Determination of diuron, terbuthylazine, and terbutryn in wastewater and soil by micellar liquid chromatography

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Abstract

An analytical method for the quantification of the herbicides and algaecides diuron, terbuthylazine, and terbutryn in wastewater and soil by micellar liquid chromatography was developed. The sample preparation was expedited to reduce the number of intermediate steps and the use of chemicals. The analytes in soils were recovered by ultrasonication in the mobile phase. The obtained supernatant and the water samples were directly injected, thus avoiding intermediate steps. The chromatographic behavior of the analytes, depending on the surfactant and alcohol was studied, in order to optimize the chromatographic run, by a chemometrical approach. The herbicides were resolved in <16 min using a C18 column and a mobile phase of 0.07 M sodium dodecyl sulfate/6% 1-pentanol phosphate buffered at pH 3, running under isocratic mode at 1 mL/min. The detection absorbance wavelength was set to 240 nm. The method was successfully validated in terms of selectivity, detection limit (0.06 mg/L in water and 0.3 mg/kg in soil), quantitation range (0.2 to 2 mg/L in water and 1 to 10 mg/kg in soil), trueness (-6.1 to +5.0%), precision (<9.4%), and ruggedness (<8.3%). The procedure was reliable, practical, easy-to-handle, available, short-time and ecofriendly and useful for routine analysis. Its applicability to real samples was evaluated by analyzing several wastewater, decorative reservoir, and soil samples from agricultural and urban sources.

Keywords: Herbicide; Micellar liquid chromatography; Soil; Validation; Wastewater

1. Introduction

The growth of wild weeds and algae causes economic, esthetic, and practical disturbances in many human activities. Indeed, they compete with useful plants for water and nutrients, grow out of control, poison humans and livestock, provide unpleasant odors, or hinder the passage of pedestrians and vehicles. Therefore, the pesticide industry manufactures a wide range of synthetic chemicals to fight against these pests [1,2]. The phenylurea diuron (DIU; without acid/basic activity, log Po/w = 2.9, solubility in water 35.6 mg/L) and the triazines terbuthylazine (TBA; pKaTBA-H+ = 1.9, log Po/w = 3.4, solubility in water = 6.6 mg/L) and terbutryn (TBT; pKaTBT-H+ = 4.3, log Po/w = 3.7, solubility inwater = 25 mg/L) are broad-spectrum herbicides, microbiocides, and algaecides with strong and rapid effects, which acts by the inhibition of the photosynthesis [3]. The structures can be seen in Fig. 6.1. They are used to prevent and control the growth of weeds, grasses, and mosses in agricultural crops, forestry, plant nursery, gardens, urban woodland and landscaped areas, lawns, and rights of ways. They are applied by spraying on the leaves and soil, and by aerial drift, and absorbed by roots and foliage. They are also used as aquatic herbicides for control of submerged and free-floating weeds and slime-forming algae in industrial and ornamental watercourses, reservoirs, fountains, water cooling systems, aquaria, and ponds as well as in fisheries. In these cases, they are directly added to water [3-7].

Despite their beneficial effects, the prolonged use of DIU, TBA, and TBT is a great concern since they are an important source of contamination of the environment and humanactivity zones. Indeed, these herbicides are moderately to highly persistent in water and soils, and toxic for plants (other than weeds), animals, and humans. Diuron, terbuthylazine, and terbutryn are able to remain in agricultural, residential, and public soils for a long time after use [3-6, 8, 9]. They can arrive to urban sewerage waters, due to the discharging of wastewater from green, domestic, and industrial areas [10], and in agricultural gutters, where they arrive by drainage [11]. These wastewaters are usually purified in wastewater treatment plants (WWTP) in order to remove the contaminants and then released to the aquatic ecosystem. However, some areas even lack of effective wastewater sanitation services [12]. In the surface water, they tend to adsorb on sediments and then pollute river and estuary sludge [4,8,13]. Besides, diuron has the potential to leach into the soil and may also pollute groundwater, water springs, and water wells [3,4,9,14].

The presence of these herbicides in natural water and soils represent a major threat for the autochthonous flora and fauna. In addition, they can induce serious diseases in the population, which is exposed to these compounds either directly from a polluted source by dermal, eye, and oral way or by food chain, as they bioaccumulate in edible tissues of some consumed living beings [3-9]. These negative effects are enhanced in densely populated areas [15]. Therefore, DIU, TBA, and TBT have been classified as emerging pollutants, hazardous compounds which occurrence must be monitored and regulated in all the potentially contaminated locations [16]. Because of its higher danger, terbutryn was banned in 2003 for use in the European Union (EU), but its occurrence is suspected because of its persistence or current illegal utilization [7]. Local authorities, supported by the scientific community, have implemented actions and policies to reduce the pollution and monitor the quality of wastewaters, treated waters, and soils in their area [17]. Therefore, diuron, terbuthylazine, and terbutryn must be simultaneously screened in water and soils using a reliable a practical analytical method.

Nowadays, hydoorganic gas- and reverse-phase liquid chromatography (RP-HPLC) coupled to mass spectrometry have become the technique of choice for the multiresidue analysis of organic pesticides in water and soil samples [17]. Indeed, liquid chromatography-mass spectrometry (LC-MS) has been used to quantify diuron, terbuthylazine, and terbutryn in urban wastewater [18], diuron and terbuthylazine in agricultural wastewater [11], diuron [11] and terbuthylazine [11, 19] in soils, and terbutryn in sediments [13]. In spite of its performances, this instrumentation is quite expensive, considering both acquisition and maintenance, and delicate. Therefore, it requires an extremely careful sample purification, to avoid the introduction of solid particles and solved salts, and highly specialized staff [20]. Several authors have proposed the use of liquid chromatography-absorbance detection (LC-UV) to determine these three herbicides in urban and agricultural wastewater [21], diuron in urban [22] wastewater, as well as diuron [14,22-28], terbuthylazine [23,29] and terbutryn [29-31] in soils and sludge, to use an affordable detector with a reasonable level of sensitivity and selectivity.

Wastewater samples must be treated to remove interfering and harmful compounds before the HPLC analysis. The most used procedures are based on cartridge solid-phase extraction [11,13,22], solid-phase microextraction [30], and liquid/liquid [26] extraction. Soils must be processed to extract the herbicides from the particles to a liquid environment as

clean as possible. The most common methods involve ultrasonication [11,13,22,30], mechanical shaking [14,23,28], stand-overnight [26], microwave-assisted [27,31], Soxhlet [24,29], simple flowing-through-sample [25], simple pressurized solvent [28] extraction using a solution containing a high proportion of organic solvent (60-100%), and QuEChERS [19]. Some of them also include a further purification by cartridge solid-phase extraction [13,14,18,25,26,28], molecular imprinting solid-phase extraction [29], and column switching [27]. However, these procedures are large, tedious, and complex and require multiple operations and specific equipment. These characteristics increase the sources of variance and enlarge the analysis time and price, as well as the use of reagents and toxic solvents. These drawbacks have been partially solved in water analysis by the introduction of automated purification procedures, such as on-line solid-phase extraction [18], in-tube solid-phase microextraction and direct column switching [21]. Therefore, the development of reliable, easy-to-operate, cost-effective, and green methods based on more available instrumentation is quite interesting for routine analysis [32].

Micellar liquid chromatography (MLC), using a C18 column and a hybrid mobile phase, containing sodium dodecyl sulfate (SDS) as surfactant, running under isocratic mode has been used as an interesting alternative to hydroorganic RP-HPLC to determine toxic organic pollutants both polar and hydrophobic in water, such as melamine [33], phenols [34,35], imidazoles [34, 35], organophosphates [34], anilinopyrimidines [35], and carbamates [36], as well as carbaryl in soil [36]. Under these conditions, the retention behavior of compounds with average hydrophobicity is highly reproducible in SDS mobile phases. Hence, it can be modeled using appropriate and well-established equations, which can be adjusted by testing a few mobile phases [37]. The micellar environment also enhances the spectrophotometric activity. Besides, hydrophobic compounds dispersed in water can be easily solubilized in the micellar medium. Therefore, filtered aqueous solutions and suspensions can be directly injected without risk of column degradation or clogging, expediting the sample preparation. The pesticides can also be recovered in an aqueous micellar liquid phase by a simple stirring, due to their affinity for the micellar pseudophase. As micellar mobile phases use a low proportion of hazardous and volatile organic solvent (maximum of 30%), they are relatively stable, harmless, non-pollutant, and non-flammable [20].

The aim of the work was the development of a reliable method for the determination of the herbicides diuron, terbuthylazine, and terbutryn in wastewater and soils by micellar liquid chromatography. The method should hold practical performances (easy-to-conduct, rapid, ecofriendly, and inexpensive) in order to be applicable for routine analysis. Its analytical quality must to be proven by validation following the recommendations of the Validation and Peer Review of US Environmental Protection Agency (EPA) Chemical Methods of Analysis guideline, in terms of selectivity, calibration and quantitation range, sensitivity, trueness, precision, and ruggedness [38]. Finally, it should to be applied to determine the concentration of DIU, TBA, and TBT in several wastewater and soil samples from diverse sources (agricultural, urban, input WWTP streams, and decorative water reservoirs and right-of-way) in order to verify its suitability for pollution monitoring.

2. Materials and methods

2.1 Standard and reagents

The powdered standards of terbuthylazine, terbutryn, and diuron (purity >99.0%) were supplied by Dr. Ehrenstorfer-Schäfers (Augsburg, Germany). SDS (>99.0%), 1-butanol, and 1-pentanol (HPLC grade) were purchased from Scharlab (Barcelona, Spain). Hydrochloric acid (37.0%), sodium dihydrogen phosphate monohydrate (analytical grade) and 1-propanol (HPLC grade) were bought from Panreac (Barcelona, Spain). Ultrapure water was produced in the laboratory from distilled water, provided by the University Jaume I (Castelló, Spain) using an ultrapure water generating device Simplicity UV (Millipore S.A.S., Molsheim, France). This ultrapure water was used to prepare all the aqueous solutions.

2.2 Apparatus and instrumentation

The solid standards were weighted using an analytical balance Mettler-Toledo (Greifensee, Switerland). A GLP potentiometer (Crison, Barcelona, Spain) equipped with a

191 combined Ag/AgCl/glass electrode was used to measure the pH of the aqueous solutions. The
192 vortex shaker and the Ultrasons-H ultrasonic bath were from Selecta (Abrera, Spain).
193 The chromatograph was an Agilent Technologies Series 1100 (Palo, Alto, CA, USA)
194 equipped with an isocratic pump, a degasser, an autosampler, and a UV-Visible absorbance
195 diode array detection (DAD), connected to a personal computer. The software Chemstation
196 version B.01.01 (Agilent Technologies) was used to control the instrument and the
197 acquisition and processing of the signal. The experimental dead time was ≈1.0 min.
198
199 *2.3 Solutions and mobile phases*
200

Individual stock solutions containing 100 mg/L of each pesticide were prepared by weighting the appropriate quantity and solving it in methanol. The working solutions were prepared by successive dilutions with ultrapure water. These solutions were kept at +4 °C for a maximum of 2 weeks.

Mobile phases were prepared by solving the adequate amount of SDS and $NaH_2PO_4 \cdot H_2O$ in ultrapure water, and then drops of HCl solutions were added to reach the desired pH. Furthermore, the appropriate volume of alcohol was added, and then the flask was filled up with ultrapure water. Afterwards, the mobile phases were ultrasonicated for 5 min and filtered through a 0.45-µm-Nylon membranes (Micron Separations, Westboro, MA, USA) with the aid of a vacuum pump. All the mobile phases were stored in amber bottles.

2.4 Chromatographic conditions

The stationary phase was in a Kromasil C18 column (Sigma-Aldrich, Saint-Louis, MO, USA) with the following characteristics: length, 150 mm; internal diameter, 4.6 mm; particle size, 5 µm; pore size, 10 nm). The mobile phase was an aqueous solution of 0.07 M SDS/6% 1- pentanol buffered to pH 3 with 0.01 M phosphate salt. It runs at 1 mL/min under isocratic mode. The injection volume was 20 µL, and the absorbance detection wavelength was set to 240 nm. Standard solutions and samples were filtered through 0.45-µm Nylon membranes before introduction into the vials. The analyses were carried out without controlling the temperature. The special care required when dealing with micellar mobile phases can be seen in [39].

2.5 Sample treatment

The samples were taken in several towns in from densely populated areas, belonging to the Castellón Province (Spain). Wastewater samples were collected and supplied to the laboratory by FACSA (Castellón). This company is in charge of the control of the quality of waste, sewerage, and surface water, as well as the management of the integrated water cycle in the studied area. The soil samples were collected at the surface by the laboratory staff. Both kinds of samples were taken and stored in amber bottles. The source and characteristics of the samples can be seen in Tables 6.1 and 6.2, respectively. The samples were kept in a fridge and analyzed at a maximum of 2 weeks after collection.

Samples were thawed 30 min at room temperature before processing. Water samples were filtered and directly injected. Soil samples were air-dried for 30 min and powdered in an agate mortar, and then 1 g was weighed and mixed with 5 mL of mobile phase (sample/supernatant ratio 1:5 w/v). The mixture was stirred for 1 min and ultrasonicated for 5 min to extract the studied herbicides. After decantation, the supernatant was filtered and injected. In spiking samples, the appropriate volume of standard solution was introduced in the soil before adding the mobile phase. The resulting sample was stored 1 day to assure the evaporation of the solvent and the deposition of the herbicides on the solid particles. These artificially herbicides-added samples imitated those "naturally" contaminated [40].

Table 6.1. Characteristics and herbicide concentrations (mg/L) in the several water samples.

Source	Nº	Location	DIU	TBA
Public fountains	1	Villarreal	1.4	2.1
	2	Castelló	0.98	1.3
Private aquaria	3	Villarreal	0.84	0.76
	4	Almassora	0.52	0.68

Agricultural gutters	5	Villarreal	0.43	0.62
	6	La Vilavella	0.24	n.d.
	7	Betxí I	0.32	0.48
	8	Betxí II	0.28	<0.2
	9	Onda	<0.2	0.43
Urban sewage water streams	10	Alcora	<0.2	n.d.
	11	Nules	0.44	0.36
	12	Vilarreal I	n.d.	0.45
	13	Alcora	<0.2	n.d.
	14	Vilarreal II	n.d.	0.22
	15	Onda	<0.2	0.34
Influent wastewater	16	WWTP Vora Riu (Villarreal)	<0.2	0.31
Influent wastewater	17	Joint WWTP OBVA (Onda/Betxí/Vilareal/Alquerías)	0.51	<0.2
Influent wastewater	18	WWTP Nules, La Vilavella	0.31	0.25

The places refer to several towns from the Castelló Province (Spain) - n.d. = not detected

Table 6.2. Characteristics and herbicide concentrations (mg/kg) in the several soil samples

Source	Nº	Location	DIU	TBA
Agricultural fields	19	Villarreal	5.7	2.1
	20	La Vilavella	8.5	5.4
	21	Alcora	4.1	1.9
Private gardens	22	Villarreal	2.2	3.2
	23	Burriana	2.1	5.1
Public gardens	24	Castelló	2.1	4.5
	25	Villarreal	4.3	2.8
Soil near to a road	26	Onda	5.8	n.d.
Soil near a railroad	27	Villarreal	3.7	n.d.

The places refer to several towns from the Castelló Province (Spain) - n.d. = not detected

3. Results and discussion

3.1 Optimization of the chromatographic conditions

The principal chromatographic conditions (injection volume, 0.020 mL; stationary phase, C18; flow-rate, 1 mL/ min running under isocratic mode; surfactant, SDS, buffer, a phosphate salt; and pH 3) were taken from previously published papers related to the determination of pesticides in water [34,35]. The concentration of SDS, the nature and proportion of the organic solvent, and the absorbance wavelength detection were optimized. These studies were performed using a standard solution of 1 mg/L DIU, TBT, and TBA in ultrapure water.

3.1.1 Selection of the organic solvent

The studied pesticides are moderately hydrophobic (log Po/w 2.9-3.7). Using a C18 column, the use of hybrid mobile phases was envisaged to obtain useful retention times, and additionally, improve the shape of the peak. The shortchained monoalcohols 1-propanol, 1-butanol, and 1-pentanol were tested as organic solvents [20].

The analytes were analyzed using three mobile phases containing 0.01 M SDS and 7.5% 1-propanol, 5% 1-butanol, and 4% 1-pentanol. The herbicides were less retained and the efficiency was enhanced by increasing the length of the carbon chain, as expected in MLC [20]. The retention time of TBT was too high using 1-propanol (44.9 min) and 1-butanol (65.4 min). Therefore, 1-pentanol was selected for the analysis.

3.1.2 Modeling of the retention behavior

The effect of the concentrations of SDS and 1-pentanol in the retention factor was studied by an interpretative strategy, using a complete factorial design plus the central point. Therefore, the retention factor of DIU, TBA, and TBT were measured by five mobile phases containing a combination of the minimum and the maximum concentration of SDS (M) and 1-pentanol (%, v/v) recommended for MLC and the following average values: 0.05/2, 0.15/2, 0.10/4, 0.15/2, and 0.15/6 [20].

In all the mobile phases, the elution order was the same: t_R(TBT) > t_R(TBA) > t_R(DIU). The three herbicides show a binding behavior face to the SDS-micelles, as the retention factor decrease at increasing values of SDS in the mobile phase. Otherwise, the addition of 1-pentanol enhances the elution strength of the mobile phase, as expected in RP-HPLC.

An empirical hyperbolic model was used to describe the retention behavior of each herbicide. Equation 6.1 allows to predict the retention factor (dependent variable) at several concentrations of SDS ([SDS] in M) and 1-pentanol (φ in %, v/v) in the mobile phase (factor), for moderately hydrophobic compounds [37]:

$$1/k = c_0 + c_1\,[SDS] + c_2\varphi + c_{12}[SDS]\varphi \qquad (6.1)$$

The constants c_1, c_2, and c_{12} represent the influence of each factor and their interaction, respectively. The equation was adjusted taking the experimental values of the retention factors of the analytes obtained at the five mobile phases above indicated, by curve-fitting non-linear least-square regression [41]. The concentration of SDS and the proportion of 1-pentanol were normalized from 0.05 to 0.15 M and 2 to 6% to (-1)-(+1), respectively, in order to better compare the calculated constants. The finally fitted equations were as follows:

DIU $1/k = 0.174 + 0.090\ [SDS] + 0.042\ \varphi + 0.037\ [SDS]\varphi$ $R^2 = 0.995$ (6.2)

TBA $1/k = 0{:}1329 + 0.0677\ [SDS] + 0.0293\ \varphi + 0.0304\ [SDS]\ \varphi$ $R^2 = 0.9997$ (6.3)

TBT $1/k = 0.097 + 0.073\ [SDS] + 0.034\ \varphi + 0.021\ [SDS]\ \varphi$ $R^2 = 0.98$ (6.4)

These equations are able to provide an accurate theoretical retention time of the three herbicides at values of SDS and 1-pentanol concentrations in the 0.05-0.15 M and 2–6% range, respectively, by interpolation (Fig. 6.1), according their adequate multiple determination coefficient (R^2). The order of the predicted retention times (TBT > TBA > DIU) was maintained in the whole interval of SDS/1-pentanol amounts.

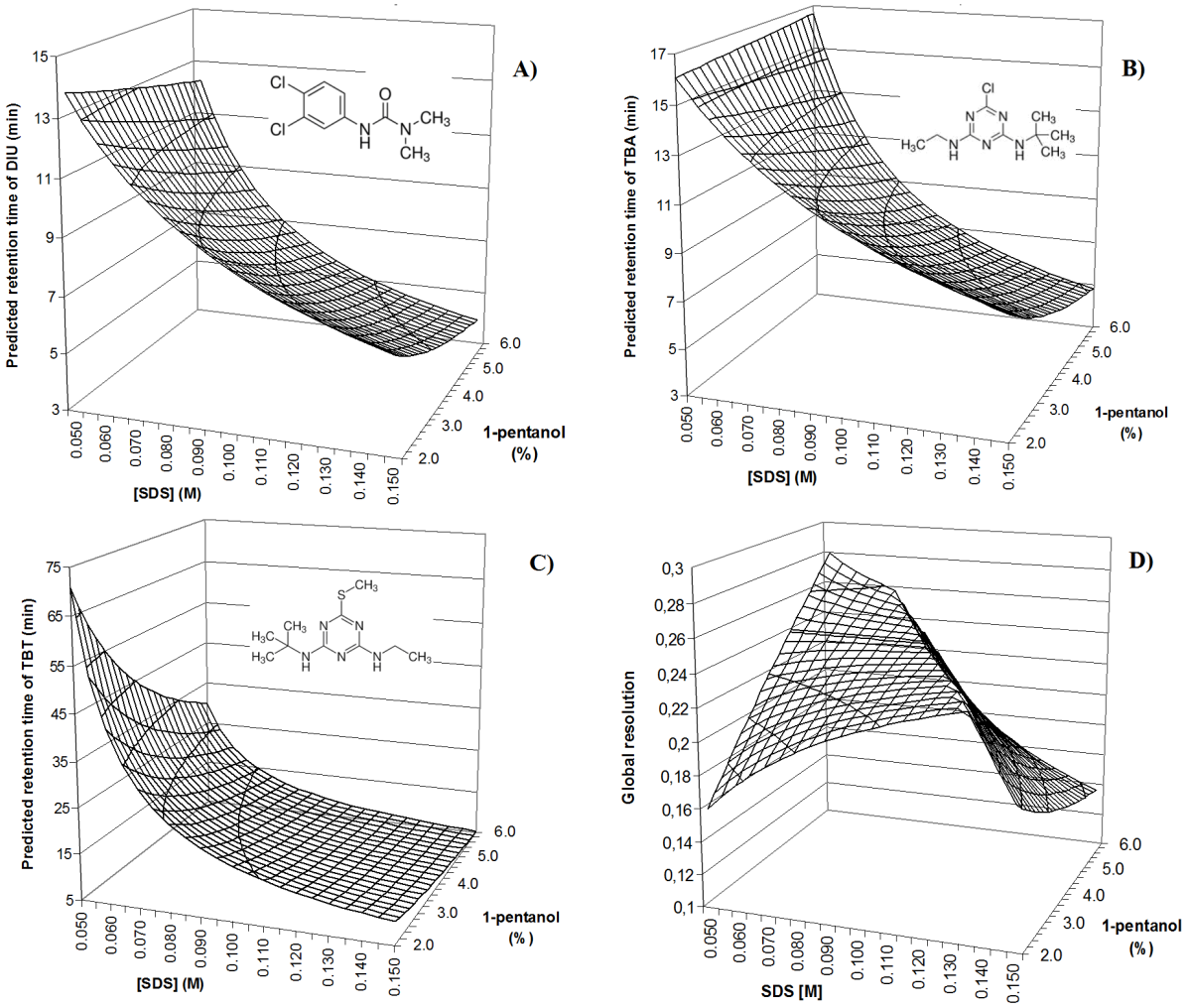


Figure 6.1. 3-D plot of the predicted retention time of A) diuron, B) terbuthylazine, and C) terbutryn, and D) global resolution, v.s. the concentration of sodium dodecyl sulfate and 1-pentanol in the mobile phase.

The influence of each factor and their interaction of the retention factor can be inferred from the empirical constants. According to the positive values of all the constants, the augmentation of the concentration of the surfactant and the alcohol effectively improves the elution strength of the mobile phase. These assessments match to those extracted from the qualitative interpretation of the unprocessed values obtained by the experimental design. Besides, the diminishing of the retention caused by the increase of the SDS concentration is enhanced at higher values of 1-pentanol, and vice versa. Indeed, the presence of the alcohol causes a diminishing of the critical micellar concentration and then the increase of the number of micelles [42]. The relative influence of each factor is as follows (in decreasing order): SDS concentration, 1-pentanol proportion, and their interaction. These findings were applicable for the three studied herbicides.

3.1.3 Optimization of the SDS/1-pentanol concentration

The concentrations of SDS/1-pentanol in the mobile phase were selected by surface response methodology using the mathematical model developed in the section 3.1.2, to maximize the global resolution and minimizing the analysis time.

The elementary resolution between each pair of consecutive peaks (DIU and TBA, and TBA and TBT), described as the modified selectivity (α′) [37], was calculated as follows (Eqs. 6.6 and 6.3, respectively):

$$\alpha'(\text{DIU-TBA}) = 1-k(\text{DIU})/k(\text{TBA}) \quad (6.5)$$

$$\alpha'(\text{DIU-TBA}) = 1-(0.1329+0.0677[\text{SDS}]+0.0293\varphi+0.304[\text{SDS}]\varphi)/(0.174+0.090[\text{SDS}]+0.042\varphi+0.037[\text{SDS}]\varphi$$

$$\alpha'(\text{TBA-TBT}) = 1-k(\text{TBA})/k(\text{TBT}) \quad (6.6)$$

$$\alpha'(\text{TBA-TBT}) = 1-(0.097+0.073[\text{SDS}]+0.034\varphi+0.021[\text{SDS}]\varphi)/(0.1329+0.0677[\text{SDS}]+0.0293\varphi+0.0304[\text{SDS}]\varphi$$

The global resolution (*Z*) was modeled as the minimum elementary resolution (Fig. 6.1D) [37]. The maximal theoretical Z (0.280) was reached at 0.05MSDS/6%1-pentanol, but with a too long retention time of TBT (30.0 min). We can see in Fig. 6.1, that the augmentation of [SDS] from this point provokes a reduction of the retention time, without excessively diminishing the global resolution. Finally, the mobile phase SDS/1-pentanol 0.07 M SDS/6% was selected, with a theoretical Z of 0.261 and a retention time of 14.6 min. In addition, the retention time of the first eluted (DIU) is 8.2 min, which is relatively far from the front of the chromatogram, and then would not be disturbed by the less retained compounds of the matrix.

A mixture of the three herbicides (1 mg/L each one) was analyzed using the optimized mobile phase. The experimental retention times (min) were as follows: DIU, 8.0; TBA, 10.8, and TBT, 14.6 min, and then each chromatographic run would take ≈16 min. The error in the prediction of the retention time was <3%.

3.1.4 Optimization of the detection conditions

The absorption spectrum of each herbicide was on-line taken using the DAD of the chromatograph, by adjusting the other instrumental parameters at their previously fixed

values. The optimized mobile phase was selected, in order to consider the influence of the solvent in the absorptivity of the chromogenes. Under these conditions, the wavelengths of maximum absorbance were as follows: diuron, 235 nm; terbuthylazine, 265 nm; and terbutryn, 230 nm. The wavelength detection was set to an average value of 240 nm, at which the three herbicides show a high absorptivity, in order to avoid changes in the detection conditions during the chromatographic run.

3.2 Sample preparation

3.2.1 Wastewater

Wastewater can contain variable amounts of solid particles and oily liquids suspended in the aqueous phase, depending on its source and cleanliness degree. These aggregates are the responsible of the turbidity, usually noticeable at a glance, of the aqueous sample. Besides, the oily phase is potentially harmful for the column, as it can be adsorbed on the stationary phase (because of its high hydrophobicity) and may slowly modify its properties [43]. The optimization of the experimental protocol was performed on the basis of eliminating the potential danger of these compounds without including an extraction step. The studies were performed using a waste water sample directly taken from the urban sewerage system, which was especially dirty (blank sample). This sample was collected for a previous study and does not contain the studied pesticides.

A filtration was proposed to remove the sludge and solid particles suspended in the waste water. In order to verify the behavior of wastewater after injection, the filtered sample was diluted in optimummobile phase, and then the oily drops were solubilized and only one phase was observed.We suppose that the hydrophobic phase was introduced inside the micelles.

This blank sample was filtered and directly injected to investigate the chromatographic behavior of these oily compounds. Only the front of the chromatogram (dead time to 3.0 min) was observed, with a relatively low signal. We suppose that the oily compounds bounded to the micelles scarcely interact with the stationary phase. Anyway, they did not interfere with the studied pesticides, thus assessing the selectivity of the method.

The effect of the direct injection of filtered dirty waste water on the column was examined by 50 successive analyses of the blank sample, spiked at 1 mg/L DIU, TBA, and TBT. The chromatogram obtained by the first analysis can be seen in Fig. 6.2A. No variation of the system pressure and retention time of the pesticides was observed, indicating that the injection of the oily drops is harmless for the chromatographic system. Therefore, no dilution was required to maintain a reasonable sensitivity level. Finally, the sample processing was limited to filtration and direct injection in the chromatographic system.

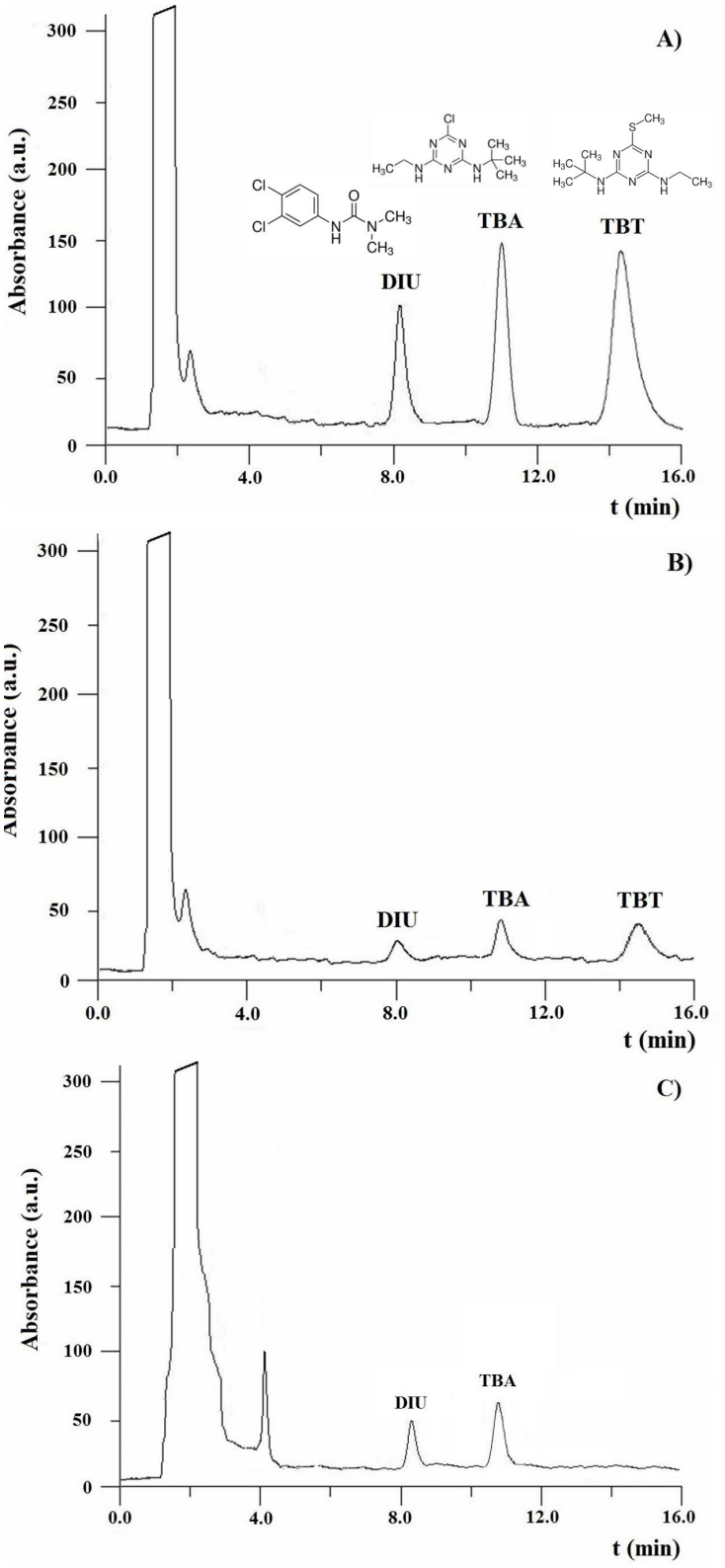
3.2.2 Soil samples

The optimization of the main extraction conditions (extraction solution, ultrasonication time, and sample/supernatant) was carried out using a soil sample taken from a private garden, where the studied herbicides have been never used.

The extraction solvent and the time of ultrasonication and the sample/supernatant ratio were optimized to maximize the recovery, which was evaluated by comparing the area of the chromatographic peaks corresponding to each herbicide. The ability of the mobile phase to recover the herbicides was compared to that of methanol, which is usually used to extract pesticides from soil samples [23, 31]. In both vases, clear chromatograms were obtained, and the recovery was similar. Therefore, the mobile phase was selected, due to its lower content of toxic organic solvent to improve the normality of the peak shape [44]. The ultrasonication time was tested from 1 to 10 min, at 1-min steps. For the three herbicides, the recovery increased up to 5 min and remained nearly constant at higher values. Therefore, 5 min was considered as the most adequate ultrasonication time.

The soil/supernatant (So/Su) ratio (w/v) considered were 1:1, 1:2, 1:5, 1:10, and 1:20. In all cases, the number of solid particles was not significant, and no obstruction of the filter was noticed during the filtration of the supernatant. In this case, the product peak area times the So/Su was used to compare the extraction capacity. This value increased from 1:1 to 1:5 and remained nearly constant from 1:5 to 1:20. Indeed, at values under 1:5; the mobility of the soil particles in the supernatant was hindered, reducing the contact between the two phases. This slows down the transfer of the herbicides from the solid particles to the supernatant. No advantage was found to use higher So/Su ratios higher than 1:5, and then this value was selected, in order to avoid an excessive diminishing of the sensitivity.

184 Finally, the blank sample was analyzed, in order to check the presence of contaminant.
185 No peak was observed at and near to (±2.00 min) the window time of the analytes, thus
186 demonstrating the selectivity of the procedure. This was confirmed by the analysis of the
187 blank soil sample spiked at 5 mg/kg of each analyte (Fig. 6.3A).



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189 **Fig. 6.2**. Chromatograms obtained by the analysis of a blank wastewater sample spiked at A) 1 mg/L and B)
190 LLOQ, of the three studied herbicides, and C) sample 11, taken from an urban sewerage water stream, and
191 containing DIU 0.44 and TBA 0.36 mg/L

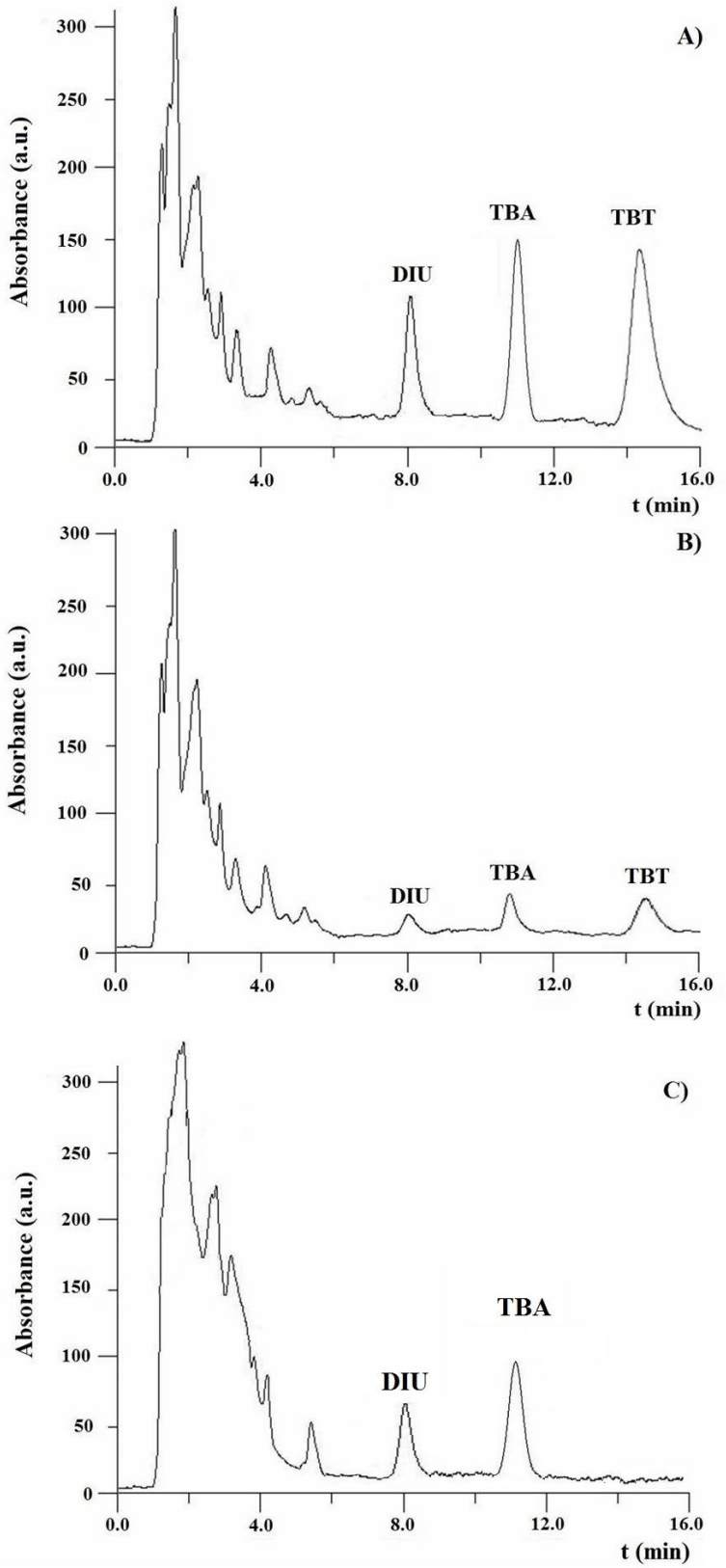

Figure 6.3. Chromatograms obtained by the analysis of a blank soil sample spiked at A) 1 mg/kg and b) LLOQ of each herbicide, and C) sample 22, taken from a private garden and containing 2.2 and 3.2 mg/kg of DIU and TBA, respectively

3.3 General comments about the procedure

The developedmethod holds several practical advantages over those based on hydroorganic RP-HPLC, because of the use of micellar mobile phases. These characteristics make the method meets the requirements of "green analytical chemistry", which fits the current trend in analytical chemistry [32].

The main feature of the method is, undoubtedly, the strong simplification of the sample preparation, which was limited to a one-step treatment. The transference of the herbicides from the solid particles to the liquid phase was a single ultrasonication. The wastewater and the soil extract were directly injected (after filtration), involving a minimal participation of the operator, thus avoiding long and tedious cleanup steps with variable recovery. Otherwise, it reduces the risk of exposure to other chemicals and the contamination of the processed sample.

The soil extract solution and the micellar mobile phase contain innocuous and biodegradable reagents (SDS and phosphate salts) and require less proportion of toxic, volatile, and flammable solvent (<6%) than typically used in hydroorganic RP-HPLC (up to 100%). Besides, the interaction of 1-pentanol with SDS-micelles even decreases its evaporation rate. No chemicals are involved in the treatment of wastewater. Therefore, the operator is barely exposed to hazardous chemicals and the waste contains a minimum amount of pollutants. This makes the developed procedure ecofriendly and relatively safe for the laboratory staff.

Another interesting advantage is the shortening of the analysis time. Samples can be processed in nearly 1-2 and 6-7 min, for water and soil, respectively. Moreover, as the mobile phase flows in isocratic mode, the column does not require a stabilization time between two successive injections, as in gradient programs. These characteristics facilitate the study of a high number of samples per day.

The developed procedure only required basic laboratory material and chromatographic instrumentation, a small amount of common chemicals, and the participation of a unique operator, which can even coordinate perform this activity with other tasks. In addition, a high number of samples per day can be analyzed and the expenditure related to segregation and waste treatment is inferior. Therefore, the analysis of wastewater and soils potentially contaminated with these herbicides can be carried out at an affordable price, which is reasonably advantageous in the current context of economic crisis.

3.4 Method validation

The developed analytical method was *in-lab* validated following the directives of the Validation and Peer Review of the US EPA Methods of Analysis. The evaluated parameters were instrument calibration, trueness, precision, ruggedness (in ultrapure water), quantitation range and limits, detection limits [38], and recovery in blank wastewater and soil.

3.4.1 Instrument calibration

Five solutions containing increasing concentrations of DIU, TBA, and TBT (calibration range from 0.2 to 2 mg/L) were analyzed (n = 3). For each herbicide, the corresponding peak area was plotted versus the concentration by least-squares regression [45]. The slope, y-intercept, determination coefficient, and the Cook's squared distance (CD^2), calculated for each level to detect possible outliers, were (concentrations in mg/L):

DIU	A = (175.1±2) [DIU]+(8.6±3)	$r^2 = 0.9996$	$CD^2 = 0.69$	(6.7)
TBA	A = (315.6±5) [TBA]+(10±5)	$r^2 = 0.9996$	$CD^2 = 0.74$	(6.8)
TBT	A = (684±10) [TBT]+(25±11)	$r^2 = 0.9996$	$CD^2 = 0.31$	(6.9)

The values of the goodness of fit criteria ($r^2 > 0.99$ and $CD^2 < 1$) demonstrate the high reliability of the statistical model.

3.4.2 Trueness and precision

These parameters were evaluated at the extremes of the calibration range (0.2 and 2 mg/L) and 0.5 mg/L.

For the intraday measurements, a standard solution of DIU, TBA, and TBT at the appropriate concentration was analyzed six times by consecutive injections. The trueness was calculated as the bias (difference of the average of the found concentrations and the true value, divided by the true value), while the precision (repeatability) was the closeness of agreement (relative standard deviation, RSD) of the peak areas. To determine the interday values, the same procedure was carried out five different days with renewed standard solutions over a 2-month period. The interday trueness was the average of the five intraday bias values, whereas the interday precision (reproducibility) was the RSD of the five daily averages of the peak areas. The results are shown in Table 6.3. The low bias (-6.1 to +5.0%) and low variability of the measures (<9.4%) indicate the high reliability of the chromatographic determination.

Table 6.3. Intra- and inter-day trueness and precision for the studied herbicides.

		Intra-day[a]		Inter-day[b]	
Herbicide	Concentration (mg/L)	Trueness (error, %)	Precision (RSD, %)	Trueness (error, %)	Precision (RSD, %)
DIU	0.2	- 5.2	8.9	- 3.0	7.4
	0.5	+4.2	4.2	+2.1	5.4
	2	+0.1	1.0	+0.5	2.8
TBA	0.2	-5.3	9.4	- 6.1	7.4
	0.5	+3.7	3.3	+2.9	4.2
	2	+0.8	1.1	+1.9	3.0
TBT	0.2	+5.0	8.3	+4.1	5.9
	0.5	+0.8	3.0	+3.1	1.4
	2	+0.5	0.4	-0.2	3.1

[a]n=6; [b]n = 5

3.4.3 Ruggedness

The real composition of the mobile phase may fluctuate in a short range around the indented value, because of the random errors arising during its preparation. In order to evaluate the effect of this annoyance on the instrumental response, the variation of the retention time and the peak area for each analyte was examined at minor deviations of the main components of the mobile phase from the optimal value: SDS concentration, 70 ± 5 mM; 1-pentanol proportion, 6.0 ± 0.2%; and pH, 3.0 ± 0.2%. The effect of each one was separately investigated.

A working standard solution of 1 mg/L of each herbicide was analyzed by triplicate, using three mobile phases, containing the selected value, the selected value minus the studied deviation, and the selected value plus the considered deviation, respectively, for the examined constituent, whereas the other ones were maintained at their optimal value. The results are shown in Table 6.4. Insignificant variations of the retention times (RSD <8.3%) and peak area (RSD <5.8%) were noticed, and then the instrument responses are robust enough to be unaffected by possible oscillations of the mobile phase in the studied range.

Table 6.4. Evaluation of the robustness of the MLC-method (n=3)

Herbicide	Chromatographic parameter	Level	Retention time (R.S.D.; %)	Peak area (R.S.D; %)
DIU	SDS concentration (mM)	65 – 75	8.3	4.6
	1-pentanol amount (%)	5.8 – 6.2	7.4	5.8
	pH	2.8 – 3.2	3.4	2.9
TBZ	SDS Concentration (mM)	65 – 75	7.5	3.8
	1-pentanol amount (%)	5.8 – 6.2	6.6	4.2
	pH	2.8 – 3.2	2.0	2.1
TBT	SDS Concentration (mM)	65 – 75	6.4	2.6
	1-pentanol amount (%)	5.8 – 6.2	5.9	4.5
	pH	2.8 – 3.2	1.5	1.5

3.4.4 Quantitation range

The lower limit of quantitation (LLOQ) was taken as the smallest concentration that can be quantified with a reasonable level of trueness and precision. The higher limit of quantitation (HLOQ) was the highest expected concentration of contaminants in water. For wastewater, the quantitation range was the same as for the calibration range, as neither dilution nor preconcentration steps are included in the sample preparation. For soils, the amount in soils (mg/kg) was five times the concentration in the injection solution, because of the solid/liquid extraction. Therefore, the lower/upper quantitation ranges were 0.2/2 mg/L and 1/10 mg/kg for wastewater and soils. The chromatograms obtained from blank samples of wastewater and soil spiked with DIU, TBA, and TBT at their corresponding LLOQ can be seen in Figs. 2B and 3B, respectively.

The detection limit (LOD) is the lowest concentration that provides a signal clearly above the baseline noise, but not accurately quantifiable [45]. The detection limit was measured by the 3.3 s criterion (3.3 times the standard deviation of the blank divided by the sensitivity). The standard deviation of the blank was calculated as the standard deviation of the residuals, and the sensitivity was the slope of the calibration curve, divided by the dilution ratio. For the three herbicides, the LOD were 0.06 mg/L and 0.3 mg/kg for wastewater and soils, respectively. Therefore, the sensitivity is sufficient to detect moderate quantities in wastewaters and soils.

3.4.5 Recovery

The effect of the matrix on the bias of the measurements was evaluated by analyzing blank samples of wastewater and soils (same as used in section 2.5), spiked at several amounts of diuron, terbuthylazine, and terbutryn. The analysis was performed six times within the same day, repeating at each case the whole experimental procedure. The average and the RSD of the quotient found concentration/true value were measured for each level. The results can be seen in Table 6.5.

The developed procedure shows good recovery (89.4 to 103.2%) through the whole quantitation range, indicating that the matrix barely affects the results. This is mainly due to simplification and the short time of the sample preparation, which minimizes the risk of loss or chemical change of the analyte and the arising of random errors.

Table 6.5. Recovery (%) for the studied herbicides in wastewater and soil (n=6)

	Wastewater		Soil	
Herbicide	Concentration (mg/L)	Recovery	Amount (mg/kg)	Recovery
DIU	0.2	93.6±7.3	1	90.1±7.7
	0.5	101.4±4.1	5	95.4±5.5
	2	99.1±1.1	10	97.1±3.0
TBA	0.2	94.8±8.4	1	89.4±8.8
	0.5	96.8±4.8	5	94.2±3.9
	2	98.4±2.1	10	93.8±4.1
TBT	0.2	103.2±7.5	1	97.4±8.0
	0.5	102.0±2.9	5	94.5±4.1
	2	99.1±0.9	10	93.9±3.5

3.5 Analysis of wastewater and soil samples

The method was applied to samples from several kinds of wastewater (agricultural gutters, urban sewerage), influent WWTP streams, decorative water pools, and soils (garden and right-of-way) from the Castelló area (Spain). If necessary, the quantitation range was extended by augmenting the dilution ratio. The results are shown in Tables 6.1 and 6.2 for water and soil samples, respectively.

Terbutryn was not found in any sample, thus indicating that the users comply with the regulation, and there is no residue from the time before the ban. DIU and TBAwere detected in the studied samples, indicating the suitability of the method to the quantification of the studied herbicides in these samples. The chromatograms obtained by the analysis of samples 11 (urban sewerage wastewater) and 22 (soil from a private garden) are shown in Figs. 6.2C and 6.3C, respectively.

A high amount was detected in the primary sources of pollution, soils, and decorative water reservoirs, mainly because their intense use. Indeed, the local climate (Mediterranean) is characterized by warm temperatures and sunny days in the major part of the year and stimulates the growing of weeds in soils, especially those irrigated, and algae in outdoor reservoirs, which must be frequently fumigated to avoid spoilage. In addition, their persistence motivates their accumulation in soils. The concentration of DIU and TBA in the

356 decorative water pools is limited, because the treated water is frequently renewed, and then
357 the addition of low amount of herbicides each time is preferable. Besides, there is a major
358 risk of involuntary contact with the population. The concentration found in aquaria was lower
359 than in fountains. Indeed, a less amount of herbicides must be added, to reduce the danger to
360 the fishes.
361 Agricultural, sewerage, and WWTP-influent wastewaters show lower concentrations
362 of DIU and TBA. Indeed, when a treated water stream discharges to the wastewater system, it
363 results in its dilution with other herbicide-free spills. Otherwise, in the fields, the herbicides
364 have a slow tendency to move from soils to water. Besides, the herbicides tend to move from
365 the wastewater stream to the walls and sediments of the channels. According to the results,
366 these water streams must be treated before releasing into the environment.
367
368 **4. Conclusions**
369
370 MLC-FLD was demonstrated a suitable technique for the routine monitoring of
371 diuron, terbuthylazine, and terbutryn in water and soil. The samples were injected directly
372 (for water) or after an uncomplicated one-step extraction (for soils), despite of the complexity
373 of the matrices, due to the particular properties of the micellar media. Therefore, the sample
374 preparation was as simple as possible, thus minimizing the sources of variance. A
375 mathematical relationship between the retention factor of the herbicides and the composition
376 of the mobile phase (SDS and 1-pentanol) was found, expediting its optimization by surface
377 response methodology. The analytes were resolved without interferences in <16 min, using
378 the isocratic mode. The method was validated by following the guidelines of the
379 Environmental Protection Agency in terms of selectivity, calibration and quantitation range,
380 limit of detection, trueness, precision, ruggedness, recovery, and uncertainty. The method is
381 short-time, easy-to-handle, eco-friendly, relatively safe for the operator, inexpensive, and
382 useful for routine analysis, if compared to other procedures based on hydroorganic RP-
383 HPLC. Finally, the method was successfully applied to quantify DIU, TBA, and TBT in
384 different kinds of water (agricultural and urban wastewater, input WWTP streams, and
385 decorative water pools) and soil (agricultural, garden, and right-of-way) samples, suspected
386 to be polluted with these herbicides.
387

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The authors declare that they have no conflicts of interest.

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