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Development of a dispersive liquid-liquid microextraction coupled with magnetic dispersive microsolid phase extraction for determination of triazines in wastewater samples

Karina Aguilar-Arteaga¹ · Cynthia Hernández-Mera¹ · Luis Díaz-Batalla¹ · Araceli Castañeda-Ovando² · Alida Elizabeth Cruz-Pérez³ · Enrique Barrado-Esteban⁴ · Manuel Carrillo-Cárdenas⁵

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Abstract

The combination of two microextraction techniques (dispersive liquid-liquid microextraction [DLLME] and magnetic dispersive microsolid phase extraction [MDMSPE]) was developed and reported for atrazine and simazine preconcentration from wastewater samples. The proposal methodology involved the use of magnetite supports functionalized with different alkyl or phenyl groups. The magnetic adsorbents were synthesized by the solvothermal method assisted by microwave, characterized, and used in the sample preconcentration of atrazine and simazine. The method validation included parameters such as the wastewater matrix effect, repeatability, and recovery. The analyte separation and quantification were performed by high-performance liquid chromatography with ultraviolet detection (HPLC-DAD). Parameters, such as the polarity and mass of magnetic solids and pH, were evaluated to provide better extraction performance. The highest recoveries (> 95%) were obtained with 50 mg of the phenyl group support (CS2) at pH 5, using 5 mL of the sample and carbon tetrachloride and methanol, as extraction and dispersive solvents, respectively. The lowest limits of detection (LOD) achieved were 13.16 and 13.86 ng L⁻¹, and the limits of quantification (LOQ) were 43.89 and 46.19 ng L⁻¹ for simazine and atrazine, respectively, with repeatability (expressed as %RSD) below 5% in all cases. The developed method is simple, easy, and low cost for the analysis of two herbicides potentially dangerous for environmental and human health.

Keywords Coupled microextraction techniques · Magnetic adsorbents · Triazines · Dispersive liquid-liquid microextraction · Magnetic dispersive microsolid phase extraction

✉ Araceli Castañeda-Ovando
ovandoa@uaeh.edu.mx

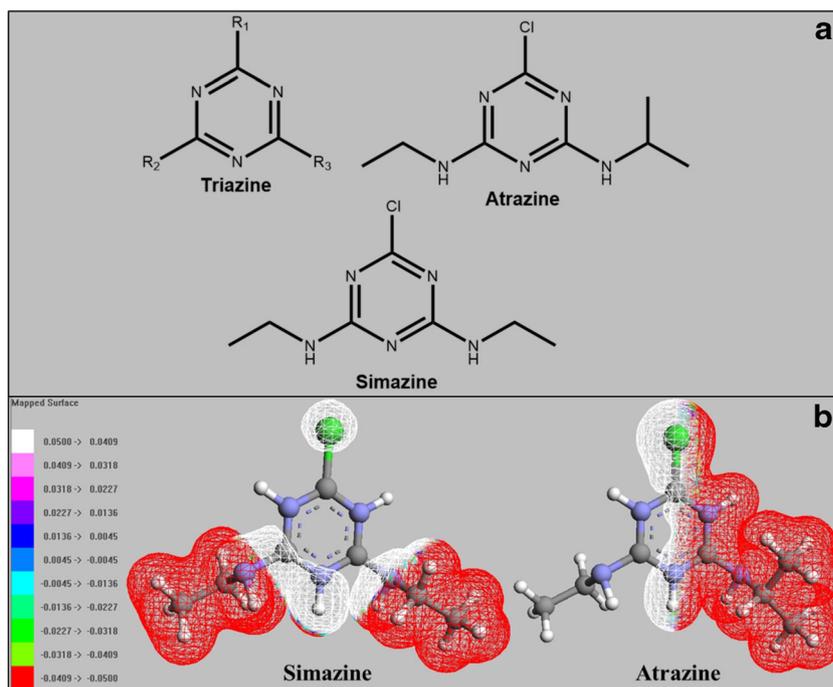
- ¹ Agroindustry Engineering, Universidad Politécnica de Francisco I. Madero, Carr. Tepatepec-San Juan Tapa km. 2, 42660, Tepatepec, Hidalgo, Mexico
- ² Chemistry Department, Universidad Autónoma del Estado de Hidalgo, Carr. Pachuca- Tulancingo km. 4.5, 42184, Mineral de la Reforma, Hidalgo, Mexico
- ³ Academic Division of Engineering and Architecture, Universidad Juárez Autónoma de Tabasco, Carr. Cunduacán-Jalpa de Méndez km. 1, 86690 Villahermosa, Tabasco, Mexico
- ⁴ Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid, Campus Miguel Delibes, Paseo de Belén 7, 47011 Valladolid, Spain
- ⁵ Universidad Politécnica de Huejutla. Energy Engineering, Carr. Huejutla-Chalahuyapa km 3.5 Col. Tepoxtequito, 43000 Huejutla de Reyes, Hidalgo, Mexico

Introduction

Herbicides have improved crop production; however, their excessive use has also increased the risks of environmental pollution and health damage because they enter the food chain. The triazine group is one of most used, which was introduced in 1954, after proving clorazine efficacy for the control broadleaf and grassy weeds in tomato, corn, onion, potato, and carrot crops [1]. Subsequently, different derived compounds were applied, such as pre- and post-treatment, to control weeds in crops, mainly in corn, sorghum, and wheat [2].

The main compounds that belong to the group of triazines and which derive from s-triazine (Fig. 1A) are heterocyclic compounds with symmetric atoms which are substituted in positions 2, 4, and 6 [3]. These herbicides have variable life expectancy depending on the environmental conditions. Atrazinic pesticides are stereochemically stable; thus, their persistence in the environment can be prolonged from

Fig. 1 (A) Chemical structure of the triazine compounds. (B) Mapped surface of electron density-electrostatic potential from simazine and atrazine



months to years; in addition, these compounds are degraded depending on their solubility in water.

Most of these pesticides have been banned in many countries, due to the high risk to human health and environmental pollution that represent [4]. However, they are still used indiscriminately in Mexico; according to FAO reports, more than 30,000 tons of these herbicides were used in 2013 [5].

In Mexico, herbicide use occurs most frequently in areas for crops irrigated with wastewater (treated or untreated); one of these areas is Irrigation District 03 (DDR03) located in Mezquital Valley, Hidalgo, Mexico. DDR03 receives and uses wastewater from Mexico Valley [4]; in this area, several crops are grown like corn, wheat, oats, beans, green chili, and triticale, among others.

The most applied herbicides used in DDR03 are 6-chloro-4-N-ethyl-2-N-propan-2-yl-1,3,5-triazine-2,4-diamine (atrazine) and 6-chloro-2-N,4-N-diethyl-1,3,5-triazine-2,4-diamine (simazine). Atrazine has been reported as an endocrine disruptor by Hayes and coworkers [6] and together with simazine have been included in that group by the Environmental Protection Agency of the USA [7].

Atrazine is one of the most used pesticides in the world for weed control. In 2001, atrazine was the most detected pesticide in drinking water in the USA, and its use has increased. According to the EPA, atrazine was the second most widely used herbicide after glyphosate. Simazine is widely used as a residual nonselective herbicide. The use of atrazine and simazine has been banned in the European community; however, countries such as the USA and Mexico still use them.

Tolerance levels for atrazine ($3 \mu\text{g L}^{-1}$) and simazine ($4 \mu\text{g L}^{-1}$) have been suggested by national primary drinking water regulations of the USA [8].

In order to avoid serious pollution problems, the levels of these herbicides must be monitored constantly. Several methods of sample preparation have been reported for triazine residue analysis, such as liquid-liquid extraction (LLE), solid-phase extraction (SPE) and matrix solid-phase dispersion (MSPD). LLE is the most used; however, SPE and MSPD are current preferred methods, which are characterized by their use of small amounts of organic solvents. Nevertheless, these methods are time-consuming and costly. A good alternative to minimize these disadvantages is the implementation of microextraction methods and their coupling, which has many advantages, like simplicity of operation, short extraction time, low amount of organic solvents used, low cost, and high enrichment factors. Recently, several methods for the determination of triazines have been proposed. A fluorescence-based method has been reported, which needs previous derivatization with ammoniacal 2-cyanoacetamide, reporting a LOD and a LOQ of $0.07 \pm 0.023 \mu\text{g mL}^{-1}$ and $0.23 \pm 0.023 \mu\text{g mL}^{-1}$, respectively, with RSD percentage $< 12.1\%$ for atrazine [9].

Mei et al. [10] proposed a method for the determination of triazines, which was based on microextractions using capillary tubes packed with magnetite with LODs and LOQs of $0.074\text{--}0.23 \mu\text{g L}^{-1}$ and $0.24\text{--}0.68 \mu\text{g L}^{-1}$, respectively. The method was applied to agricultural, lake, and river water samples, showing recovery percentages between 70.7 and 119%.

On the other hand, assisted microwave extractions of triazines have shown to have similar results to microextraction methods based on adsorbent materials, with LODs and LOQs in a range of 0.24 and 0.80 $\mu\text{g mL}^{-1}$ [11]. Other methods based on microextractions have used nanoparticulated materials of complex synthesis, such as the application of single-walled carbon nanotubes, obtaining LOD below ng L^{-1} [12].

Therefore, the aim of this work was to develop a proposal for the determination of simazine and atrazine, based on the coupling of two microextraction techniques (dispersive liquid-liquid microextraction [DLLME] and magnetic dispersive microsolid phase extraction [MDMSPE]) and its subsequent analysis by HPLC-DAD. The developed method was applied in the quantification of these herbicides in wastewater samples from DDR03.

Materials and methods

Materials

All reagents and chemicals used were of analytical grade. Triethoxyphenylsilane (TEPS), triethoxyethylsilane (TEES), triethoxyoctylsilane (TEOOS), KBr, triazine, and simazine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of triazines were prepared at a concentration of 1 mg L^{-1} in ultrapure water. These solutions were stored under refrigeration (4 °C) and in darkness until use to avoid possible decomposition. Calibration standards were prepared by mixing adequate volumes of each standard solution in ultrapure water or wastewater like an analytical matrix.

Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), trisodium citrate, ammonium acetate, ethylene glycol, ethanol, hydrochloric acid (HCl), sodium hydroxide (NaOH), carbon tetrachloride, and methanol were acquired from J.T. Baker (Center Valley, PA, USA).

Synthesis and coating of magnetic supports

Silanes of different polarities were used to coat magnetic solid (magnetite). The synthesis was performed by the microwave-assisted solvothermal method reported by Hernández-Hernández and coworkers [13] with some modifications.

First, 590 mg of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 350 mg of ammonium acetate (anti-caking agent), 400 mg of sodium citrate (pH buffer), and 6 mL of ethylene glycol were mixed; then, it was homogenized by ultrasound for 10 min. Subsequently, the microwave reaction was performed in a MONOWAVE Extra device from Anton Paar (Graz, Austria), under the following conditions: the temperature was increased from 20 to 235 °C in 7 min, maintaining the final temperature for 10 min; the stirring rate was 600 rpm. The obtained solid (magnetite, Fe_3O_4) was separated with a neodymium magneto of 30,000 G, washed with ethanol, and oven-dried at 60 °C.

The magnetite coating was also carried out by microwave heating, using 100 mg of Fe_3O_4 , 5 mL of ethanol, and 1 mL of the corresponding silane (TEPS, TEES and TEOOS). The reaction conditions were as follows: 125 °C for 5 min and 600 rpm. At the end of the reaction time, an ethanol rinse was performed, and the coated magnetite was recovered using a neodymium magneto of 30,000 G. The coated solids (CS) obtained were labeled as CS1 ($\text{Fe}_3\text{O}_4 @ \text{TEES}$), CS2 ($\text{Fe}_3\text{O}_4 @ \text{TEPS}$), and CS3 ($\text{Fe}_3\text{O}_4 @ \text{TEOOS}$); for their use, they were oven-dried at 60 °C.

Characterization of magnetic supports

The magnetic solids that were characterized were those which presented the best preconcentration conditions of simazine and atrazine, labeled previously as CS2.

X-ray diffraction

The X-ray diffraction analysis (XRD) was carried out on a Bruker AXs diffractometer model D2 phaser; a Cu tube with a wavelength of 1.5418 Å from 10 to 80 on a 2 θ scale was used.

Fourier transform infrared spectroscopy

An IRAffinity-1 spectrometer from Shimadzu (Maryland, USA) was used to perform Fourier Transform Infrared spectroscopy (FT-IR) analysis. The coated magnetic support was mixed with KBr at 5% w/w concentration and pressed to obtain pellets. The analysis was performed in a range of 340–4700 cm^{-1} to 40 scans.

Scanning electron microscopy

The characterization by scanning electron microscopy (SEM) was performed in a JEOL (Massachusetts, USA) JSM-6010LA equipment at 20-kV acceleration voltage, under high vacuum conditions, with coupled energy dispersive detector (EDS). For the morphological analysis, the sample was mounted on double-sided carbon conductive tape in an aluminum sample holder, then the micrographs were obtained at different magnifications ($\times 500$, $\times 15,000$, and $\times 30,000$). The semiquantitative analysis by EDS was carried out to obtain the distribution of elements on the surface of the samples. The images were processed with the InTouchScope™ Software.

High-resolution transmission electron microscopy

A JAN-2100 JEOL equipment (Massachusetts, USA) of 200-kV acceleration and electron source of lanthanum hexaboride (LaB_6) was used for the high-resolution transmission electron

microscopy (HRTEM) analysis. For the HRTEM analysis, 10 mg of the magnetic support (CS2) was dispersed in 2 mL of isopropyl alcohol and the mixture was placed in an ultrasonic bath for 15 min, a drop of the dispersion was extracted and deposited in a copper rack with a cover of amorphous carbon (50 nm). Finally, the rack with the magnetic support was left in a desiccator for 24 h.

Nitrogen physical adsorption

The nitrogen physical adsorption was done in a Quantachrome ASiQwin Automated Gas Sorption Data Acquisition and Reduction equipment from Quantachrome Instruments (Florida, USA). For the analysis, 200 mg of the magnetic support was weighed and degassed for 20 h at 250 °C.

Extraction method development

The extraction method was carried out in two steps: (1) DLLME and (2) MDMSPE. The matrix used for the development of the method was wastewater enriched with two concentration levels, 50 and 100 $\mu\text{g L}^{-1}$ of the herbicides (atrazine and simazine).

DLLME

The effect of pH was evaluated (3, 5, 8 and 11) for DLLME, in which, carbon tetrachloride and methanol were used as extractant and disperser solvents, respectively [14]. 5 mL of samples enriched with atrazine and simazine at a concentration of 50 and 100 $\mu\text{g L}^{-1}$ was taken, and pH was adjusted (3, 5, 8 and 11) with HCl or NaOH 0.01 mol L^{-1} . Then, 30 μL of extractant solvent and 300 μL of disperser agent were added. The mixture was kept in vortex agitation for 5 min. Subsequently, the non-polar phase was separated by centrifugation during 2 min at 5000 rpm (Fig. 2). The organic phase was kept into the tube until the second extraction step was continued.

Development of MDMSPE

In this step, the coating polarity of the magnetic support was evaluated. The different synthesized supports (CS1, CS2, and CS3) were evaluated in order to obtain the best MDMSPE conditions. Later, the best support was chosen and three different amounts of this (10, 50, and 100 mg) were used to establish the highest recoveries.

The extraction procedure by MDMSPE was done according to Rodriguez et al. [3] with some modifications. The magnetic support (CS1, CS2, or CS3) was added to the organic phase obtained from DLLME; the mixture was homogenized into an ultrasonic bath for 10 min. Later, the magnetic solids were separated attracting them with a neodymium magnet

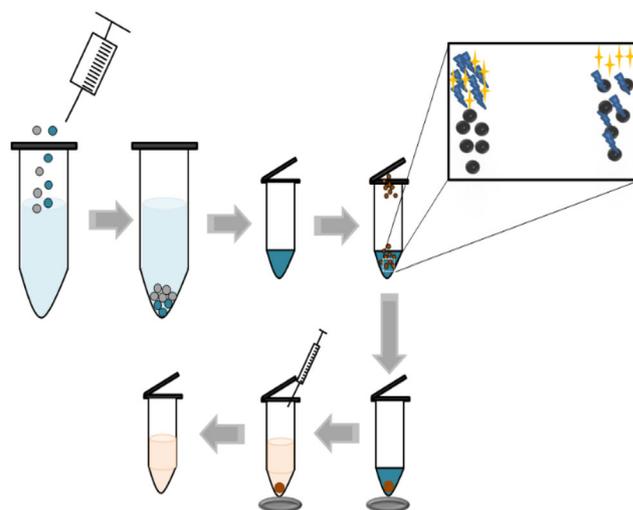


Fig. 2 Coupled methodology for atrazine and simazine extraction based on dispersive liquid-liquid microextraction and magnetic-based dispersive microsolvation extraction (DLLME-MDMSPE)

placed on the outside of the container. The magnetic support was washed with deionized water. The adsorbed analytes on the magnetic supports were eluted with 1 mL of methanol [2]. Finally, methanolic eluates were filtered with a membrane with a pore size of 0.45 μm and injected for their analysis by HPLC-DAD (Fig. 2).

Separation and quantification by HPLC-DAD

The determination and quantification of simazine and atrazine were performed in a HPLC Surveyor Thermo Scientific equipment (Massachusetts, USA), using a RP-C18 column (250x4mm, 5 μm) from Supelco (St. Louis, MO, USA), and an isocratic elution. The mobile phase was methanol:water (2:1) at 1 mL min^{-1} . Twenty-five microliters of the sample was injected and the detection was carried out at 220 nm [3].

Recovery percentages and enrichment factor (EF) were calculated using Eqs. 1 and 2, respectively. These parameters were used to evaluate the final extraction efficiency.

$$\text{Recovery (\%)} = \frac{c_1}{c_2} \times 100 \quad (1)$$

Where c_1 was the analytes concentration measured at the end of the extraction processes and c_2 is the analytes concentration added in the sample.

$$EF = \frac{C_f}{C_i} \quad (2)$$

where C_f was the analyte concentration in final methanolic phase (after the preconcentration treatment by coupled microextraction techniques) and C_i was the initial analyte concentration in the sample solution.

Validation of the extraction method

Samples

Wastewater samples were collected from 18 points localized into the DDR03, in the state of Hidalgo, Mexico. Sampling was carried out during September 2018. Two liters of each sample was collected and stored at 4 °C in polypropylene bottles with hermetic closure (previously conditioned by filling with 2% v/v H₂SO₄) until analysis.

The wastewater samples were filtered through 0.45- μ m cellulose acetate filters by vacuum filtration. Bottles were filled with the sample, wrapped in hermetic plastic bags, and transported to the laboratory in iceboxes. Subsequently, samples were stored not longer than 5 days at 4 °C until their analysis.

pH, electrical conductivity (EC), and temperature were measured in situ, while turbidity, chemical oxygen demand (COD), and total soluble solids (TSS) were determined in the laboratory.

Method validation

Linearity, recoveries, precision (expressed as repeatability and reproducibility), limits of detection (LODs), and quantification (LOQs) were evaluated for the method validation. In order to obtain these analytical parameters, calibration curves were constructed.

Calibration curves were constructed by a external and standard addition method, with the purpose of determining the matrix effect. Wastewater samples were spiked with the herbicides (atrazine and simazine) in the range of 0 to 250 μ g L⁻¹. The extraction method was carried out as described in “DLLME” and “Development of MDMSPE,” but pH was fixed at 5 for DLLME, and 50 mg of CS₂ magnetic solid was used for MDMSPE. The determinations of simazine and atrazine were done using the methodology described in “Separation and quantification by HPLC-DAD.”

The SPE procedure for comparison was performed as described Ma et al. [15]; C18 SPE cartridges (500 mg, Bound Elut, Varian, Netherlands) were conditioned using 5 mL of ethyl acetate, 5 mL of methanol and 5 mL of deionized water at a flow rate around 2 mL min⁻¹. Water samples (1 L) were flowed through the cartridges with a flow rate between 10 and 15 mL min⁻¹ under vacuum and the loaded cartridges were rinsed with 3 mL of methanol:water (5:95, v/v). The elution was performed with three aliquots (1 mL) of ethyl acetate at a flow rate of about 1 mL min⁻¹. The combined aliquots were evaporated to dryness by a gentle stream of nitrogen and the residues were dissolved in 50 μ L of methanol and injected into the HPLC system.

Statistical analysis

The statistical analysis was performed using the Minitab software (v.17).

Results and discussion

Characterization of magnetic supports

The diffractogram of the magnetic support Fe₃O₄@TEPS (CS₂) is showed in Fig. 3A, where the characteristic diffraction lines of the magnetite can be observed, and no diffraction lines of maghemite, which is an iron oxide that is also obtained in other methods as a product of the synthesis of magnetic supports, among which coprecipitation stands out, are shown [16]. According to the Debye-Scherrer equation, the calculated particle size, based on the diffraction pattern, was 10 nm, which is consistent with the HRTEM analysis performed.

The FT-IR analysis allowed the verification of the presence of SiO₂ in the magnetic solids, since it presented the vibrations of its functional groups. The FT-IR spectrum is shown in Fig. 3B, in which characteristic absorption bands are observed, among which stand out the stretching vibration of the OH bond (3398 cm⁻¹) and the stretching vibration C=C of the aromatics (1420, 1559, and 1624 cm⁻¹). The characteristic band for Fe-O was observed at 581 cm⁻¹, while for Si-O and Si-O-Si was observed at 1330 and 1075 cm⁻¹, respectively [13].

The SEM micrograph analysis is shown in Fig. 3C, in which the presence of magnetic supports agglomerations is observed, which are due to the magnetic saturation that the material possesses. The EDS analysis (Fig. 3D) allowed knowing the semiquantitative composition of the magnetic support, which was as follows: Fe (52.24 \pm 0.69%), O (26.69 \pm 0.01%), Si (1.29 \pm 0.01%), and C (20.04 \pm 0.33%). The presence of Si and C evidenced that silane coated the magnetic support.

High-resolution transmission electron microscopy (HRTEM) showed the morphology of the nanometric supports. Figure 4 shows the HRTEM images, in which the presence of quasi-spherical Fe₃O₄@TEPS nanoparticles with a range size between 5 and 10 nm was observed.

Dark-field HRTEM image is shown in Fig. 4A, in which it is observed an agglomeration of 200 nm, the nanoparticulated centers composed of magnetite were highlighted in white color and are also observed in the bright-field of HRTEM image (Fig. 4B). A Fe₃O₄@TEPS nanoparticle HRTEM image is obtained (Fig. 4C), in which the inset is the corresponding FFT pattern. Lattice-resolved image (Fig. 4D) of single Fe₃O₄@TEPS showed lattice fringes. The ring patterns were identified to be 220, 311, 400, and 511.

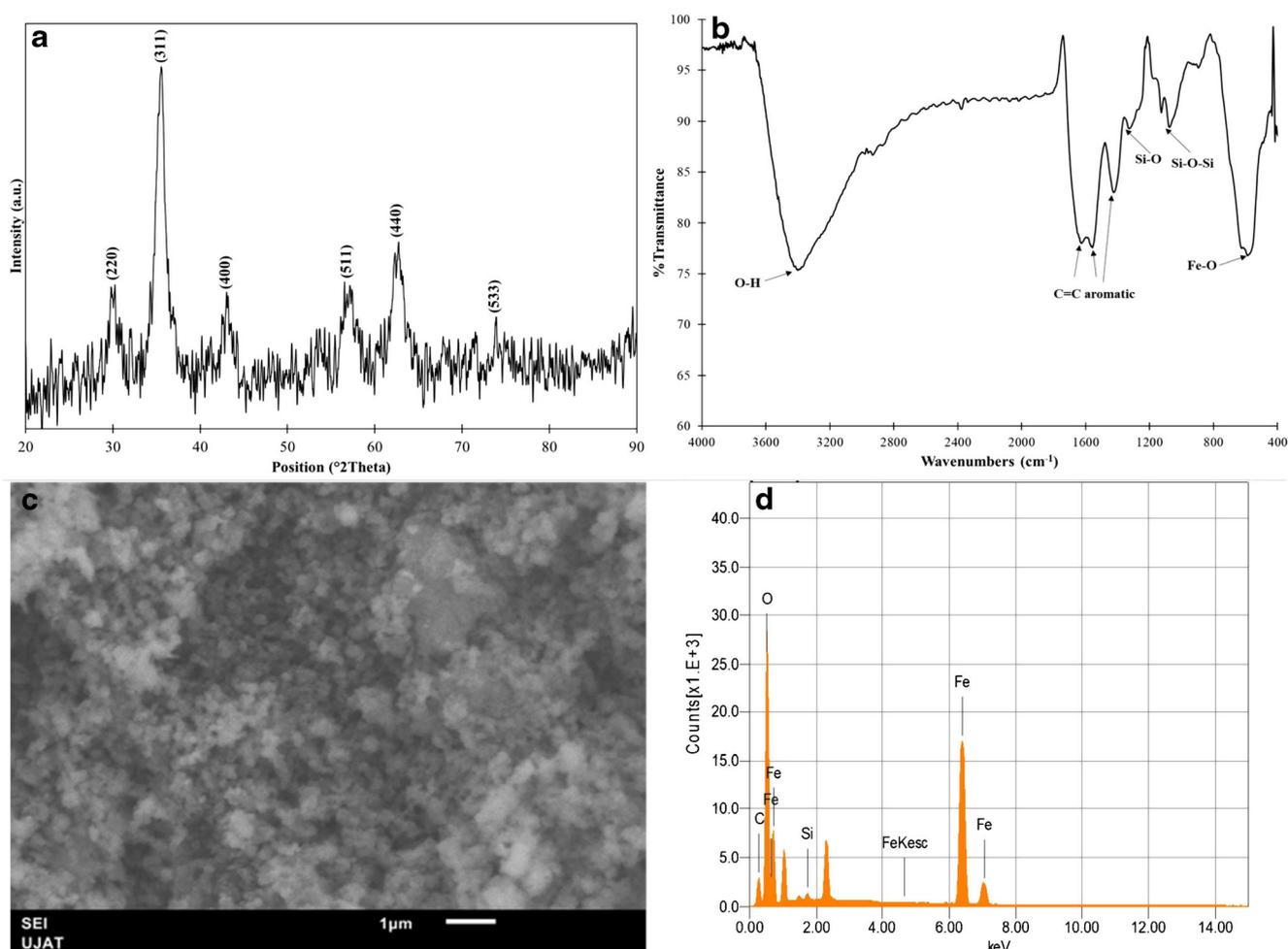


Fig. 3 Characterization of CS2 magnetic support ($\text{Fe}_3\text{O}_4@TEPS$). (A) XRD spectrum. (B) FT-IR analysis. (C) SEM micrograph. (D) EDS analysis

The solvothermal synthesis assisted by microwave allowed obtaining solids with high surface area ($140.257 \pm 0.050 \text{ m}^2 \text{ g}^{-1}$), high pore volume ($0.262 \pm 0.001 \text{ cm}^3 \text{ g}^{-1}$), and radius of $18.363 \pm 0.005 \text{ \AA}$. These parameters are very important, since they indicate that the solids obtained are good magnetic materials capable of adsorbing analytes of interest and could be used as adsorbent materials in solid-phase magnetic extraction processes [17].

Development of the extraction method

The generalized trend towards the development of analytical procedures that allow the determination of contaminants with advantages, such as reduction of toxic waste generation, energy consumption, and sample pretreatment times, has increased the development of coupled microextraction methodologies [9]. The coupling of microextraction techniques evaluated in the present work (DLLME-MDMSPE) provides the mentioned before advantages for the determination of simazine and atrazine concentrations in wastewater samples.

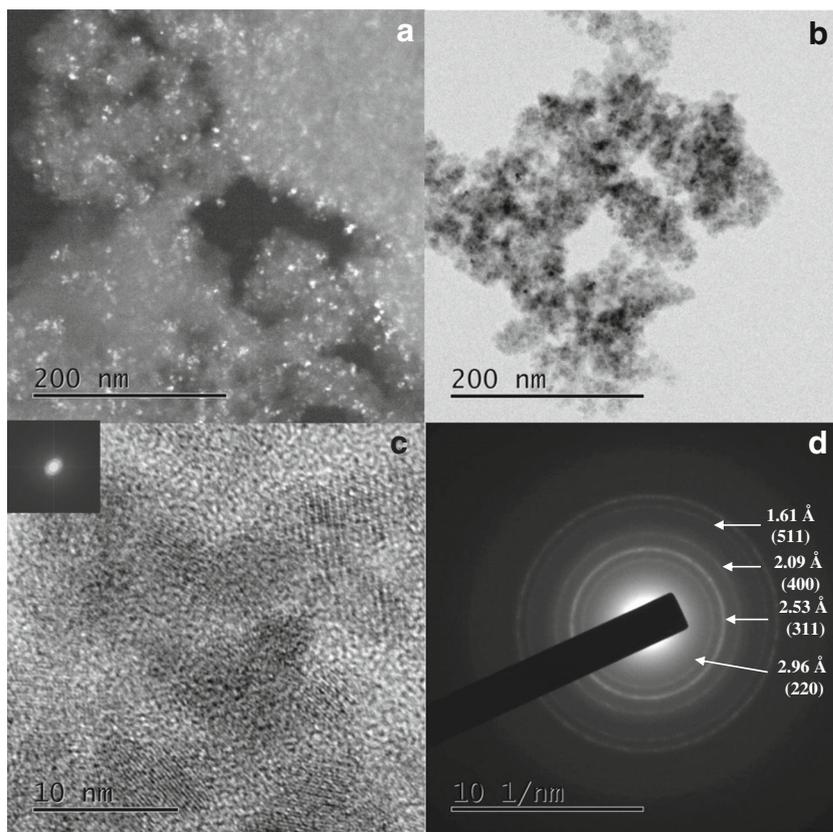
Effect of magnetic supports polarity and pH

Firstly, the proposal extraction method (DLLME-MDMSPE) was performed using CS1, CS2 and CS3 as magnetic supports. This procedure was done at different pH levels (3, 5, 8 and 11). Magnetite was coated with three non-polar silanes (ethyl, phenyl, and octyl) and was chosen because the analytes of interest (simazine and atrazine) have the same nature.

The calculation of the recovery percentage for each herbicide was performed. Higher percentages of recovery were observed for CS2, which corresponded to $\text{Fe}_3\text{O}_4@TEPS$; this silane can be considered as the one with the most non-polar nature, compared with TEES or TEOOS. On the other hand, CS1 proved to be the worst support for extracting herbicides, with recovery percentages between 10 and 35%, even with basic pH values.

Figure 5A shows the percentages of recovery of simazine, using the three synthesized solids (CS1, CS2, and CS3), at different pH values. CS2 recovery percentages were higher throughout all pH range, due to the non-polar nature of the adsorbent. The percentage of recovery depended on pH, since

Fig. 4 HRTEM analysis of CS2 magnetic support ($\text{Fe}_3\text{O}_4@TEPS$). (A–C) micrographs. (D) Graph showing the electron diffraction of an ensemble of magnetic nanoparticles



simazine obtained the highest percentage of recovery at pH 5 (100%) and at pH 3 and 11 the lowest (75%).

Simazine extraction using CS3 (Fig. 5A) also showed high recovery percentages at pH 5 (90%) and pH 8 (85%). Likewise, atrazine recovery percentages were determined (Fig. 5B) using the three solids (CS1, CS2, and CS3). Again, when CS1 was used as an adsorbent, the recovery percentages were low (15–35%), making it unsuitable for use in the method validation stage.

The best adsorbent for atrazine extraction was CS2 (Fig. 5B), showing recovery percentages between 70 and 95%, getting the highest percentages at pH 5. When CS3 was used, lower values of recovery than simazine (30–85%) were observed throughout all the working pH range.

The behavior presented by the three evaluated adsorbents depended on their polarity ($\text{CS3} < \text{CS2} < \text{CS1}$). CS2 presented better percentages of analyte recovery, because its surface favored both hydrophobic interactions (van der Waals

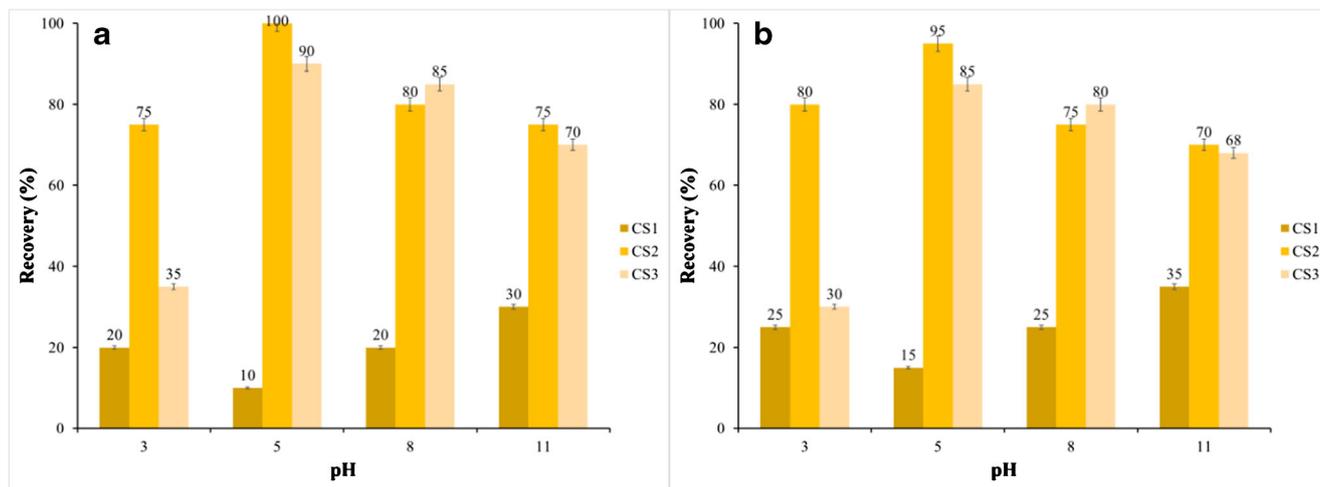


Fig. 5 Recovery percentages for (A) simazine and (B) atrazine, by a coupled methodology based on DLLME-MDMSPE, and using magnetite coated with silanes: CS1 ($\text{Fe}_3\text{O}_4@TEES$), CS2 ($\text{Fe}_3\text{O}_4@TEPS$) and CS3 ($\text{Fe}_3\text{O}_4@TEOOS$)

Table 1 Recovery percentages for atrazine and simazine by a coupled methodology based on DLLME-MDMSPE and using different amounts of CS2 (Fe₃O₄@TEPS)

Amount of CS2 (mg)	%Recovery Atrazine	1 Simazine
10	64 ± 2.92 ^b	54 ± 3.08 ^b
50	96.80 ± 0.84 ^a	99.20 ± 0.84 ^a
100	97.60 ± 1.52 ^a	98.60 ± 0.89 ^a

Values are given as mean ± standard deviation ($n=5$). The different letters (a, b) in the same column represent a significant difference with $p < 0.05$ among each group

interactions) and π - π interactions (due to the presence of phenyl group) with the triazinic herbicides, which are compounds of aromatic nature.

The difference in the percentages of recovery between simazine and atrazine is related to the polarity of the analytes, which even when both belong to the same family, their electrophilic sites make the difference, as shown in the mapping of electronic density and electrostatic potential (Fig. 1B).

Effect of amount of magnetic supports

According to the results, CS2 was chosen as the best magnetic support, and the highest recoveries were obtained at pH 5 for atrazine and simazine (Fig. 5). Then, the extractions were repeated under these conditions (CS2 and pH 5), but the amount of magnetic support was changed. Recoveries reached for each amount are showed in Table 1, in which 10 mg of CS2 is insufficient to extract both herbicides, and desorption can occur. On the other hand, there are no significant differences between 50 and 100 mg of magnetic support. Therefore, 50 mg of magnetic support was chosen as the best condition.

Table 2 Analytical parameters of the DLLME-MDMSPE method ($n = 3$)

Coating solid (CS)	Intercept	Slope	R ²	LOD $\mu\text{g L}^{-1}$	LOQ $\mu\text{g L}^{-1}$	Repeatability		Reproducibility	
						S1	S2	S1	S2
Simazine									
CS1	223	32.3	0.9807	20.60	43.01	> 10	> 10	> 10	> 10
CS2	19,276	7362.4	0.9976	0.01	0.04	2.10	2.60	4.80	2.80
CS3			0.9957	7.30	24.34	2.40	6.70	5.80	7.50
Atrazine									
CS1	2965	206.5	0.9207	68.68	143.60	> 10	> 10	> 10	> 10
CS2	22,493	9065.8	0.9964	0.01	0.05	1.20	1.50	3.20	2.60
CS3			0.9929	0.82	2.73	6.10	5.60	6.50	6.30

Calibration curves 0–250 $\mu\text{g L}^{-1}$. Wastewater matrix. Solid mass: 50 mg. Where CS is coating solid, CS1 is Fe₃O₄@TEES, CS2 is F₃Oe₄@TEPS, and CS3 is F₃Oe₄@TEOS. Repeatability and reproducibility were evaluated for 2 concentration levels. Where S1 is standard at 5 $\mu\text{g L}^{-1}$ and S2 is standard at 250 $\mu\text{g L}^{-1}$.

Validation of the extraction method

The analytical parameters of the three solids (CS1, CS2, and CS3) were calculated in order to determine the limits of detection (LOD) and the limits of quantification (LOQ), as well as the repeatability and reproducibility of the method (Table 2).

Calibration curves were constructed from 0, 25, 50, 75, 100, 125, 150, 175, 200, 225, and 250 $\mu\text{g L}^{-1}$ of atrazine and simazine, wastewater samples were used as matrix, and the extraction process was performed at pH 5 with 50 mg of magnetic support. The samples have similar physicochemical characteristics (Table 3), so that a composite sample was used for the construction of the calibration curves.

Even when CS1 showed low recovery percentages, it was decided to be used to evaluate its efficiency with wastewater as the matrix. According to the data presented in Table 2, CS1 as adsorbent of simazine and atrazine showed the highest LOD and LOQ, and the lowest determination coefficient (R^2). In fact, the precision parameters (reproducibility and repeatability) are the highest. Therefore, CS1 was inappropriate for obtaining the best analytical parameters.

The best extraction support was CS2, since it presented the lowest LOD and LOQ (Table 2). The LODs were 10 ng L^{-1} for both herbicides and the LOQs were 40 and 50 ng L^{-1} , for simazine and atrazine, respectively. This adsorbent presented the best precision parameters (reproducibility and repeatability), which RSD values were below 5%; in addition, its R^2 was very close to 1. Besides, the use of CS2 allowed obtaining enrichment factors of 1250 and 1000, for simazine and atrazine, respectively. Matrix effect was estimated for CS2 by comparison of slopes (external calibration and standard addition methods) [18]. Results are shown in Table 3, in which $t_{\text{exp}} > 2$ for both herbicides; therefore, there was a matrix effect and the standard addition method is most suitable for the quantification of simazine and atrazine.

Table 3 Estimation of matrix effects

Analyte	External calibration		Standard addition		R	U_R	t_{exp}
	Slope	u_s	Slope	u_s			
Simazine	13,062.1	632.4	7362.4	244.3	1.77	0.059	13.19
Atrazine	11,852.5	611.4	9065.8	315.5	1.31	0.062	4.94

where u_s is uncertainty, R is the ratio of the slopes (external calibration and standard addition), u_R is uncertainty of the ratio of the slopes

CS3 presented good linear adjustment; its reproducibility and repeatability were greater than 5%; consequently, its use would limit the method precision.

Although, DLLME-MDMSPE methods have not been previously reported for the analysis of triazines, results in the present work show that coupled microextraction techniques, with the use of magnetic materials as adsorbents, are viable alternatives for the pre-concentration of triazines in complex samples, for their subsequent analysis with chromatographic techniques, which are characterized as easy-to-access and low-cost methodologies (HPLC or GC).

Sample analysis

Physicochemical analysis

Physicochemical parameters were measured to know the nature and complexity of the analyzed wastewater samples, as well as to establish analysis prior stages (buffer pH, filtration, sedimentation, among others). The results are shown in Table 4.

The pH values were in a range between 7.9 and 9.1, which indicated a certain degree of alkalinity. Because the desired pH was 5, all samples were acidified. The results shown in situ for EC from the different sampling points were high (920–2245 $\mu\text{S cm}^{-1}$) demonstrating high concentrations in ionic species and that they are related to salinity.

Because the values of turbidity, COD and TSS, were high, it was decided to work with the samples previously sedimented and filtered. The steps of sedimentation and filtration did not affect the distribution of the analytes, because they were in solution, stabilized by the soluble organic matter, which was estimated in the parameters of COD and TSS. Salazar-Ledesma et al. [4] studied the mobility of atrazine in

Table 4 Physicochemical parameters determinate for the wastewater samples collected into DDR03

Sample parameter	pH	Conductivity ($\mu\text{S cm}^{-1}$)	Temperature ($^{\circ}\text{C}$)	Turbidity NTU	COD (mg L^{-1})	TSS (mg L^{-1})
Mean	8.36	1679	20.62	243	210	171
Mínimum	7.87	920	18.80	13	144	34
Maximum	9.13	2245	22.00	534	389	287

$n = 18$. COD chemical oxygen demand, TSS is total soluble solids

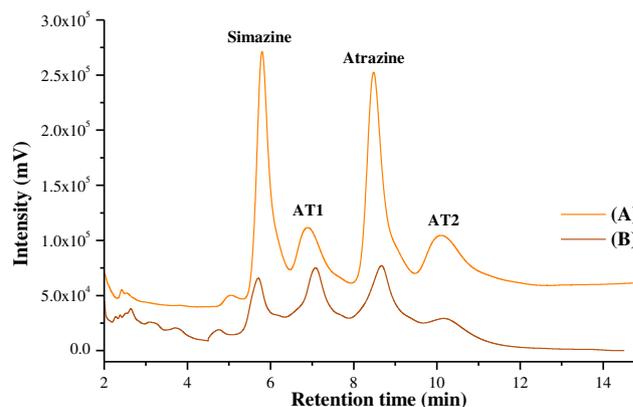


Fig. 6 Chromatograms of triazine analysis under best conditions. (A) Standard solution ($10 \mu\text{g L}^{-1}$ for each herbicide (simazine and atrazine)). (B) Wastewater sample, which was treated by the coupled pre-concentration method (DLLME-MDMSPE), simazine and atrazine were found at 5.11 ± 0.016 and $5.95 \pm 0.002 \mu\text{g L}^{-1}$, respectively. AT1 and AT2 were labeled as possible triazine derivatives

soils irrigated with wastewater, which depends on the organic matter concentration in water and soils, concluding that atrazine is mostly in organic fractions.

Analysis of simazine and atrazine

Real samples were collected from DDR03, having 18 wastewater samples. DLLME was performed at pH 5 and MDMSPE was carried out with 50 mg of CS2 adsorbent. The calibration parameters are shown in Table 2.

Figure 6 shows chromatograms for the standard solution (Fig. 6A) and wastewater sample (Fig. 6B), which were pre-concentrated by microextraction coupled techniques (DLLME-MDMSPE) and analyzed by the same HPLC method. Retention time of simazine was 5.8 ± 0.1 min, while atrazine showed a retention time of 8.5 ± 0.2 min. Other species of triazine nature are labeled AT1 and AT2; these could be associated with degradation metabolites or other triazine compounds widely used in the irrigation district.

Table 5 shows the concentrations of simazine and atrazine found in the analyzed wastewater samples. Simazine concentrations ranged from 0.31 to $8.24 \mu\text{g L}^{-1}$, while atrazine ranged between 0.07 and $12.83 \mu\text{g L}^{-1}$; these concentrations are considered high compared to those allowed in other countries like Japan, where the maximum permissible limit is of

Table 5 Triazinic herbicide concentration in wastewater samples of DDR03 (%RSD, $n = 3$)

Sample	Simazine $\mu\text{g L}^{-1}$ (%RSD)	Atrazine $\mu\text{g L}^{-1}$ (%RSD)
1	7.01 (0.45)	11.60 (0.67)
2	5.11 (0.31)	5.95 (0.04)
3	0.58 (1.65)	9.71 (0.80)
4	1.34 (0.72)	8.67 (0.09)
5	0.89 (0.01)	3.90 (0.20)
6	3.51 (2.63)	12.83 (0.62)
7	7.34 (2.06)	11.85 (0.98)
8	7.02 (2.81)	12.47 (0.34)
9	8.24 (0.05)	10.48 (0.07)
10	< LOD	4.78 (2.30)
11	< LOD	0.07 (0.05)
12	< LOD	2.96 (2.30)
13	< LOD	3.45 (0.05)
14	0.31 (0.05)	< LOD
15	< LOD	< LOD
16	< LOD	< LOD
17	< LOD	9.24 (0.94)
18	< LOD	11.08 (0.09)

0.003 $\mu\text{g L}^{-1}$ [19] and the European Union with a limit concentration of 0.05 $\mu\text{g L}^{-1}$ [20].

Besides, the SPE-HPLC method was used for comparing the proposed method, in which the concentrations ranged (mean and %RSD, $n = 5$) were 0.32 $\mu\text{g L}^{-1}$ (2.0) to 8.35 $\mu\text{g L}^{-1}$ (3.0) for simazine and 0.06 $\mu\text{g L}^{-1}$ (1.5) to 11.85 $\mu\text{g L}^{-1}$ (2.3) for atrazine. The average of each analyte (determined by both methods) was compared by a *t* test for comparison of means, assuming comparable variances (verified by a *F* test). *t* experimental values were compared with *t* theoretical value for 8 degrees of freedom and a significance level of 0.05 ($t = 2.30$). Thus, the null hypothesis was accepted, meaning that there were no significant differences between the results provided by both methods.

Conclusions

The solvothermal synthesis and the coating of magnetic supports (microwave-assisted processes) allowed obtaining nanoparticles with a high surface area and a quasi-spherical core-shell type morphology. The best recovery percentages for atrazine and simazine were obtained with $\text{Fe}_3\text{O}_4@\text{TEPS}$ support. The selectivity of the proposed method and the preconcentration of the wastewater samples were achieved by the coupling of two microextraction techniques (DLLME-MDMSPE), for further analysis by HPLC-DAD. The coupling of microextraction techniques allows obtaining higher enrichment factors compared to separated techniques.

The described methods have been adjusted to evaluate the remanence of triazine herbicides in aquatic bodies, as well as their mobility; however, the proposed method makes it a routine alternative for the quantification in environmental samples, which are exposed at least twice a year to high doses of triazines, which would allow the evaluation of the risks that these pollutants represent for the aquatic environments. The content of simazine and atrazine in the samples analyzed represented a high risk for human health, since they were found above the maximum limits allowed by international regulations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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