. This is the first method for the simultaneous determination of the major degradation hydroxy and dealkylated products of TER in aquatic samples with one SPE procedure in one chromatographic run. The sample pretreatment was carried out by SPE with Oasis MCX cartridges, and the samples were analyzed by HPLC using photodiode-array detection (DAD).

2. EXPERIMENTAL PROCEDURES

2.1. Reagents and Chemicals. TER, DET, DIA, HT, DEHA, and DIHA were kindly provided by Syngenta Crop Protection (Munchwilen, AG). Ammonium acetate was obtained from Panreac (Barcelona, Spain). Acetonitrile (AcN) and methanol (MeOH) used throughout the experiments were purchased from Merck (Darmstadt, Germany). HPLC water used was doubly purified using reversed osmosis and an ultrapure water production system (Barnstead nanopure infinity).

2.2. Stock Standard Solutions. Stock standard solutions of all analytes were prepared in methanol at the 1 mg mL^{-1} level. Regarding the hydroxy metabolites protonation of the amine moiety was necessary

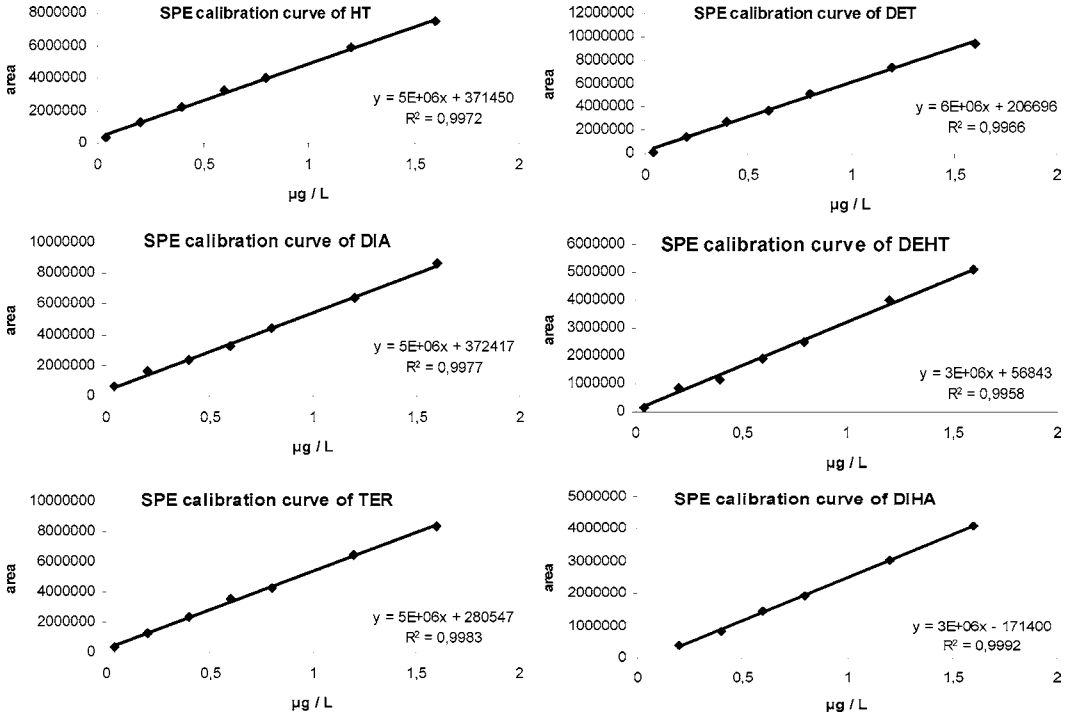

Figure 2. Calibration curves and regression equations of DEHT, DIA, HT, DET, and TER (fortified wetland water samples) in 0.04, 0.2, 0.4, 0.6, 0.8, 1.2, and 1.6 μg L^{-1} and 0.2–1.6 μg L^{-1} concentration levels for DIHA.

for their dissolvation. Thus preparation of the hydroxy metabolites standard solutions was carried out as follows: the corresponding solid was suspended in methanol (in a volumetric flask) followed by dropwise addition of 37% (v/v) HCl, and the volume was finally adjusted with methanol. All of the stock standard solutions are stored in a refrigerator at 4 °C. Working standard solutions (0.1, 0.2, 0.5, 1, and 5 mg mL^{-1}) were prepared every week by serial dilution of stock standard solutions in 10% acetonitrile/H_2O (v/v) (initial mobile phase composition of the HPLC gradient elution program). Mixed working standard solutions were prepared in the same way as the working standard solutions.

2.3. Sample Preparation. The wetland water samples were vacuum filtered through Whatman filter papers no. 1 prior to analysis, and an exact volume of 500 mL was fortified with appropriate volumes of the corresponding mixed working solution. The sample was then acidified at pH 1.5 with 1 M HCl prior to the application on the cartridge.

2.4. Instrumentation. An HPLC system comprising a Spectra system P4000 (Finnigan, Riviera Beach, FL), a quaternary pump equipped with a 7725i injector (Rheodyne, Rohnert Park, CA) fitted with a 100-μL loop, coupled to a Finnigan Spectra system UV 6000LP DAD, and a Finnigan on line degasser was utilized. The whole process was computer controlled by the ChromQuest v.2.51 software through the Finnigan SN 4000 controller. Chromatographic separation was performed on a C_8 reversed-phase column (250 × 4.6 mm, i.d. 5 μm), (Kromasil - Rigas Labs, Thessaloniki, Greece).

2.5. HPLC. Separation of the six substances was performed using gradient elution at a flow rate of 1 mL min^{-1}. Initial conditions were 90% ammonium acetate (AMA) buffer 0.01 M, pH 6.7 (buffered with glacial AcOH) and 10% AcN. Linear gradient to 100% AcN within 10 min is followed. After that, isocratic conditions for 5 min are retained, and then linear gradient to 10% AcN within 2 min is followed. At the end of each run, i.e., 17 min, the column was left to equilibrate at the starting mobile phase composition (i.e., 90% A−10% B) for an additional 3 min, giving a total chromatographic analysis time of 20 min. However it should be noted that all substances are eluted within 11 min.

All mobile phases were vacuum filtered through a 0.2 μm Titan membrane filter (Scientific Resources, U.S.A.) and degassed in an ultrasonic bath prior to HPLC analysis. The column was maintained at 40 °C throughout all experiments with the aid of an electronically controlled oven.

2.6. Detection. UV spectra of all substances were recorded with the aid of the DAD system, and the maxima of absorbance was set at 220 nm for TER, DET, DIA, HT, and DEHT and 235 nm for DIHA; therefore, the recording of the chromatogram was performed at the two aforementioned wavelengths. Additional confirmation of the UV maxima was obtained by recording the spectrum of each separate substance using a Unicam UV-300 UV−vis spectrophotometer with a pair of 10-mm optical path length quartz cuvettes. Identification of the eluting peaks was performed by comparing their retention time values (t_R) and the corresponding UV-DAD spectra with those of the standards. The time window for software peak identification was set to be 1% of t_R.

2.7. Solid-Phase Extraction. Sample extraction was performed using Oasis MCX SPE cartridges (60 mg, 3 mL) obtained from Waters (Massachusetts, U.S.A.). The cartridges were equilibrated initially with 2 mL of methanol and subsequently rinsed with 2 mL of HPLC-grade water. Using an applied vacuum of about 0.01 kPa, water samples were drawn through the cartridges at a flow rate of about 10 mL/min. The cartridges were then washed with 2 mL of 0.1 M HCl and subsequently with 2 mL of methanol, dried under vacuum for 30 min, and finally eluted using 3 mL of 4% ammonium hydroxide in AcN. The extract was then dried under a gentle stream of N_2 at 37 °C and reconstituted to 200 μL with 10% AcN in 0.1 M HCl.

2.8. Stability of the Analytes. In order to minimize possible degradation of the analytes, the substances were stored in dark colored vials and kept refrigerated at −35 °C. All substances were found to be stable for at least 6 months.

2.9. Validation of the Proposed Method. At the beginning of every laboratory day two mixed working standards of the six substances at the 1 and 5 μg L^{-1} levels, serving as system suitability standards, were injected for assessing the performance of the chromatographic procedure in terms of retention time stability and signal sensitivity.

2.9.1. Linearity. For the linearity study seven water samples from a constructed wetland fortified with TER, DET, DIA, HT, and DEHT at the levels of 0.04, 0.2, 0.4, 0.6, 0.8, 1.2, and 1.6 μg L^{-1} were analyzed, and the linearity of the data was checked by performing linear regression analysis. It should be noted that the corresponding levels for DIHA were 0.2, 0.4, 0.6, 0.8, 1.2, and 1.6 μg L^{-1}.

2.9.2. Precision. The intra-assay precision of the method was evaluated by performing the overall assay (SPE and chromatographic

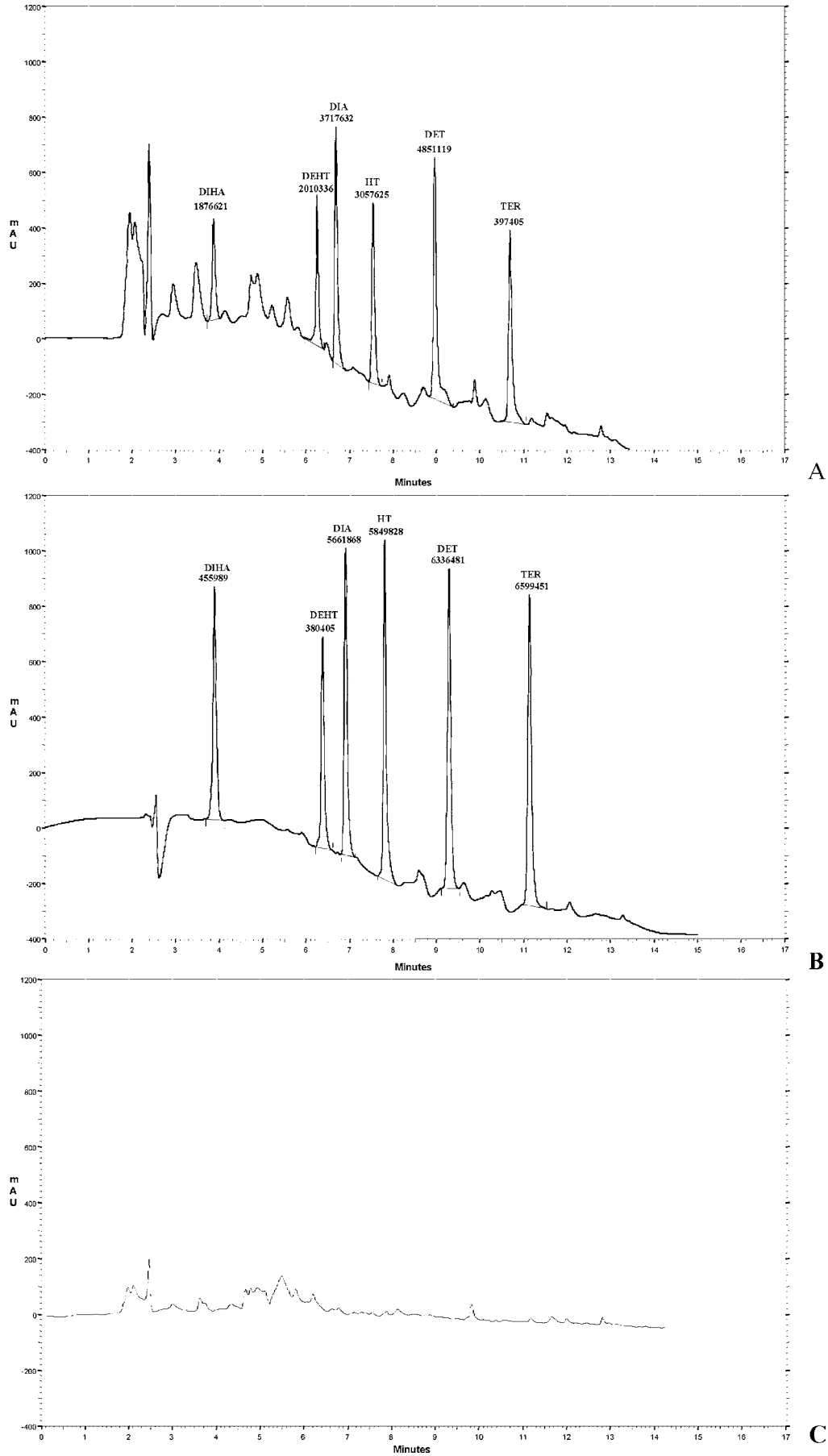

Figure 3. Chromatographic analysis of TER and its metabolites ($\lambda = 220$ nm) of A. a fortified (0.8 μg L^{-1}) wetland water sample recovered by the SPE procedure, B. a standard solution for TER and metabolites at the 0.8 μg L^{-1}, and C. a blank wetland water sample subjected to the same SPE procedure as the fortified wetland water sample.

Table 1. Intra-Assay and Inter-Assay Precision and Accuracy Data from Fortified Wetland Water Samples in Two Levels (0.2 μg L^{-1} and 0.8 μg L^{-1}) of the DIHA, DEHT, DIA, HT, DET, and TER

	intra-assay precision $n = 5$		accuracy
	0.2 μg L^{-1}	% RSD	% Er
DIHA	0.191 ± 0.0256	12.8	–4.2
DEHT	0.194 ± 0.028	14.0	–2.7
DIA	0.184 ± 0.0092	4.6	–7.9
HT	0.197 ± 0.0268	13.4	–1.3
DET	0.197 ± 0.0152	7.6	–1.5
TER	0.196 ± 0.0282	14.1	–1.9

	intra-assay precision $n = 5$		accuracy
	0.8 μg L^{-1}	% RSD	% Er
DIHA	0.723 ± 0.116	14.5	–9.6
DEHT	0.778 ± 0.0744	9.3	–2.7
DIA	0.746 ± 0.0376	4.7	–6.7
HT	0.766 ± 0.008	1.0	–4.2
DET	0.745 ± 0.0864	10.8	–6.8
TER	0.770 ± 0.0864	10.8	–3.7

	inter-assay precision $n = 5$		inter-assay precision $n = 5$	
	0.2 μg L^{-1}	% RSD	0.8 μg L^{-1}	% RSD
DIHA	0.191 ± 0.025	12.5	0.723 ± 0.1128	14.1
DEHT	0.194 ± 0.015	7.5	0.778 ± 0.1144	14.3
DIA	0.184 ± 0.0288	14.4	0.746 ± 0.0936	11.7
HT	0.197 ± 0.0182	9.1	0.766 ± 0.0776	9.7
DET	0.197 ± 0.0144	7.2	0.745 ± 0.0624	7.8
TER	0.196 ± 0.0246	12.3	0.770 ± 0.1168	14.6

analysis) at two levels (0.2 and 0.8 μg L^{-1}) in five replicates ($n = 5$) each and calculating the corresponding % RSD values. The inter-assay precision was calculated by performing the whole analytical procedure on five different laboratory days ($n = 5$).

2.9.3. Accuracy. The accuracy of the method was assessed at two concentration levels, i.e., 0.2 and 0.8 μg L^{-1}, and are expressed as the relative percentage error defined as

$$\% \text{ Er} = \frac{\text{assayed concn} - \text{nominal concn}}{\text{nominal concn}} \times 100\%$$

2.9.4. Mean Recovery. In order to evaluate the mean recovery across all the linear range of both TER and metabolites, wetland water samples were fortified at all concentration levels of the calibration curve and then analyzed employing the described method. Peak areas were employed for the calculation of concentration, and mean recovery was evaluated as the ratio of the slope of the calibration curve of each substance in the fortified sample against that of the corresponding calibration curve of the standards.

2.9.5. Limits of Detection and Quantitation. Limits of detection (LOD) and quantification (LOQ) were calculated by measuring the standard deviation of the analytical background response of six blank wetland water samples. The signal-to-noise (*S*/*N*) ratio of 3:1 (peak area ratio of the analyte vs baseline noise) and 10:1 were used for the calculation of the LOD and LOQ, respectively.

3. RESULTS AND DISCUSSION

3.1. Chromatography. The concentration level for the AMA buffer in the mobile phase was determined in order to achieve optimum separation and reasonable baseline. Three concentration levels of AMA buffer, 0.050, 0.025, and 0.010 M, were evaluated in order to achieve optimum chromatographic conditions. The optimum result was achieved with 0.010 M of AMA (Figure 3), whereas a significant drift of the baseline was observed for 0.025 and 0.050 M, even though the resolution essentially remained unaffected.

Table 2. Concentrations of TER and Its Metabolites over the Time in Four Different Water Samples from Constructed Wetlands Collected after 7, 12, 30, and 44 Days of TER Application.

		μg L^{-1}					
sample	days	DIHA	DEHT	DIA	HT	DET	TER
1	7	2.34	1.44	0.06	79.41	3.01	148.64
2	12	1.76	1.53	0.01	124.71	2.49	138.83
3	30	3.84	1.00	0.02	92.33	5.21	128.54
4	44	1.44	2.68	0.20	14.88	5.17	62.86

3.2. System Suitability. The system suitability of the method was evaluated by analyzing five replicates of standard solutions at one concentration level (1 μg/mL) and expressed as the relative standard deviation (% RSD) of retention time, area, and height of the chromatographic peak of each substance. The % RSD ranged from 1.1 to 2.3 (TER and DEHT) for the retention time and 2.0 to 6.3 (HT and DIHA) for the area. The % RSD for the height of the peak ranged from 4.6 to 6.7 for the HT and DEHT, respectively.

3.3. Extraction Procedure. Four different SPE cartridges [Thermo-Hyper SEP C_{18} (500 mg, 3 mL), Varian Chrompack-Bond Elut PRS (500 mg, 3 mL), Waters Oasis HLB (60 mg, 3 mL), and Waters Oasis MCX] were evaluated for establishing an adequate extraction procedure. Regarding the MCX (mixed mode sorbent) cartridges, a fortified wetland water sample was acidified (pH 1.5) by dropwise addition of 37% HCl prior to the SPE step in order to convert the amine moiety of the molecules to the corresponding amine salt, thus increasing the absorption of analytes to the cation mode of sorbent. The MCX cartridge was subsequently conditioned with 2 mL of methanol and washed with 2 mL of water. The water sample was then loaded onto the cartridge and washed with 2 mL of 0.1 M HCl in order to further bind the amine substances to sorbent by the ion-exchange mechanism. The cartridge was then washed with 2 mL of methanol to remove interferences retained by hydrophobic interaction. The methanolic fraction was analyzed, and no TER and any of the metabolites were found in detectable traces. When employing AcN (2 mL) instead of MeOH for the washing step, the degree of interference to the chromatogram increased significantly. After drying for 30 min under vacuum, the substances were eluted with 4% NH_4OH in AcN. When performing this last step with MeOH instead of AcN the recoveries were significantly decreased. Finally the SPE eluate was dried under a nitrogen stream and light heating (37 °C), and the dry residue was reconstituted with 200 μL of 10% AcN in 0.1 N HCl. It was found that the recoveries were slightly improved, when the reconstitution solution was acidic. Due to the acidification of amine moiety of triazines with the above reconstitution solution, the analytes became more soluble, and generally the recovery was improved.

The recovery results were expressed as the ratio of areas of blank wetland water samples, fortified with the analyzed substances and subjected to the SPE procedure (with the relevant cartridge) to that of a corresponding standard sample. The corresponding recoveries of TER ranged from 76% for MCX to 95% for PRS cartridges. For DET the recovery ranged from 67% for C_{18} to 98% for the PRS cartridges. For DIHA, DEHT, and HT the corresponding recoveries were nearly 0% for the C_{18} cartridges. Similarly, DIHA and DEHT showed nearly 0% recoveries for the PRS cartridges. HLB cartridges have per-

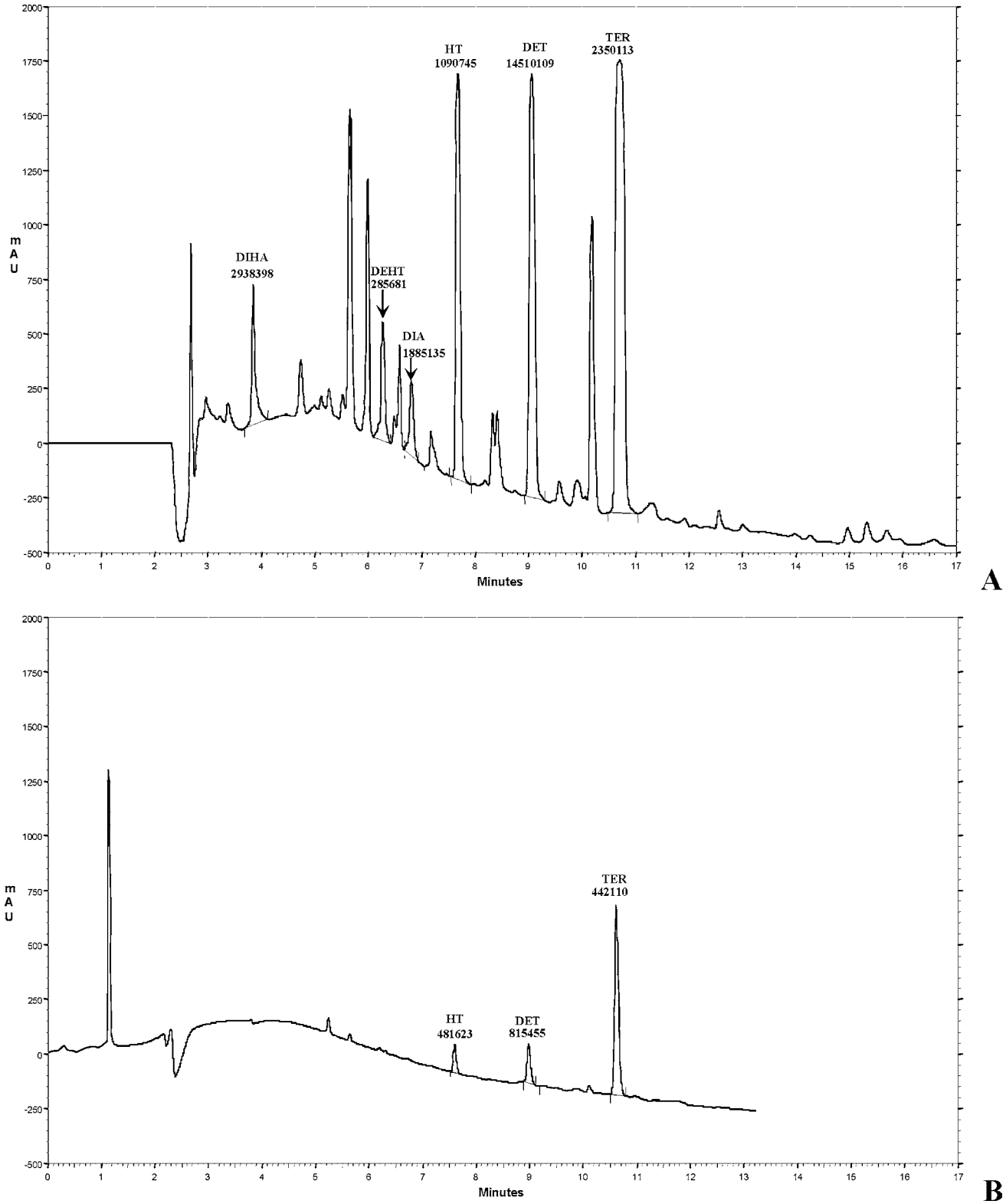

Figure 4. Chromatographic analysis of TER and its metabolites ($\lambda = 220$ nm) of A. a water sample from a constructed wetland at the 44th date after the initial application. The saturated peak of TER is due to the high concentration which has been applied in the constructed wetland. B. A water sample from a constructed wetland (diluted 1/50).

formed well for the recoveries of DIA, HT, DET and TER, but they showed low recoveries for DIHA and DEHT, i.e., 12% and 8%, respectively. Therefore the MCX cartridges were selected, because they exhibited good recovery for all substances (80, 70, 72, 71, 77, and 76% for DIHA, DEHT, DIA, HT, DET, and TER, respectively). The MCX cartridges combine the strong cation exchange mode of PRS with the lipophilic reversed phase mode of HLB cartridges, hence they are efficient for analyzing both lipophilic and positively charged species. In conclusion, the SPE procedure using MCX cartridges was found to be advantageous in terms of maximum recovery for all substances of interest.

3.4. Method Validation. The whole SPE-chromatographic procedure was validated for its linearity, precision, accuracy,

specificity, sensitivity, and recovery in accordance with the guidelines of ICH Q2 (revision 1).

3.4.1. Linearity. The linearity of the method was evaluated by linear regression analysis using seven concentrations of DEHT, DIA, HT, DET, and TER and six for DIHA. The concentration levels of TER and its metabolites in the calibration standards injected ranged from 0.04 μg L^{-1} to 1.6 μg L^{-1} and 0.2 μg L^{-1} to 1.6 μg L^{-1} for DIHA (linearity region). The results reported apply to the overall procedure, i.e., both the SPE and the chromatographic separation. The calibration curve was neither forced through nor included the 0.0 point. Good linearity was achieved for all of the analytes as indicated by the equations listed in Figure 2. The quantitation was based on the external calibration method. The calibration curves showed that the method is linear over the calibration range for all substances.

3.4.2. Mean Recovery. Water samples were fortified with standard amounts of DIHA, DEHT, DIA, HT, DET, and TER at the calibration curve concentrations ($n = 5$). The mean recovery was estimated as the ratio of the SPE calibration curve slope versus the standard calibration curve slope for every analyte injected the same day. The recoveries were found to be 80% for DIHA, 70% for DEHT, 72% for DIA, 71% for HT, 77% for DET, and 76% for TER.

3.4.3. Recovery Precision. The intra-assay precision of the overall procedure was determined by analyzing five replicates of fortified water samples at two concentration levels, low and middle (0.2 and 0.8 μg L^{-1}). Table 1 shows raw data and the relative standard deviation (% RSD) data. The results show acceptable values, ranging from 1.0 to 14.5%. The interassay precision was determined by analyzing five replicates of fortified water samples at 0.2 and 0.8 μg L^{-1} levels, prepared on five different days. The interassay precision ranged from 7.2 to 14.6% (Table 1). These RSD values indicate the suitability of the method for environmental purposes.

3.4.4. Accuracy. Two concentration levels 0.2 and 0.8 μg L^{-1} were analyzed in order to determine the accuracy of the method. The accuracy ranged from 3.7 to 9.6% as shown in Table 1. The estimated accuracy values of the proposed method are within acceptable levels for all six substances.

3.4.5. Selectivity and Specificity. The *selectivity* of the method was assessed by comparison of the acquired full UV spectrum scan (200−400 nm) using the DAD system of the peak eluting at the corresponding retention time of every substance under analysis to that of authentic standards, and its peak purity has been assessed. That was used as an additional confirmation to the chromatographic retention time data. The specificity of the method was determined by checking for possible interferences at the corresponding retention time for each substance. For that reason several blank water samples were analyzed with the proposed method and indicated no interfering peaks at the expected retention times of the analytes. Therefore, the method's ability to efficiently separate the analytes from interferences indicates the specificity of the developed assay.

3.4.6. Sensitivity. The sensitivity of the method, expressed by its LOD, was found to be 0.05 μg L^{-1} for DIHA and 0.01 μg L^{-1} for all the other substances. The LOQ was 0.2 μg L^{-1} for DIHA and 0.04 μg L^{-1} for the other substances. The signal-to-noise ratio (*S*/*N*) was 10/1 for the LOQ and 3/1 for the LOD. The baseline noise was evaluated by performing five consecutive injections of a blank wetland water sample.

3.5. Application of the Method. The method has been applied to water samples from a constructed wetland planted with *Typha latifolia* (Table 2). The samples have been collected 7, 12, 30, and 44 days after the application of TER in wetland, according to an experimental design protocol. [The selected time intervals reflect the full factorial experimental design protocol which included time, concentration, plant density, and the presence of zeolite as the main factors.] Chromatographic analysis of these samples using the proposed method shows the presence of DIHA, DEHT, DIA HT, DET, and TER in concentration levels ranging from 0.01 μg L^{-1} for DIA to 148.64 μg L^{-1} for TER (Figure 4). The later results prove that TER can be metabolized by *Typha latifolia* under the experimental conditions used. More results employing different conditions applied to the wetland are under evaluation in our laboratory.

3.6. Conclusions. An HPLC method has been developed and validated for the determination of TER and its major degradation products DIHA, DEHT, DIA, HT, and DET in water samples from constructed wetlands. The method includes an SPE pretreatment step with adequate recoveries (70−80%) for all substances. The developed assay has been shown to be accurate, specific, sensitive, and precise. The SPE method shows good recovery for the determination of both lipophilic and polar TER metabolites. The MCX cartridge is the only one that exhibits acceptable recovery for all substances analyzed. The advantage of the proposed method is that it uses a single SPE cartridge for both polar and lipophilic substances with mixed mode cation exchange and reversed-phase mechanisms. On the other hand, our method is not laborious and time-consuming as opposed to methods that require a derivatization procedure. The proposed method has the ability of analyzing both polar and nonpolar metabolites together with the parent molecule using only one extraction step (MCX extraction) followed by one chromatographic run without the need of the derivatization step rendering thus the method as easy and cheap both by means of resources and instrumentation as well as human effort. The sensitivity of the method (0.01 μg L^{-1} for DEHT, DIA, HT, DET, and TER and 0.05 μg L^{-1} for DIHA) is comparable with that of previously reported GC-MS methods (1 μg L^{-1}) (*10*, *29*). This can be attributed to the good SPE extraction procedure that reduces the background noise in the chromatogram, thus resulting in higher *S*/*N* ratio and improved sensitivity. In conclusion, this method can be used in phytoremediation programs for monitoring TER and its main degradation products. This method is suitable for the biodegradation studies of dealkylated and hydroxylated degradation products of TER in water samples from constructed wetlands, which is an ongoing program in our laboratory. Nevertheless, there is still the need for more sensitive determination of all substances and improvement of their already low LOQ values, which could be achieved with the incorporation of a mass spectrometric detector into the developed assay.

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