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Optimization and Validation of the Preconcentration Technique with SBSE Coupled HPLC-UV/DAD for the Identification of Atrazine and Two of its Metabolites, 2-Hydroxyatrazine (2-HA) and Desethylatrazine (DEA) in Aqueous Samples

L. A. García-Villanueva[1†], J. R. Martínez-Castro[1], G. Fernandez-Villagomez[1], G. L. Andraca-Ayala[2], J. M. Yáñez-Campuzano[3] and O. Zamora Martínez[4]

[1]Faculty of Engineering, Geomatic and Civil Engineering Division, Sanitary and Environmental Engineering Department, National Autonomous University of Mexico. Av. Universidad 3000, Ciudad Universitaria, Coyoacán, Mexico City, Mexico

[2]Institute of Atmospheric Sciences and Climate Change, National Autonomous University of Mexico. Av. Universidad 3000, Ciudad Universitaria, Coyoacán, Mexico City, Mexico

[3]Faculty of Chemistry, National Autonomous University of Mexico. Av. Universidad 3000, Ciudad Universitaria, Coyoacán, Mexico City, Mexico

[4]Institute of Geology, Department of Environmental Sciences and Soil, National Autonomous University of Mexico. Av. Universidad 3000, Ciudad Universitaria, Coyoacán, Mexico City, Mexico

†Corresponding author: L. A. García-Villanueva; lagvillanueva@unam.mx

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ABSTRACT

The purpose of this work is to address an environmental problem in Mexico, which uses significant amounts of water for agricultural activities, where atrazine is frequently used as a pesticide for weed control. Currently, there is no law prohibiting its use, even though it is considered an endocrine disruptor in some mammals and harmful to health. Due to the difficulty in the direct quantification of several herbicides, which present a low concentration in water, the present work aims to develop the optimization and validation of the preconcentration with magnetic stir bars (SBSE) in aqueous samples for the quantification of atrazine and two of its metabolites: 2-hydroxyatrazine (2-HA) and desethylatrazine (DEA), coupled to High-Performance Liquid Chromatography (HPLC-UV/DAD). For the optimization of the preconcentration technique, the nature and quantity of the solvents used in each step, contact time for retention and quantitative extraction of the analyte, as well as the effect of the concentration of the analyte on its retention on the bar were considered. Finally, it was determined that the presence of the metabolites 2-HA and DEA does not affect the sorption of atrazine on the sorption bar used. The analytical methodology can be considered as an efficient method of atrazine preconcentration for subsequent quantification via HPLC-UV/DAD in the range of 0.03 to 0.25 mg/L and in the absence of matrix interferences; its limits of detection and quantification are respectively 0.0014 mg/L and 0.0016 mg/L.

INTRODUCTION

Agriculture is the science of cultivating the land to obtain raw materials. The main reasons that have led to agricultural production have been to solve the problem of world hunger through the extensive use of agricultural practices (Carmona 2004).

Mexico uses 76% of its renewable water for agriculture, which mainly produces sugarcane, corn and sorghum. During its cultivation, atrazine is used as a pesticide for weed control. Studies worldwide consider atrazine as an endocrine disruptor, even low doses of pesticides can alter the biochemical profile, resulting in oxidative stress within the population's brains causing hormonal imbalance, and is classified as a carcinogenic substance, it also poses various risks to the aquatic environment and its propagation in it, so it has been banned in some countries (CICOPLAFEST 2005, IARC 2014, Sharma et al. 2023).

There are studies where residual pesticides are detected in soil, water, food, biological fluids, and tissue samples. In food, different pesticides have been found in cereal grains, cacti, shrimp, vegetable oils, tomatoes, and grapes, among others (Ahmed et al. 2009, Aldana et al. 2008, Alsayeda et al. 2008). Hence the interest in studying the persistence of pesticides in the environment.

Atrazine is one of the most widely used pesticides in the world and Mexico is the second country where it is used in the greatest quantity, and where there is no law prohibiting or regulating its use. It should be noted that some Mexican standards provide maximum permissible limits in a preventive manner, mainly those referring to drinking water, wastewater discharges, and food (Bello 2016, FAO 2016, González-Márquez & Hansen 2009).

In the practice of chemical analysis, there are countless reasons to perform tests, measurements, and examinations in laboratories worldwide, for example, to control the quality of drinking and irrigation water, food, medical analysis, high value-added products among many others, which require high reliability since important decisions depend on the results that these measurements produce (Eurolab 2016). That is why it is considered necessary that the methods of analysis, as well as the analytical results, are subjected to some acceptance criterion that allows them to document their quality and verify that the experimental procedures meet the quality criteria requested by the customer and are suitable for the specific use for which they were developed. This procedure is known as method validation and is applied by countless laboratories around the world (CDER 1994).

MATERIALS AND METHODS

Equipment

- Liquid chromatographic system consisting of two model 1525 high-pressure binary pumps, a model 717 plus autosampler, and a model 2998 UV-DAD diode array detector, all from Waters, controlled by Empower 2 data acquisition software (Build 2154), also from Waters Co.
- Analytical balance with a capacity of 210.0 g and a precision of 1.0 mg, Ohaus, model Explorer.
- Ministart® syringe filters, 25 mm diameter, and 0.45 pm, Nylon (PA), 4.8 cm^2, 0.15 mL, Sartorius.
- Four-position magnetic stirring grill, without heating, model MS-01 from ELMI Ltd.
- Magnetic stirring grid model Speedsafe™ from HANNA Instruments.
- Milli-Q® plus water purifier and deionizer, model 185 from Merck-Millipore.

Materials

- Glass Twister® magnetic stirring rods with dimensions of 10 mm length x 3.2 mm diameter and a thickness of 0.5 mm of GERSTEL polydimethylsiloxane (PDMS) non-polar film coating.
- C18 Spherisorb ODS2 column, 80 A, (4.0 mm x 250 mm, i.d.), 5 pm particle size, from Waters Co.
- Cellulose nitrate membrane filters, 47 mm diameter, 0.45 pm pore size, Sartorius brand.
- Chromatographic amber glass vials, certified, Waters brand, dimensions 8 × 40 mm, 1 mL volume.
- Headspace® amber glass vials, round bottom, spiral cap, 20 mL.

Reagents and solvents

- Acetonitrile, HPLC grade (> 99.93%), Honeywell.
- Atrazine, analytical standard (99%), 100 mg ampoule, Sigma-Aldrich.
- Atrazine-2-hydroxy, analytical standard (99%), PESTANAL ®, Sigma-Aldrich.
- Atrazine-desethyl, analytical standard (99%), PESTANAL ®, Sigma-Aldrich.
- Ultra pure water, resistivity 18.2 Mfl-cm, at 25°C.
- Phosphate buffer pH = 7.2, grade R.A. (98.9%), Sigma-Aldrich. (98.9%), Sigma-Aldrich.
- Methanol, HPLC grade (> 99.9%), Honeywell.

Preparation of Solutions

Standard solutions and standards: To carry out the development of the preconcentration methodology, all solutions were prepared with chromatographic grade solvents, analytical grade reagents, and deionized water. For the analyte of interest, as well as for its potentially interfering metabolites, standard (stock) solutions of ~500 mg/L in methanol were prepared and each was transferred to an amber glass bottle to avoid photochemical degradation and stored under refrigeration. All the standards used for the development of the method were prepared by diluting the different standard solutions with deionized water.

Chromatographic analysis: All working samples were analyzed by HPLC-UV/DAD as reported by Cortes (2016). A Waters® C18 Spherisorb ODS2, 80 A, (4.0 mm x 250 mm, i.d.), 5 pm particle size column was used as stationary phase. A mixture containing acetonitrile (AcCN) and phosphate buffer of concentration equivalent to 0.005 M pH = 7.2, in a 40:60 (v/v) ratio, maintaining a flow rate of 1 mL/min in isocratic mode, was used as mobile phase. Analyte monitoring was performed at 220 nm, using an injection volume of 10 pL.

Methodology validation: Once the previously optimized working conditions were selected, we proceeded to obtain the typical parameters of an analytical validation, which are:

linear and working range, precision, limits of detection and quantification, as well as the evaluation of the effect of the study matrix.

Linear and working range: To select the working range, dilutions of atrazine concentration standards ranging from 0.005 to 5.0 mg/L were prepared from a stock solution of ~ 500 mg/L by diluting it in deionized water. Linearity was observed starting at a concentration of 0.03 mg/L and ending at 0.25 mg/L. Each calibration curve was prepared in triplicate and at least seven concentration levels were required for proper evaluation.

Precision and accuracy: Seven concentration levels (0.03 and 0.25 mg/L) were selected for the evaluation of these parameters. Each concentration level was prepared independently in triplicate. Data analysis was performed according to Miller & Miller (2002) by calculating the percentage coefficient of variability (% CV):

$$\% CV = \left| \frac{s}{\bar{x}} * 100 \right| \quad \ldots(1)$$

where $\bar{x}$ is the mean or average of the measurements and is the standard deviation of the measurements.

The standard deviation, in turn, is calculated by the following equation:

$$s = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2} \quad \ldots(2)$$

Where x_i is the value of the i-th measurement and n is the number of measurements taken.

Limits of detection (LOD) and quantification (LOQ): For this stage, the quantification of seven dilutions of a very low concentration of the analyte (0.005 mg/L) was performed. With the values determined, in absorbance units, the mean (x) and its standard deviation (s) were calculated.

The detection limit was reported according to the equation:

$$LOD = \bar{x} + 3s \quad \ldots(3)$$

The limit of quantification is reported as:

$$LOQ = \bar{x} + 10s \quad \ldots(4)$$

Where $\bar{x}$ is the average of the measurements and s is the standard deviation of the measurements.

Both the limit of detection and the limit of quantification were reported according to Miller & Miller (2002). Once calculated (Eqs. 3 and 4), with the regression equation of the calibration curve, they were transformed into concentration units.

Study matrix: The evaluation of the matrix effect was carried out with five surface water samples from the Montebello Lagoons, Chiapas, which were sampled in August 2014. These samples came from the lagoons: Balamtetic, Bosque Azul, San Lorenzo, Vuelta el Agua and Yalmus.

For each sample, two liters of surface water were taken at a depth of one meter. The samples were subjected to experimental measurements in the field, such as pH and electrical conductivity with a multiparameter meter, model 9812 from HANNA Instruments. The samples were transported in a cooler and then kept under refrigeration at 4 °C, stored in polypropylene jars with lids. Before working with them, the samples were treated using a filtration system, using nitrocellulose membranes of 0.45 pm pore size.

For the evaluation of possible interferents, the standard additions method was applied to these water samples.

Fortification of samples: The five water samples from the Montebello Lagoons were fortified in duplicate with atrazine at five concentration levels between 0.03 and 0.30 pg/mL. The fortifications were carried out starting from the previously prepared ATZ standards and diluting the five samples with water for each corresponding level.

Recovery or Recoveries: This parameter was determined by direct analysis of five water samples from the Montebello Lagoons, which were subsequently fortified with atrazine at five concentration levels, ranging from 0.03 to 0.25 mg/mL. The percentage of recovery (% R) is determined by the following equation:

$$\% R = \left[\frac{C_F - C_U}{C_A}\right] \cdot 100 \quad \ldots(5)$$

Where C_F is the concentration of analyte measured in the fortified sample, C_U is the concentration of analyte measured in the unfortified sample, C_A is the concentration of analyte added to the fortified sample.

With the area values determined during the analysis and the regression parameters calculated from the respective external calibration curves, the concentrations corresponding to the fortification processes were determined. The fortification processes were carried out in duplicate.

RESULTS AND DISCUSSION

Optimization of the SBSE stages

Conditioning: Since the sorption rods used for SBSE are coated with a polydimethylsiloxane (PDMS) film, which acts as a non-polar adsorbent, it was decided to use a volume of 5.0 mL of methanol, which was sufficient to cover the entire working rod. The selection of the appropriate solvent was made based on the elution power and the affinity of the solvent for atrazine to ensure efficient extraction. In addition, the use of methanol is recommended by the manufacturer, since it guarantees that the film covering the rod will have exposed the sites that interact with the molecules of the analyte to be retained, and therefore, carry out satisfactorily its sorption.

The agitation speed was selected based on the recommendations provided by the manufacturer (GERSTEL 2014, Baltussen et al. 2015), in addition to that mentioned by the developers of the SBSE methodology (Baltussen et al. 1999) and in works by other authors (Leon et al. 2003, Neng et al. 2007, Popp et al. 2001, Prieto et al. 2010). These speeds range from 500 to 1200 rpm, so it was decided to work at an average value of 750 rpm (both for the loading and desorption stages), to avoid degradation of the working rod, and given the specifications and limitations of the equipment used for this purpose.

For the contact time, it was decided to use 15 minutes and although, according to the manufacturer's description, the activation of the film takes a few minutes, this time was selected to guarantee the complete disposition of the sorption sites and, therefore, the highest retention of atrazine once the bar was in contact with the solution containing the working analyte. Finally, the sorption rods were washed with deionized water.

Evaluation of atrazine sorption

Affinity of atrazine for sorption bar: It was necessary to know if the atrazine could be retained on the working sorption bar, so in this stage, we first worked with an aqueous solution of atrazine of very low concentration, which could be quantified by HPLC, we chose to use a volume of 5.0 mL of a solution containing approximately 0.5 mg/L of atrazine. This experiment was performed in quintuplicate, and both the atrazine solution without having been in contact with the bar, identified as initial [ATZ], and that resulting from contact with the bar, indicated as [ATZ] after sorption, were analyzed using HPLC-UV/DAD, to determine their numerical values using an external calibration curve. Table 1 summarizes the conditions and values determined.

The results indicate that a certain amount of the atrazine molecules present in the initial solution were retained on the polymer film of the sorption rod, since the concentration values before and after the experiment were not equal, the concentration of atrazine determined in the solution that was in contact with the PDMS rod being lower. The ratio of the concentration after sorption to the initial concentration of atrazine was 0.73, which is equivalent to 73% of atrazine that was not retained, which, in turn, indicates that the remaining 27% corresponds to the amount of atrazine retained on the bar under these working conditions.

Evaluation of the presence of 2-hydroxyatrazine and desethylatrazine on atrazine retention: Because once atrazine (ATZ) is applied in the crop fields, it can undergo both chemical and microbiological degradation, being its main decomposition metabolites 2-hydroxyatrazine (2-HA) and desethylatrazine (DEA), it was decided to evaluate the presence of known concentrations of these two compounds, on the retention of ATZ on the sorption bar. As in the previous case, the concentration values of the three analytes were determined with the use of external calibration curves. The working conditions and numerical results are summarized in Table 2.

The results obtained indicate that the presence of 2-HA and DEA did not affect the sorption of ATZ when the sorption experiment was carried out, since the same amount of atrazine molecules were retained in the solution after sorption as those determined in the sorption experiments carried out in the absence of the two metabolites, again obtaining a percentage of atrazine retained of 27%, with a non-retained amount of 73%. Under these working conditions, the retention of 2- HA and DEA on the rod was not observed, since their concentrations were the same before and after sorption, so the ratio of concentrations of both species was practically unity. The non-retention of DEA and 2-HA can be attributed to the fact that these substances have a more polar character than atrazine, so they exhibit a greater affinity for the water used as a solvent, thus inhibiting their sorption on the working rod.

Effect of contact time on atrazine retention on the surface

Table 1: Atrazine concentration values determined, before and after sorption for a contact time of 60 min (n=5).

Contact time (min)	Volume dissolution of ATZ (mL)	$[ATZ]_{initial}$ (mg/L)	$[ATZ]_{then\ sorption}$ (mg/L)	$\frac{[ATZ]_{then\ sorption}}{[ATZ]_{initial}}$
60	5.0	0.56	0.41	0.73

Table 2: Evaluation of ATZ sorption on the presence of its two main metabolites (n=5).

Analyte	Contact time (min)	Volume dissolution of ATZ (mL)	$[ATZ]_{initial}$ (mg/L)	$[ATZ]_{then\ sorption}$ (mg/L)	$\frac{[ATZ]_{then\ sorption}}{[ATZ]_{initial}}$
2-HA	60	5.0	0.44	0.45	1.02
DEA	60	5.0	0.45	0.45	1.00
ATZ	60	5.0	0.48	0.35	0.73

Table 3: Effect of contact time on ATZ sorption. The initial concentration of atrazine is 0.46 mg/L, which is equivalent to 2.28 pg ATZ (n=5).

Volume dissolution (mL)	Time of ATZ contact (min)	$[ATZ]_{after\ sorption}$ (mg/L)	Mass in solution after sorption (pg)	ATZ mass retained (pg)
5.0	15	0.44	2.19	0.09
5.0	30	0.43	2.15	0.13
5.0	60	0.40	2.01	0.27
5.0	120	0.41	2.03	0.25

of the sorption rod: Since the contact time drastically influences the retention of analytes in sorption experiments, it was mandatory to determine the minimum contact time necessary to reach equilibrium on the atrazine retention process. For this purpose, a volume of 5.0 mL of atrazine solution of a concentration of approximately 0.5 mg/L was used, varying the working contact times. Table 3 shows the results obtained for the concentration of atrazine remaining in solution after the sorption experiment was carried out for each of the contact times, as well as the respective amount of atrazine retained on the polydimethylsiloxane rod.

Table 3 shows that, as the contact time increases, the amount of ATZ in the solution decreases, which indicates that a greater amount of this compound has been deposited on the surface of the sorption bar (more efficient sorption), it is possible to verify that after 60 minutes, the sorption remains constant, i.e. reaches equilibrium, so this contact time was selected for the following experiments.

Effect of contact time for quantitative desorption of atrazine: Considering the nature of atrazine, a solvent for which this compound presented a good affinity was sought. Initially, methanol was selected since atrazine is highly soluble in this solvent, in addition to the fact that this solvent acts as a strong eluent when working with reverse-type phases, such as the one used as the coating of the working sorption bar. For this stage, 1.0 mL of methanol was used as the extractant solvent and because desorption depends on the contact time, the minimum time necessary was sought to achieve the greatest extraction of the ATZ retained on the bar. The results obtained are presented in Table 4. As can be observed, in the contact times evaluated, there was practically no greater amount of ATZ in the solution as the contact time between the methanol and the stir bar increased, so, to increase the productivity of the methodology, it was decided to use the shortest contact time of the study (10 minutes) to carry out the desorption of the analyte.

Influence of the amount of extraction solvent: To verify if the volume of the extractant, in this case, methanol, influences the desorption process of atrazine, it was decided to evaluate five different volumes of this solvent, with n=3; the results obtained are presented in Table 5.

Although the concentration of desorbed atrazine decreases as the volume of methanol used for desorption increases, the parameter that directly indicates the effect of this variable on ATZ extraction is not the concentration determined, but the amount of atrazine in solution after desorption, since this involves the dilution effect that the analyte undergoes when exposed to different volumes of extractant.

The results indicate that the amount of atrazine extracted is practically the same for all methanol study volumes, so it was decided to perform the ATZ extraction from the bar with the smallest volume of MeOH (1.0 mL), which guarantees the least dilution effect on the concentration of atrazine once it is in solution, for its adequate quantification via HPLC-UV/DAD. The resulting preconcentration factor (5.0 mL loading/1.0 mL elution) is 5, indicating that the methodology allows the sample to be concentrated five times.

Effect of analyte concentration present on its retention on the sorption bar: Because the concentration in a sample is usually totally uncertain, it was necessary to evaluate the retention behavior of atrazine as a function of its initial concentration in solution. The concentrations used were selected based on the limit of quantification of the HPLC-UV/

Table 4: Effect of extractant (MeOH) contact time on ATZ desorption (n=3).

Volume MeOH (mL)	Contact time (min)	Mass of ATZ in solution after desorption (pg)
1.0	15	0.23
1.0	30	0.25
1.0	60	0.25
1.0	120	0.25

Table 5: Effect of MeOH volume on ATZ desorption (n = 3).

Volume (mL)	MeOH Contact time (min)	Concentration (pg/mL)	Of desorption ATZ mass in dissolution after desorption (gg)
0.5	10	0.66	0.33
0.8	10	0.34	0.28
1.0	10	0.33	0.33
1.5	10	0.18	0.27
2.0	10	0.13	0.25

Table 6: Effect of analyte concentration on its retention on the sorption bar (n = 3).

ATZ load concentration (gg/mL)	Adsorbed mass			%CV
	1	2	3	
0.03	0.028	0.025	0.022	12.54
0.05	0.035	0.040	0.035	7.80
0.08	0.054	0.054	0.059	4.90
0.10	0.068	0.066	0.068	1.70
0.15	0.098	0.094	0.101	3.65
0.20	0.125	0.132	0.128	2.82
0.25	0.154	0.154	0.157	1.00

DAD methodology (lowest initial concentration that could be quantified) and the highest initial ATZ concentration that was fully retained on the sorption bar. Table 6 summarizes the results of this experiment.

Each experiment was performed in triplicate, so Table 6 also shows the percentage values of the coefficient of variation (% CV), which indicate the degree of repeatability for each of the working concentration levels. As can be seen, in all cases acceptable CV values are presented, following the criteria for the validation of physicochemical methods (< 20%), established in document CCAyAC-P-058 (COFEPRIS 2011). Thus, the accuracy of the developed methodology is evaluated in terms of repeatability.

The graph presented in Fig. 1 shows the correlation between the initial concentration of atrazine present in the solution and the amount of analyte that was retained on the sorption rod used. This graph exhibits a clear linear dependence of the amount of atrazine retained by the bar as a function of the initial concentration of the dissolved analyte ($R^2 = 0.9968$, r = 0.9984), which is confirmed by the study of the nonparametric test of streaks, whose results indicate that the residuals of the data pairs were random and therefore these fit a straight line.

The knowledge of this behavior is of vital importance in the use of the sorption bar for the preparation of real samples, since the sorption of atrazine is conditioned by the concentration present in the solution and it is necessary to know the values of the parameters slope and ordinate to the origin, to determine the concentration of atrazine in the original sample.

Limits of detection and quantification: The determination of the detection and quantification limit values was carried out by measuring seven atrazine solutions of known concentration (0.005 mg/L) independently. Likewise, this concentration was selected based on the minimum differentiable signal/noise ratio of the chromatographic analysis system. Table 7 shows the values of the limit of detection (LOD) and limit of quantification (LOQ), which were calculated according to Miller & Miller (2002) and, employing the regression parameters of the external calibration curve, were obtained in units of concentration.

Finally, these parameters were significantly reduced compared to the direct analysis method, where values of LOD = 0.050 mg/L and LOQ = 0.070 mg/L are reported and whose methodology does not employ a sample preparation technique.

Evaluation of the effect of the study matrix: To evaluate the presence of interferents from the study matrix, five water samples from the Montebello Lagoons were fortified in

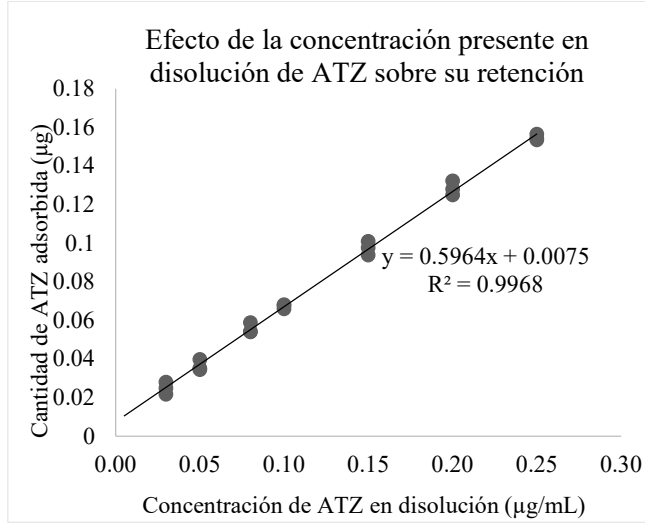

Fig. 1: Dependence of the amount of ATZ adsorbed as a function of the initial concentration present in solution (n=3).

Table 7: Detection and quantification limits of the developed methodology.

Parameter	Value (mg/L)
LOD	0.0014
LOQ	0.0016

duplicate with atrazine at five concentration levels ranging from 0.03 to 0.30 pg/mL. In all the chromatograms obtained by directly analyzing the water samples from the Montebello Lagoons, as a blank (background), the null presence of ATZ (whose retention time is 7.8) was observed; this occurred in all cases. Consequently, the slope values obtained for the five lake samples were as expected, being very similar to each other, compared to that observed for the deionized water. Since the slope values are practically the same (m ~ 0.6), it is clear that there are no interferences affecting the quantification of atrazine. For the ordinate to the origin (b), for all cases values very close to zero were obtained, while the correlation coefficients indicate the existence of a linear behavior of the adsorbed amount of atrazine as a function of its initial concentration in solution.

On the other hand, to confirm the similarity between the values of the slopes of the study samples and the value obtained for the calibration curve with deionized water, five hypothesis tests were carried out, where the value of the slope of the respective sample was compared with that of the external calibration curve, using Student's t distribution statistic, at 95% reliability (a = 0.05).

Alternatively, the matrix effect was evaluated qualitatively by graphical analysis of the amount of analyte adsorbed for each lake sample against the amount adsorbed in deionized water, as described by Thompson & Ellison (2004); for all cases, the slope obtained was practically equal to unity.

Recoveries: When observing the values of the recovery percentages for each concentration level, both for the deionized water matrix (Table 8) and for each of the fortified samples (Table 9), it can be concluded that these meet the acceptance criteria for the recovery parameter established in the manual of the Ministry of Health (COFEPRIS 2011).

Table 8: Recovery percentages as a function of analyte concentration for the water matrix deionized.

ATZ load concentration (pg/mL)	Recoveries (%) Deionized water	Acceptance criteria (%)	Result
0.03	90.00	70 - 120	Complies
0.05	102.27	70 - 120	Complies
0.08	94.39	70 - 120	Complies
0.10	105.14	70 - 120	Complies
0.15	116.82	70 - 120	Complies
0.20	115.58	70 - 120	Complies
0.25	108.25	70 - 120	Complies

Additionally, the recovery percentages can be evaluated by comparing the slopes of the external calibration curves of each of the fortified samples from five water samples from the Montebello Lagoon System with that of the deionized water matrix (Table 10).

When analyzing the values of these slopes, it can be determined that they are practically the same; therefore, there is no significant difference between the working matrices.

Finally, it can be affirmed, based on the above, that the instrumental method developed in the present methodology, once validated, is robust for the matrix.

Reproducibility: The retention times for the analyte atrazine, obtained in the present methodology, are very similar to those obtained by the direct analysis technique developed by Cortes (2016), i.e.: t_R ATZ ≈ 7.8; that is why it can be stated that the instrumental method of HPLC-UV/DAD analysis is reproducible for that substance.

CONCLUSIONS

For the preconcentration of atrazine in aqueous samples, a methodology was developed based on stir bar sorption extraction (SBSE), the following optimal conditions were found: Extraction volume (loading): 5.0 mL, Stirring speed: 750 rpm, for all cases (loading and elution), Contact time: 60 minutes, Desorption solvent or eluent: methanol

Table 9: Recovery percentages as a function of analyte concentration for each of the analyte concentrations for each of the five samples from the Montebello Lagoon Park.

Concentration Charge ATZ (pg/mL)	Recoveries (%)					Criteria Acceptance (%)	Result
	Lake 1	Lake 2	Lake 3	Lake 4	Lake 5		
0.03	76.66	102.74	88.78	93.11	107.82	70 - 120	Complies
0.05	73.66	87.91	119.87	119.16	95.19	70 - 120	Complies
0.08	119.80	91.23	112.99	88.78	83.35	70 - 120	Complies
0.20	84.86	72.76	120.80	114.80	105.69	70 - 120	Complies
0.25	86.22	84.12	113.88	81.18	118.82	70 - 120	Complies

Table 10: Percentage of recovery evaluated by comparing the slopes of the recovery curves external calibration of each of the fortified samples (from the Montebello Lagoon System) with the one from the deionized water matrix.

Matrix	Slope (m)	Recoveries (%)
Deionized water	0.5964	100.00
Lake 1	0.5905	99.01
Lake 2	0.6034	101.17
Lake 3	0.5958	99.90
Lake 4	0.5733	96.18
Lake 5	0.5826	97.69

(HPLC grade), with this the quantitative desorption of ATZ is achieved, Eluent volume: 1.0 mL, thus guaranteeing the least dilution effect and finally, Desorption or elution time: 10 minutes.

The analytical methodology developed met the parameters of linearity, precision, and accuracy, so it can be considered an efficient method of atrazine preconcentration, for its subsequent quantification in the absence of matrix interferences, by high-performance liquid chromatography, using a photodiode array detector (HPLC-UV/DAD), in the range of 0.03 to 0.25 mg/L.

The limits of detection and quantification for atrazine in this methodology are 0.0014 mg/L and 0.0016 mg/L, respectively. On the other hand, these parameters were significantly reduced compared to the direct analysis method (Cortes 2016), where values of LOD = 0.050 mg/L and LOQ = 0.070 mg/L were reported and whose methodology does not employ a preconcentration or sample preparation technique.

REFERENCES

Ahmed, A.R., Tarek, M.M., Rady, A.R. and Mohamed, Y.H., 2009. Dissipation of profenofos, imidacloprid and penconazole in tomato fruits and products. *Bulletin of Environmental Contamination and Toxicology*, 83, pp.812-817. https://doi.org/10.1007/s00128-009-9852-z.

Aldana, M.L., Valdez, S., Vargas, N.D., Salazar, N.J., Silveira, M.I., Loarca, F.G., Rodriguez, G., Wong, F.J., Borboa, J. and Burgos, A., 2008. Insecticide residues in stored grains in Sonora, Mexico: quantification and toxicity testing. *Bulletin of Environmental Contamination and Toxicology*, 80, pp.93-96. https://doi.org/10.1007/s00128-007-9302-8.

Alsayeda, H., Pascal-Lorber, S., Nallanthigal, C., Debrauwer, L. and Laurent, F., 2008. Transfer of the insecticide [14C] imidacloprid from soil to tomato plants. *Environmental Chemistry Letters*, 6, pp.229-234. https://doi.org/10.1007/s10311-007-0121-2.

Baltussen, E., Sandra, P., David, F. and Cramers, C., 1999. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles. *Journal of Microcolumn Separations*, 11(10), pp.737-747. https://doi.org/10.1002/(SICI)1520-667X(1999)11:10<737::AID-MCS7>3.0.CO;2-4.

Bello, K., 2016. Determinación del plaguicida paraquat en muestras tomadas de las lagunas de Montebello, Chiapas. *Tesis de licenciatura*, Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma México, Ciudad de México, México, 81pp.

Carmona, B., 2004. Las patentes de transgénicos como mecanismo de dominación de los países desarrollados. Análisis de la situación en México y Estados Unidos. *Tesis de licenciatura*, Licenciatura en Relaciones Internacionales, Universidad de las Américas, Puebla, México, 120pp.

CDER, 1994. Reviewer guidance. Validation of Chromatographic Methods. *Center for Drug Evaluation and Research*, US Food and Drug Administration. Manual, United States, 30 pp.

CICOPLAFEST, 2005. Acuerdo que establece la clasificación y codificación de mercancías cuya importación está sujeta a regulación por parte de las dependencias que integran la comisión intersecretarial para el control del proceso y uso de plaguicidas, fertilizantes y sustancias tóxicas. *Comisión Intersecretarial para el Control del Proceso y Uso de Plaguicidas y Sustancias Tóxicas*, Diario Oficial de la Federación, México, DOF: 12/04/2013.

COFEPRIS, 2011. CCAyAC-P-058: Criterios para la validación de métodos fisicoquímicos. *Comisión de Control Analítico y Ampliación de Cobertura*, Comisión Federal para la Protección contra Riesgos Sanitarios, Informe, Secretaria de Salud, México, 133pp.

Cortes, V., 2016. Determinación simultanea de hidroxiatrazina, desetilatrazina y atrazina por medio de CLAR-UV-DAD en muestras de agua de las lagunas de Montebello. *Boletín de la Sociedad Geológica Mexicana*, 70, pp.95-119. https://doi.org/10.18268/BSGM2018v70n1a6.

Eurolab, 2016. Guía Eurachem: La adecuación al uso de los métodos analíticos - Una Guía de laboratorio para la validación de métodos y temas relacionados. Segunda edición, P.P. Morillas y co., España, 66pp.

FAO, 2016. Pesticides as water pollutants. *Food and Agriculture Organization of the United Nations*. Available at: http://www.fao.org/docrep/W2598S/w2598s06.htm.

GERSTEL, 2014. Twister /Stir Bar Sorptive Extraction SBSE. Available from: http://www.gerstel.com/en/twister-stir-bar-sorptive-extraction.htm.

González-Márquez, L. and Hansen, A., 2009. Adsorción y mineralización de atrazina y relación con parámetros de suelos del DR 063 Guasave, Sinaloa. *Revista Mexicana de Ciencias Geológicas*, 26, pp.587-599.

IARC, 2014. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. International report 14/002. *International Agency for Research on Cancer*, 38, pp.1249-1250, Lyon, France. https://doi.org/10.1093/carcin/bgw025.

Leon, V.M., Alvarez, B., Cobollo, M.A., Munoz, S. and Valor, I., 2003. Analysis of 35 priority semivolatile compounds in water by stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry: I. Method optimisation. *Journal of Chromatography A*, 1-2, pp.91-101, Alicante, Spain. https://doi.org/10.1016/j.aca.2005.10.080.

Miller, J.N. and Miller, J.C., 2002. *Estadística y Quimiometría para Química Analítica*, 4th ed. Madrid, Spain, 296pp.

Neng, N.R., Pinto, M.L., Pires, J., Marcos, P.M. and Nogueira, J.M.F., 2007. Development, Optimisation and Application of Polyurethane Foams as New Polymeric Phases for Stir Bar Sorptive Extraction. *Journal of Chromatography A*, 1-2, pp.8-14. https://doi.org/10.1016/j.talanta.2011.12.010.

Popp, P., Bauer, C. and Wennrich, L., 2001. Application of stir bar sorptive extraction in combination with column liquid chromatography for the determination of polycyclic aromatic hydrocarbons in water samples. *Analytica Chimica Acta*, 436(1), pp.1-9. https://doi.org/10.1016/S0003-2670(01)00895-9.

Prieto, A., Basauri, O., Usobiaga, A., Rodil, R., Fernandez, L.A., Etxebarria, N. and Zuloaga, O., 2010. Stir-bar sorptive extraction: A view on method optimisation, novel applications, limitations and potential solutions. *Journal of Chromatography A*, 1217(16), pp.2642-2666. https://doi.org/10.1016/j.chroma.2009.12.051.

Sandra, P., Baltussen, E., David, F. and Hoffman, A., 2015. App. Note

2/2000: Stir Bar Sorptive Extraction (SBSE) applied to Environmental Aqueous Samples. *GERSTEL GmbH and Co. KG*, pp.1-6.

Sharma, A., Gupta, S. and Kaur, M., 2023. Postnatal Exposure to A Low Dose of Imidacloprid: Oxidative Stress in Brain Without Affecting Learning and Behavior in Swiss Albino Mice. *Nature Environment and Pollution Technology*, 22(3). https://doi.org/10.46488/NEPT.2023.v22i03.043.

Thompson, M. and Ellison, S.L., 2004. A review of interference effects and their correction in chemical analysis with special reference to uncertainty. *Accreditation and Quality Assurance*, 3, pp.82-97. https://doi.org/10.1007/s00769-004-0871-5.

ORCID DETAILS OF THE AUTHORS

L. A. García: https://orcid.org/0000-0003-3080-1282
G. Fernández: https://orcid.org/0000-0002-9748-4157
G. L. Andraca: https://orcid.org/0000-0002-7945-7294