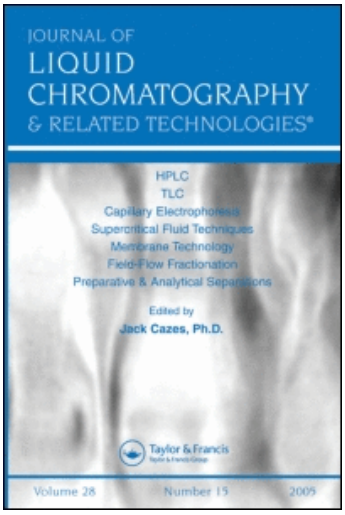

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Simultaneous Determination of Herbicide *Terbuthylazine* and Its Major *Hydroxy* and *Dealkylated* Metabolites in *Typha latifolia L.* Wetland Plant Using SPE and HPLC-DAD

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Abstract: The worldwide application of *s*-Triazines as herbicides for agricultural and nonagricultural purposes, results in a significant environmental pollution. It is necessary to develop sustainable and environmental friendly techniques in order to remediate surface water from the aforementioned organic substances. Phytoremediation with the wetland plant *Typha latifolia* L. is a technique that could potentially aid the restoration of polluted surface water. However, there is no analytical method for the determination of terbuthylazine (TER) and its metabolites in *Typha latifolia L.* and the assessment of the effectiveness of such a procedure. For this reason a method based on high performance liquid chromatography with diode array detection was developed and validated for the simultaneous determination of terbuthylazine (TER) and its major degradation products, desethyl-terbuthylazine, desisopropy-atrazine, hydroxy-terbuthylazine, desethyl-hydroxy-atrazine, and desethyl-hydroxy-terbuthylazine. This method includes both a cleanup and a solid phase extraction step (using Florisil and MCX cartridges, respectively) with adequate overall recovery efficiency (71–96%). The statistical evaluation of the

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method reveals good linearity, accuracy, and precision for the compounds determined, with RSD values not exceeding 10.5%, while the limit of detection for all analytes was found to be 17 ng g^{-1}. This method can be employed in phytoremediation studies of TER by *Typha latifolia L.* in constructed wetlands.

Keywords: Metabolites, Phytoremediation, Solid phase extraction, *Typha latifolia*, Terbuthylazine

INTRODUCTION

Phytoremediation is defined as the use of green plants to remove, contain, or render harmless environmental contaminants, from soil, water, or sediments.[1] This definition applies to all plant influenced biological, chemical, and physical processes that aid in the remediation of contaminated substrates.[2] Although the concept of plant based remediation systems has not been new, research in this area has been greatly amplified in recent years with the goal of developing successful *in situ* remediation strategies that could potentially be more cost effective and less environmentally disruptive than the corresponding conventional ex situ remediation technologies, such as excavation and burial or incineration.[3] Phytoremediation is a promising technology for the cleanup of polluted environments. It has so far been mainly used to remove toxic heavy metals from contaminated soil, but there is a growing interest in broadening its applications to degrade organic pollutants in the environment.[4] These technologies offer low cost, low maintenance, environment friendly, renewable alternatives for the clean up of polluted sites. Moreover, the use of natural agents makes them more acceptable to the public.

Constructed wetlands are commonly used for the treatment of agricultural, municipal, and industrial waste. There has been little evidence of pesticide fate in such constructed wetlands due, at least in part, to the fact that such organic compounds and their transformation products are difficult to analyze in plant tissue.[5,6] Therefore, until now there are no references on the analysis of pesticides and their metabolites in wetland plant tissues, in order to explore the potential role of tolerant wetland plants as phytoremediation agents. Nevertheless, it is generally recognized that plants can remediate organic pollutants by direct root uptake and subsequent accumulation of the nonphytotoxic metabolites in the plant tissue, by direct foliar uptake of volatile contaminants from the surrounding air, and finally, by release of exudates and enzymes that enhance biochemical transformations and/or mineralization, due to mycorrhizal fungi and microbial activity in the rhizosphere.[7,8] Recently, the research focuses on the potential use of wetland plants such as *Typha latifolia L.* (common cattails) for removing pesticides from contaminated water.[6,9] However, there is no

analytical method described in the literature for the determination of pesticides and/or their metabolites in the tissue of the aforementioned wetland plant, which would assist the investigation of the pesticide distribution and its transformation products in plant tissue.[10] On the other hand, knowledge of the uptake capacities and the distribution of pesticides within the plant are essential in planning phytoremediation methods.[11]

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, grapes, peaches, apples, and asparagus, as well as for nonagricultural purposes such as soil sterilization and road maintenance.[12] *Atrazine* (AT) is the most commonly used and the main representative of *s-Triazines*.[13] The commercial use of AT has been forbidden in the European Union for environmental reasons,[14] and it has been gradually replaced by terbuthylazine (TER).[15,16] To date, many analytical methods have been developed to

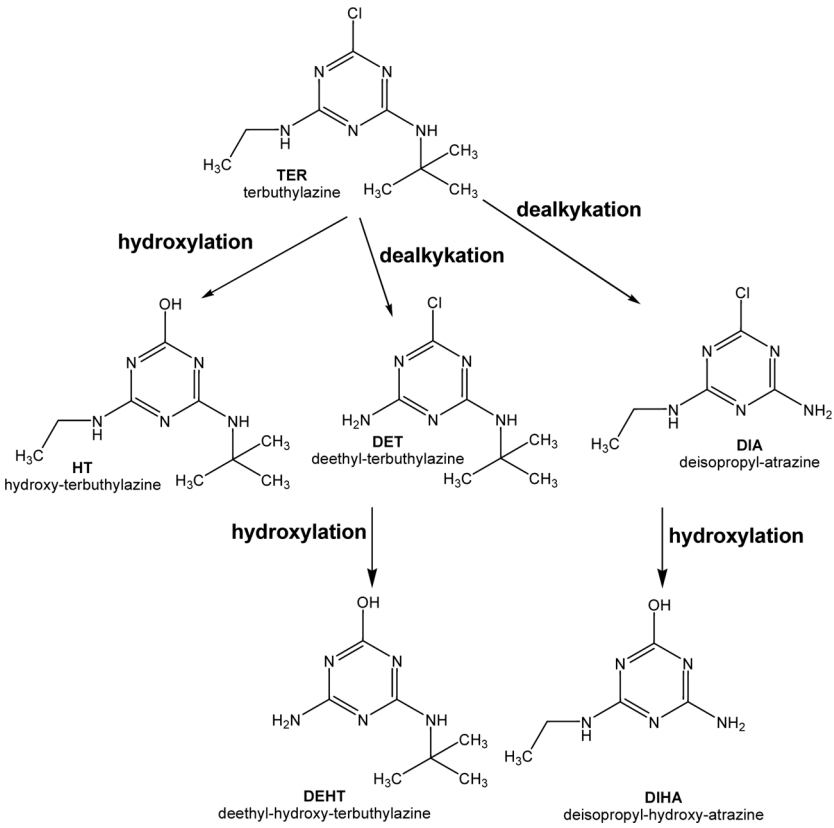

Figure 1. Terbuthylazine major metabolic pathway.

determine TER residues.[7–22] The majority of the aforementioned analytical methods dealt with the determination of TER along with its major degradation products in water samples and sediments; however, there is no analytical method for the determination of TER and its major degradation products in *Typha latifolia L.* tissue in order to study its potential use in phytoremediation techniques. Two major metabolic pathways exist for TER: dechlorination with concomitant hydroxylation and/or dealkylation of the amine groups in order to produce hydroxy-terbuthylazine (HT) and the respective dealkylated products deethyl-terbuthylazine (DET) and deisopropyl-atrazine (DIA). The combination of the two mechanisms (dealkylation and subsequent dechlorination) affords deethyl-hydroxy-terbuthylazine (DEHT) and deisopropyl-hydroxyatrazine (DIHA). The main degradation pathways of TER are summarized in Figure 1.

The goal of this study was the development of a sensitive method for the analysis of TER along with its major metabolites (both the N-dealkylated degradation products DET and DIA and the hydroxy products, HT, DEHT, and DIHA) in tissue samples from *Typha latifolia L.* planted in constructed wetlands fortified with TER. A prerequisite for the method was the simultaneous determination of TER and its aforementioned metabolites using a single SPE procedure and one chromatographic run for all metabolites (alkylated, dealkylated, and hydroxylated).

EXPERIMENTAL

Reagents and Chemicals

TER, DET, DIA, HT, DEHA, and DIHA were kindly provided by Syngenta Crop Protection (Munchwilen, Switzerland). Aq. ammonium acetate (AMA) 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). Florisil adsorbent was purchased from Sigma Aldrich (Steinheim, Germany), whereas anhydrous sodium sulfate was purchased from Riedel-de Haen (Seezle, Germany).

Instrumentation

An HPLC system comprising of a Spectra system P4000 quaternary pump (Finnigan, Riviera Beach, FL), 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.2.5.1 software through a Finnigan SN4000 controller. For the homogenization of the plant tissue an IKA blender (Wilmington, USA) was utilized.

HPLC

Chromatographic separation was performed on a C_8 reversed-phase column (250 × 4.6 mm, i.d. 5 μm), (Kromasil – Rigas Labs, Thessaloniki, Greece). Separation of the six substances was performed using gradient elution at a flow rate of 1 mL min^{-1}. Two elution solvents have been used, (AMA) 0.01 M and AcN. Initial conditions were 90% ammonium acetate (AMA 0.01 M) and 10% AcN followed by a linear gradient to 75% AcN within 10 min. After that, a slower increase rate of AcN is followed, leading to 100% within 25 min. At the end of each run, i.e., 35 min, the column was left to equilibrate at the starting mobile phase composition (i.e., 90% AMA–10% AcN) for an additional 3 min, giving a total chromatographic analysis time of 38 min. However, it should be noted that all substances were eluted within 26 min. All mobile phases were vacuum filtered through a 0.22 μm Titan membrane filter (Scientific Recourses, 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.

Detection

UV spectra of all substances were acquired with the aid of the DAD system between 200 and 400 nm. The absorbance maximum λ_{max} was found to be at 235 nm. Therefore, the recording of the chromatograms was performed at the aforementioned wavelength. Additional confirmation of the UV maxima was obtained by recording the spectrum of each 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 comparison of their retention times (t_R) and their corresponding UV-DAD spectra with those of the authentic standards. The time window for peak identification by the software was set to be 2.5% of each corresponding t_R.

Stock Standard Solutions

Stock standard solutions of each analyte were prepared in MeOH at the 1 mg mL^{-1} level. It must be noted that the protonation of the amine

moiety was necessary for the dissolution the hydroxy metabolites.[22] Thus, an appropriate amount of conc. HCl, usually not more than a drop, was added to the methanolic suspension of each metabolite in order to affect their dissolution. All of the stock standard solutions were stored in a refrigerator at 4°C. Working standard solutions (0.01, 0.05, 0.1, and 0.5 mg mL^{-1}) were prepared every week by serial dilution of stock standard solutions in 10% AcN/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. In order to minimize possible degradation of the analytes, all substances were stored in dark colored vials and kept refrigerated at −35°C. Under these conditions all substances were found to be stable for at least 6 months.

Sample Preparation

One gr of *Typha latifolia L.* tissue sample was placed in a 50 mL glass beaker, fortified with the appropriate volume of the corresponding mixed working solution and subsequently homogenized with 20 mL AcN, by means of a blender apparatus operating in 5000 rpm, in order to grind the plant tissues and for all the substances of interest to be homogeneously spread in the plant material. The homogenate was then left for 5 min in order for any insoluble material to settle down to the bottom of the beaker, the supernatant phase was collected and was vacuum filtered through a 0.22 μm membrane filter fitted on a Buchner funnel. The procedure is repeated with another 10 mL AcN and the organic extracts were combined. Two mg of anhydrous sodium sulfate were added to the above extract in order to retain any residual moisture from the plant tissue, prior to the application of the extract to the Florisil cleanup procedure.

Cleanup Procedure

The cleanup procedure was performed using custom made Florisil columns in order to exclude interferences from the matrix. Thus, a glass wool was placed at the bottom of a Pasteur pipette, 300 mg of Florisil were added, and 200 mg of anhydrous sodium sulfate were added above the layer of the Florisil. Each column was initially equilibrated with 2 mL of n-hexane. The acetonitrile fraction from the extraction procedure of the previous step was loaded onto the Florisil column, drawn through by gravity (approx. flow rate 3 mL min^{-1}) and the extract was collected in a 100 mL round bottom flask. Subsequently, the solvent has been

removed by a rotary evaporator apparatus. During this procedure, attention was paid to the temperature not to exceed 40°C. In the opposite case, severe decomposition of the analytes has been observed. The solid remainder was reconstituted with 3 mL 1.0 N HCl and used in the next sample preparation step.

SPE

The SPE procedure 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 MeOH and subsequently rinsed with 2 mL of HPLC grade water. The reconstituted solutions obtained from the cleanup procedure have been applied onto the cartridges and were drawn at a flow rate of 1 mL/min using an applied vacuum of about 0.01 kPa. Subsequently, the cartridges were washed sequentially with 2 mL of 0.1 N HCl and 4 mL of methanol, dried under vacuum for 30 min and finally eluted using 3 mL of 4% NH_4OH (v:v). Finally, the extract was dried under a gentle stream of N_2 at 37°C and reconstituted with 200 μL of 10% AcN in 0.1 N aq. HCl (v/v).

Validation of the Proposed Method

Peak areas were employed throughout the validation procedure for the calculation of the concentration.

At the beginning of every laboratory day two mixed working standards at the 1 and 5 $\mu g\,L^{-1}$ 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.

System Suitability

For assessing the system suitability of the proposed methodology, five replicates of the standard solutions at the 1 and 5 $\mu g\,L^{-1}$ levels were analyzed, and the results expressed as the relative standard deviation (% RSD) of retention time, area, and height of the chromatographic peak of each substance, as well as the asymmetry factor of each chromatographic peak.

Linearity

For the linearity study, seven *Typha latifolia L.* plant tissue samples (obtained from a constructed wetland prior to the application of TER), were fortified with TER, DET, DIA, HT, DEHT, and DIHA at

the 50, 100, 200, 500 ng g^{-1} and 1.0, 1.5, and 2 μg g^{-1} levels, and processed as described. The linearity of the data was checked by non-weighted linear regression analysis.

Precision

The intra-assay precision of the method was evaluated by performing the overall assay (sample preparation and chromatographic analysis) at two levels (0.2 and 1.0 μg g^{-1}) in five replicates (n = 5) each and calculating the corresponding % RSD values. The inter-assay precision was calculated by performing the whole procedure on five different laboratory days (n = 5) at two levels (0.2 and 1.0 μg g^{-1}).

Accuracy

The accuracy of the method was assessed at two concentration levels, i.e., 0.2 and 1.0 μg g^{-1} of plant tissue and expressed as the relative percentage error (%Er) defined as:

$$\%\ \mathrm{Er} = (\text{assayed concn} - \text{nominal concn}/\text{nominal concn}) \times 100\%$$

Mean Recovery

The mean recovery was assessed across the linear range of each substance, as the ratio of the slopes of the calibration curve of the fortified samples against that of the corresponding calibration curve constructed by injection of the standards at the corresponding concentration levels.

Sensitivity

LOD and LOQ were calculated by measuring the standard deviation of analytical background response of six blank *Typha latifolia L.* plant tissue samples at the corresponding t_R of each analyte. The signal-to-noise ratio (S/N) 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.

RESULTS AND DISCUSSION

Cleanup Procedure

In the proposed analytical method Florisil has been applied in order to retain the lipid and humic acids present in the plant wall in large

amounts. Omission of the above cleanup step results in significant interference by endogenous substances of the plant material.

The packing material was previously activated overnight at 300°C in order to expel any residual moisture that could compromise its effectiveness. The anhydrous sodium sulfate placed on the top of the Florisil layer was used in order to retain the potential residual moisture originating from the fresh plant tissue. Generally, the existence of water molecules could limit the active moieties of silica by hydrogen bonding, resulting in the reduction of retention capacity of Florisil.

The Florisil column was conditioned with 2 mL hexane in order to remove any residual lipophilic substances that might be present to both the glass fibers and the packing material. The acetonitrile extract of *Typha latifolia L.* was then loaded onto the glass column and the extract has been collected in a round bottom flask. AcN has been found to decrease the retention of TER and its metabolites by Florisil so that they can be eluted with adequate recovery, whereas acidic organic interferences are retained. Utilization of methanol instead of AcN resulted in the significant increase of the background in the chromatograms because of the capability of methanol to bind to Florisil by hydrogen bonding and displace, at least in some degree, the acidic substances retained in the case of AcN.

Extraction Procedure

Regarding the MCX based SPE procedure, the samples were treated using a modification of a procedure commonly used in our laboratory for the analysis of TER and its major degradation products.[23] Briefly, the MCX cartridges were conditioned with 2 mL of methanol and washed with 2 mL water. The acidified *Typha latifolia L.* samples were then loaded onto the cartridge and washed with 2 mL of 0.01 N HCl. As the Oasis MCX cartridges function through a mixed mode mechanism (both lipophilic and cation exchange), the acidified sample can be retained on the solvent both by a lipophilic (due to the triazine nucleus and the alkyl side chains) and a cation exchange mechanism (the protonated nitrogen atoms of the side chains). Washing with 0.01 N HCl both washes away any hydrophilic substances of the cartridge and enables further locking of the analytes on the column. The cartridges were subsequently washed with 4 mL of methanol in order to remove possible interferences retained by hydrophobic interaction. In this washing step, employing 2 mL methanol instead of 4 increased the degree of interferences in the chromatograms significantly.

Method Validation

The developed methodology was validated for its linearity, precision, accuracy, specificity, sensitivity, and recovery in accordance with the guidelines of ICH Q2 (revision 1).

System Suitability

The %RSD ranged from 1.1 to 1.5 (TER and DEHT) for the retention time and 1.8 to 2.5 (HT and DIHA) for the area. The %RSD for the height of the peak ranged from 3.2 to 4.9 for the HT and DEHT, respectively, whereas the asymmetry factor did not exceed 1.15.

Linearity

The linearity of the method was evaluated using seven concentration levels of TER, DET, DIA, HT, DEHT, and DIHA ranging from 0.05 μg g^{-1} to 2.0 μg g^{-1} (linearity region). The quantitation was based on the external calibration method. The results reported in Table 1 apply to the overall procedure, i.e., sample extraction, cleanup, SPE, and chromatographic separation. A non- weighted linear regression treatment has been used and the calibration curve neither forced through nor included the 0.0 point. Good linearity was achieved for all of the analytes as indicated by the calibration equations in Table 1.

Mean Recovery

The mean recovery was expressed as the ratio of the slopes of the calibration curves constructed from the *Typha latifolia L.* samples that were

Table 1. Regression data of herbicide TER and its major metabolites (correlation coefficient, slope, intercept and standard error) from fortified *Typha latifolia L.* samples in 50, 100, 200, 500, 1000, 1500, and 2000 ng/g concentration levels (n = 7)

Compounds	Slope	Intercept	Correlation coefficient, r^2
DIHA	5478.1	−89832	0.997
DEHT	7833.4	+709339	0.997
DIA	4199.8	+652070	0.9991
HT	6435.5	−254259	0.998
DET	4345.8	−167565	0.998
TER	5503.1	−20512	0.998

fortified with standard amounts of TER, DET, DIA, HT, DIHA, and DEHT at the calibration curve concentration levels to that of the corresponding curve constructed by the analysis of standards at the same concentration levels. The recoveries were found to be 96% for TER, 86% for DET, 93% for DIA, 71% for HT, 72% for DIHA, and 93% for DEHT, and are considered satisfactory for the analysis of TER and its metabolites in *Typha latifolia L.* samples.

Recovery Precision

The intra-assay precision of the overall procedure was determined by analyzing five replicates of fortified *Typha latifolia L.* tissue samples at two concentration levels, i.e., low and high (0.2 and 1.0 μg g^{-1}). Table 2 shows raw data along with the corresponding %RSD data. The results show acceptable values, ranging from 0.5 to 10.4%. The inter-assay precision was determined by analyzing five replicates of fortified *Typha latifolia L.* samples at the 0.2 and 1.0 μg g^{-1} concentration levels, prepared on four different days for each level. The inter-assay precision ranged from 3.1 to 10.5% (Table 2). These intra- and inter-assay %RSD values indicate that the method can be considered as precise for the quantitation study of TER and its metabolites in *Typha latifolia L.*

Accuracy

Two concentration levels, 0.2 and 1.0 μg^{-1}, were analyzed in order to determine the accuracy of the method. The %Er ranged from 0.03 to 11.37% as shown in Table 2. The estimated accuracy values of the proposed method are within acceptable levels for all analytes.

Sensitivity

The sensitivity of the method, expressed by its LOD, was found to be 17 ng g^{-1} for all substances analyzed, whereas the LOQ was found to be 50 ng g^{-1}, respectively. These values prove that the method exhibits adequate sensitivity for this analysis. Higher sensitivity would not be fit for this purpose, as the amount of TER that *Typha Latifolia L.* would uptake from the wetland would be insignificant.

Selectivity and Specificity

The specificity of the method was assessed by checking for the presence of possible interfering peaks at the corresponding t_R window for each substance. Thus, six *Typha latifolia L.* blank samples were

Table 2. Intra-assay and inter-assay precision and accuracy data from fortified *Typha latifolia L.* samples in two levels (0.2 μg g^{-1} and 1.0 μg g^{-1}) of TER, DET, DIA, HT, DIHA and DEHT

	Intra-assay precision (n = 5)		
Compound	0.2 μg g^{-1}	%RSD	accuracy %Er
TER	0,183 ± 0.007	3.26	−8.58
DET	0,183 ± 0.009	4.58	−8.39
DIA	0,195 ± 0.005	2.27	−2.40
HT	0,191 ± 0.010	4.96	−4.62
DIHA	0,179 ± 0.010	5.23	−10.43
DEHT	0,177 ± 0.021	10.47	−11.37
	Intra-assay precision (n = 5)		
	1.0 μg g^{-1}	%RSD	accuracy %Er
TER	0.987 ± 0.029	2.89	−1.26
DET	1.000 ± 0.037	3.74	−0.03
DIA	0.938 ± 0.012	1.23	−6.19
HT	0.959 ± 0.006	0.58	−4.13
DIHA	0.971 ± 0.040	4.04	−2.92
DEHT	0.983 ± 0.059	5.85	−1.69

	Inter-assay precision (n = 5)		Inter-assay precision (n = 5)	
	0.2 μg g^{-1}	%RSD	1.0 μg g^{-1}	%RSD
TER	0,183 ± 0.012	6.11	0.987 ± 0.043	4.28
DET	0,183 ± 0.011	5.40	1.000 ± 0.034	3.36
DIA	0,195 ± 0.020	9.90	0.938 ± 0.031	3.14
HT	0,191 ± 0.010	4.84	0.959 ± 0.040	3.98
DIHA	0,179 ± 0.018	8.97	0.971 ± 0.058	5.75
DEHT	0,177 ± 0.021	10.51	0.983 ± 0.071	7.10

processed and analyzed with the proposed methodology and the chromatograms indicated no interfering peaks at the retention times of the analytes. Therefore, the method's ability to efficiently separate the analytes from any possible interference, indicates the specificity of the developed assay. Furthermore, the selectivity of the method was assessed by comparison of the acquired full UV spectrum (200–400 nm) (using the DAD system) of the peak eluting at the corresponding t_R of each analyte, to that of authentic standards and the peak purity has been assessed. In every case, the peak purity value exceeded 98%. These data were used as additional confirmation to the chromatographic t_R data (Figure 2).

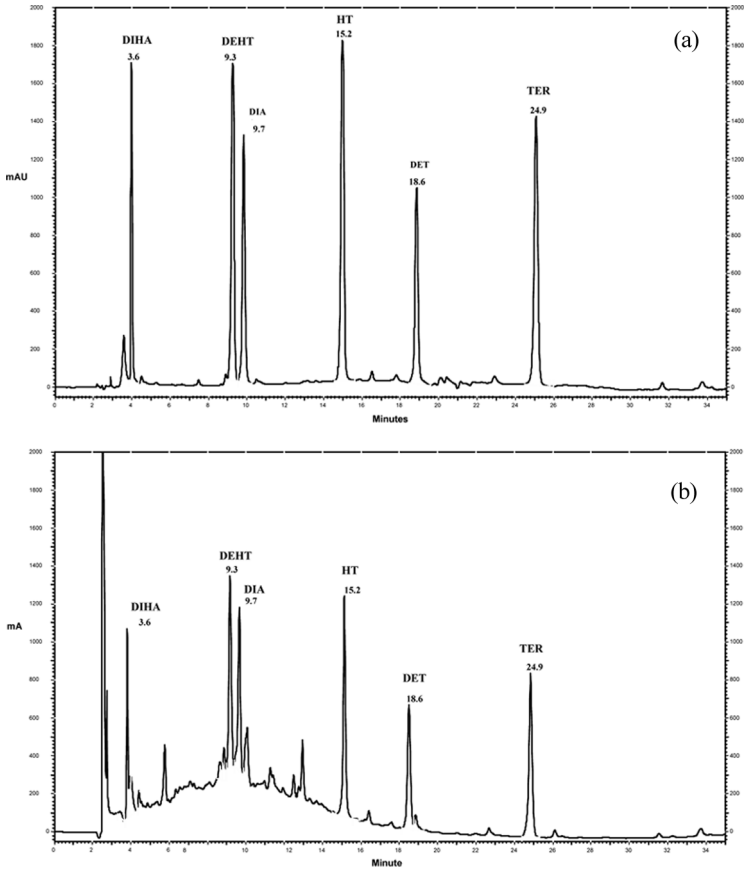

Figure 2. Chromatographic analysis of TER and its metabolites ($\lambda = 235$ nm) of a. a standard solution of TER and its metabolites at the 10 μg mL^{-1} (1000 ng on column, injection 100 μL), b. a fortified 1500 ng g^{-1} sample of *Typha latifolia L.* (750 ng on column) recovered by SPE procedure, c. a fortified 200 ng/g sample of *Typha latifolia L.* (100 ng on column) recovered by SPE procedure, d., a blank *Typha latifolia L.* sample subjected to the same SPE procedure as the fortified *Typha latifolia L.* sample.

Application of the Method

The method has been applied to the determination of TER and its metabolites in *Typha latifolia L.* from constructed wetlands, according to a phytoremediation methodology developed in our laboratory against the herbicide TER. Although the proposed method has been developed using samples for the validation procedure from the leaves of *Typha*

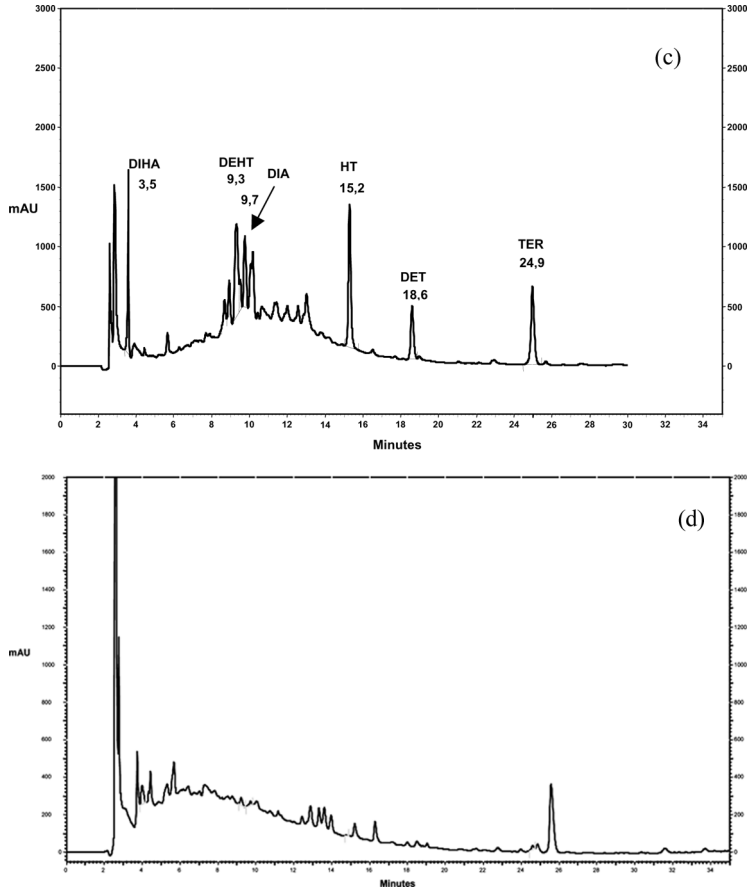

Figure 2. Continued.

latifolia L., the method was readily applied in plant tissues both from leaves and roots. The plant samples have been collected from the corresponding constructed wetlands, 44 d after the application of 1 and 2 mgL^{-1} (low and high concentration) TER in the wetland, according to an experimental design protocol. Analysis of these samples using the proposed methodology shows the presence of TER, DET, DIA, HT, DEHT, and DIHA, in concentration levels ranging from 72.9 from DIHA to 3219.5 ng g^{-1} for TER. The results show that both the parent herbicide molecule of TER and its metabolites are absorbed by *Typha latifolia L.* with significant uptake concentrations (Table 3). These results support the mechanism of accumulation of xenophobic substances in the plant tissues of *Typha latifolia L.*, thus rendering it a suitable agent

Table 3. Mean concentration of TER and its metabolites in leaves and roots of *Typha latifolia L.* samples from constructed wetlands collected after 44 days of TER application (n = 8) in phytoremediation program

	$ng\,g^{-1}$					
	TER	DET	DIA	HT	DIHA	DEHT
leaves	3219.5	145.5	321.8	524.1	197.4	199.3
Roots	1088.6	79.7	78.5	134.0	72.9	103.1

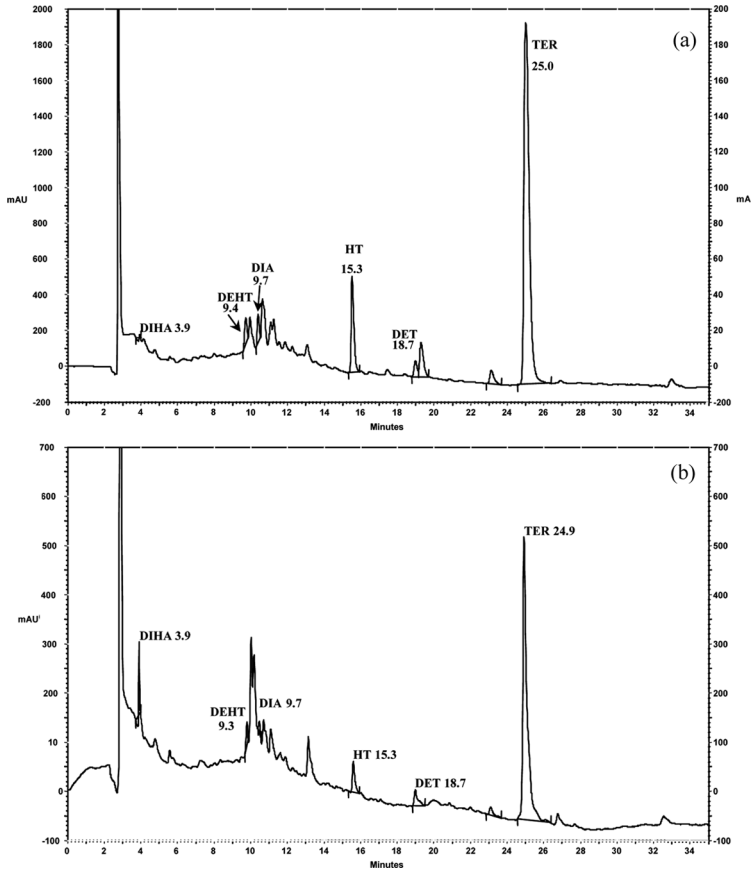

Figure 3. Chromatographic analysis of TER and its metabolites ($\lambda = 235$ nm) of a. a *Typha latifolia L.* sample from the leaves of the plant. b. a *Typha latifolia L.* sample from the roots of the plant.

for phytoremediation programs. It must be noted that the concentrations for all the analyzed substances are higher in the leaves of the plant than that in its roots.

As there are probably endogenous metabolic procedures for TER that exists mainly in the leaves of the plant, both the parent molecule and metabolites exhibit different concentration profiles between leaves and roots (Figure 3). These endogenous metabolic mechanisms of xenobiotic substances in plant leaves have been established.[23] Thus, it has been found that the root system functions as a pump that removes xenobiotic substances from the soil solution of rhizosphere to the leaves of the plant. Thus, the concentration of TER and its metabolites are higher in leaves compared to that found in the root system of the plant.[8]

CONCLUSIONS

In summary, an HPLC method has been developed and validated for the determination of TER and its major dealkylated (DET, DIA) and hydroxy (HT, DIHA, DEHT) degradation products in *Typha latifolia L.* samples from constructed wetlands that have been treated with the pesticide. The method includes a three step pretreatment procedure employing two different cartridges (Florisil and MCX) with adequate recovery efficiencies ranging from 71 to 96% for all substances, both lipophilic and polar. Furthermore, the developed methodology has been shown to be accurate, specific, sensitive, and precise.

This method is suitable for the study of TER and its metabolites uptake and metabolism by *Typha latifolia L.* in constructed wetlands during a phytoremediation program developed in our laboratory, aiming towards the sustainable management of surface water from intensive agricultural use of land in a surrounding catchment area. Incorporation of mass spectrometry detection into the developed methodology would give deeper insight to the metabolic pathways of TER, as it could facilitate the discovery of new metabolites of the pesticide.

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