chemosensors

Article

Method Validation and Determination of Ametryn Pesticide in Water Samples by QuEChERS-GC-MS

Luis Felipe Lima Guimarães [1], Maria Zillene Franklin da Silva [2], Ronaldo Ferreira do Nascimento [3] and Daniel Barbosa Alcântara [4,*]

[1] Institute of Chemistry, Federal University of Goiás, Goiânia 74001-970, Goiás, Brazil; lima.luis@mail.uft.edu.br
[2] Department of Hydraulic and Environmental Engineering, Federal University of Ceará, Fortaleza 60451-970, Ceará, Brazil; zillene@alu.ufc.br
[3] Department of Analytical Chemistry and Physical Chemistry, Federal University of Ceará, Fortaleza 60455-700, Ceará, Brazil; ronaldo@ufc.br
[4] Integrated Science Center, Federal University of Northern Tocantins, Araguaína 77813-345, Tocantins, Brazil
* Correspondence: daniel.alcantara@ufnt.edu.br

Abstract: This study developed an analytical method to monitor pesticide residues in water, ensuring compliance with maximum limits and protecting human health. While the QuEChERS method is commonly used for food matrices, its application to aqueous samples has been limited. This research aims to extend its use to water matrices by optimizing and validating chromatographic conditions for detecting Ametryn using GC-MS. Calibration curves for both the solvent and matrix extracts demonstrated satisfactory linearity. Significant matrix effects were observed, influencing the signal intensity. Detection and quantification limits were determined, with a higher sensitivity in the matrix. Precision (RSD%) and accuracy (recovery tests) met acceptable standards. Although Ametryn was not detected in the real water samples, 2,4-Di-tert-butylphenol, a possible degradation byproduct of pentachlorophenol, was found. This study advances pesticide detection methods, addressing key factors like selectivity, linearity, and matrix effects, while providing insights into degradation byproduct detection and pesticide contamination in water sources.

Keywords: herbicide; adapted QuEChERS; chromatography; wastewater

check for updates

Received: 13 November 2024
Revised: 17 February 2025
Accepted: 7 March 2025
Published: 13 March 2025

Citation: Guimarães, L.F.L.; da Silva, M.Z.F.; do Nascimento, R.F.; Alcântara, D.B. Method Validation and Determination of Ametryn Pesticide in Water Samples by QuEChERS-GC-MS. *Chemosensors* **2025**, *13*, 103. https://doi.org/10.3390/chemosensors13030103

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Herbicides are chemical compounds containing various active ingredients, such as Glyphosate, Atrazine (ATZ), Paraquat, Diuron, and Ametryn (AMT), among others, which are widely used globally for weed control. However, these compounds and their metabolites exhibit varying degrees of persistence and stability in the environment, along with potential toxicity, carcinogenicity, mutagenicity, and teratogenicity. They may also have adverse effects on the endocrine systems of non-target organisms, including humans [1].

Ametryn (AMT), chemically known as 2-ethylamino-4-isopropylamino-6-methylthio-3-1,3,5-triazine, belongs to the triazine family of chemicals. It functions as a systemic herbicide and is widely used for pre- and post-emergence weed control in various crops such as corn, sugarcane, bananas, coffee, pineapples, cotton, citrus, cassava, and grapes [2,3].

According to the Brazilian National Health Surveillance Agency (ANVISA), AMT is classified as moderately toxic (Class III), posing risks to fish, large mammals, and humans. Due to its toxicity, environmental persistence, and potential health hazards, AMT is considered a chemical pollutant that requires careful monitoring [4].

Over the years, water resources have become increasingly contaminated by chemical substances, posing significant challenges to the provision of potable water. This contamination not only raises costs but also reduces water availability, particularly in highly developed regions where pesticide use is prevalent. Consequently, monitoring programs targeting water resources, soil, and food are essential for minimizing contamination [5,6].

Water bodies play a crucial role in biogeochemical processes, both in surface and groundwater, across all regions. Pesticides typically enter these ecosystems through various transport mechanisms, including volatilization, degradation, leaching, sorption, plant uptake, and runoff [7].

Therefore, for the effective determination and control of pesticides in the environment, it is essential to develop multiresidue methods for different matrices. However, due to the low concentrations of analytes in the matrix, the complexity of different matrices, and their varied chemical properties, a sample preparation step is necessary. The main objective of sample preparation is to facilitate extraction, enrich the analytes of interest, and remove as many interferents as possible. Hence, sample preparation is of paramount importance within the entire analytical process [8].

In this context, the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction method is extensively employed in pesticide analysis. This method involves the following two main steps: solvent extraction and phase partitioning with salts, followed by extract cleaning using dispersive solid-phase extraction (d-SPE) [9]. QuEChERS stands out for its simplicity, speed, and excellent validation parameters, including impressive recovery rates compared to other techniques. It is also highly compatible with both gas and liquid chromatographic techniques [9].

Since the introduction of the original QuEChERS method by Anastassiades [10], modifications have been made to improve its performance, addressing the specific needs of different samples and increasing the recovery in complex matrices, while also reducing the matrix effects [11].

Despite its widespread use in solid matrices for pesticide quantification, some studies have explored its applications in environmental matrices such as surface and groundwater. Notably, Brondi [12] employed the QuEChERS method coupled with GC-MS for the quantification of ATZ, fipronil, and α- and β-endosulfan in water reservoirs intended for cattle supply, achieving precision, accuracy, and linearity values in accordance with regulatory criteria. After method validation, it was applied to water and sediment samples, where none of the analyzed pesticides were detected [12].

Given the above, the objective of the present study was to develop and validate an analytical method for the qualitative and quantitative analysis of the pesticide Ametryn (AMT) in water, using an adapted QuEChERS method for aqueous samples combined with gas chromatography–mass spectrometry (GC-MS). The adapted method modifies the first step of the original QuEChERS procedure by reducing the solvent volume by half and excluding the dispersive solid-phase extraction (d-SPE) step.

2. Materials and Methods

2.1. Chemicals and Reagents

Acetonitrile, 99.9% UV/HPLC grade; hydrochloric acid, 37% P.A./ACS (NEON, 1000 mL); ultrapure water, purified using the Mili-Q UV3 (Millipore, Bedford, MA, United States) system (18.2 MΩ cm); methyl alcohol (MeOH), 99.9% HPLC grade (Merck, São Paulo, Brazil); sodium chloride P.A. (NEON, 500 g); neutral-range detergent (pH 6.5 to 7.5); and anhydrous magnesium sulfate P.A. (Vetec, 500 g) were used.

The analytical standard in powder form of the AMT pesticide was obtained from Merck Brazil, where an amount was diluted in HPLC-grade methyl alcohol to obtain the

stock standard solution of 1000 mg L^{-1}. From the 1000 mg L^{-1} solution was obtained the 10 mg L^{-1} stock solution by dilution with MeOH, in which, from that, were obtained, by dilutions with MeOH, the work solutions of 100 μg L^{-1}, 150 μg L^{-1}, 250 μg L^{-1}, 500 μg L^{-1}, 1000 μg L^{-1}, and 1500 μg L^{-1}. After preparation, the work solutions were stored in penicillin vials, labeled, sealed, and cooled at a temperature of approximately 10 °C until the instrumental analysis.

These solutions were used to obtain the analytical calibration curve (concentration × area) in the solvent by the external standard method. The matrix-matched calibration was also constructed using work solutions (100 μg L^{-1}, 150 μg L^{-1}, 250 μg L^{-1}, 500 μg L^{-1}, 1000 μg L^{-1}, and 1500 μg L^{-1}) obtained through dilutions of the stock solution with the sample extracts.

2.2. Sample Collection

The developed method was applied to analyze three samples collected from the Ribeirão Grotão River, located in the Bielândia district of Filadélfia municipality, in the state of Tocantins, Brazil, with geographical coordinates of 7.539704, −47.795757. These samples encompassed water from the spring (P1), stream (P2), and lake (P3), collected on 13 November 2023, at 9:50 a.m., 10:30 a.m., and 11:10 a.m., respectively.

For the sample collection, amber glass bottles capable of holding up to 500 milliliters (mL) were utilized. Prior to use, all of the bottles underwent decontamination with 10 mL of 37% commercial hydrochloric acid, followed by thorough washing in the laboratory using neutral detergent (pH 6.5 to 7.5), distilled water, and ultrapure water. Subsequently, the sealed bottles were capped with aluminum foil and placed in a Styrofoam box for refrigerated transportation.

2.3. QuEChERS Extraction

Following sample collection, the QuEChERS method modified for water analysis was employed to extract the analytes from the matrix. A 10 mL aliquot of each sample was transferred to a 50 mL Teflon tube fitted with a screw cap. Subsequently, 5 mL of acetonitrile (ACN), being twice the volume in the original method, was added, and the mixture was homogenized for 1 min with the aid of a Vortex Mixer (VM-370, Intllab, Seri Kebangan, Malaysia) to promote the contact between the solvent and the sample. Upon homogenization, 4 g of anhydrous magnesium sulfate ($MgSO_4$) and 1 g of sodium chloride (NaCl) were added, followed by an additional 1 min shaking period.

The inclusion of the cleanup step using d-SPE, present in the original method, is not necessary for water samples due to their simpler matrix composition. In contrast, solid samples contain more matrix interferences, making a cleanup step necessary.

Finally, the sample underwent centrifugation in a centrifuge (MTD-III Plus, Rotation Technology Laboratory, São Paulo, Brazil) at 3000 rpm for 5 min. Subsequently, 1.0 mL of the supernatant phase was carefully transferred to labeled vials, designated for the chromatographic analysis.

2.4. Gas Chromatography–Mass Spectrometry (GC-MS)

The multiresidue method was developed using a gas chromatograph (Agilent Technologies, model 7890B, CA, USA) coupled with a mass spectrometer (Agilent Technologies, model 5977B, CA, USA). An HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm thickness) capillary column with helium (99.99%) was used as the carrier gas and maintained at a constant flow rate of 1.2 mL min^{-1}.

The injections were performed at a temperature of 250 °C, with a volume of 1 μL injected in splitless mode. The oven temperature was programmed as follows: initial temperature set to 50 °C for 0 min, starting at a heating rate of 20 °C min^{-1} until reaching 250 °C, and remaining for 1 min; then, increasing the temperature from 20 °C min^{-1} to

300 °C, and remaining for 1 min, totaling a running time of 14 min and 30 s. The mass spectrometer conditions were configured as follows: electron impact ionization (EI) mode at 70 eV, ion source at 230 °C, and the transfer line temperature set at 250 °C. The MS worked in both Full-Scan (SCAN) and Select Ion Monitoring (SIM) modes, monitoring the m/z range of 40–400 for the SCAN and 227 and 44 for the AMT SIM modes, which corresponded to the quantification and confirmation fragments, respectively.

2.5. Method Validation

The validation methodology used was based on the literature [13], according to the Resolution of the Collegiate Board of Directors–RDC No. 166 [14] and the document DOQ-CGCRE-008 [15]. The evaluated parameters in the present work were the selectivity, linearity, matrix effect, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision (instrument and intra-assay precision) [16,17].

Selectivity was ensured by comparing the mass spectrum of the AMT chromatograms with those in the NIST library. Additionally, for the quantitative analyses, the SIM mode was used to obtain chromatograms in the sample matrix, with ions of 227 m/z and 44 m/z set as the base peak and confirmation peak, respectively.

Limits of detection (LODs) and quantification (LOQ) were determined using the successive dilution method, starting from the lowest to the highest concentrations, until reaching signal-to-noise ratios of 3:1 and 10:1, respectively.

Instrument precision was assessed by performing each injection in the instrument in at least three replicates. Similarly, the intra-assay precision was determined from recovery studies, where each experiment was conducted in five assay replicates. The relative standard deviations (RSD%) between the replicates were then calculated based on the obtained data.

Accuracy was evaluated through recovery tests, in which water samples were spiked with known concentrations of AMT at 100, 500, and 1000 $\mu g \cdot L^{-1}$. Each contaminated sample was prepared in five replicates before undergoing sample preparation using the adapted QuEChERS method. According to the guidelines of the Brazilian National Institute of Metrology, Quality, and Technology (INMETRO) [15], recovery percentages (%) are calculated using the following equation:

$$R(\%) = \left(\frac{C_2 - C_1}{C_3} \right) \times 100 \quad (1)$$

Here, C_1 represents the concentration obtained in the unspiked sample (sample blank), C_2 is the average concentration of the analyte in the spiked sample, and C_3 is the known concentration added to the sample.

For the linearity analysis, calibration curves were prepared using both external standards and matrix-matched calibrations at concentrations of 100, 150, 250, 500, and 1000 $\mu g \cdot L^{-1}$. Each level of concentration on the calibration curve was measured in triplicate, and linearity was assessed using the linear regression method. A good linear fit for the concentration range was adopted according the approximation of the correlation coefficient (r) to 1.00.

Also, statistical tests of significance of the calibration parameters (linear-a and angular-b coefficients), using Student's t-test, and the one to verify the adjustment of the calibration curve through F-tests, were applied using the following equations [18,19]:

$$s^2{}_a = \frac{s^2{}_y \cdot \sum(x^2{}_i)}{D} \quad (2)$$

$$D = \begin{vmatrix} \sum(x^2{}_i) & \sum x_i \\ \sum x_i & n \end{vmatrix} \quad (3)$$

$$t_{calc,\ b} = \frac{|b-1|}{s_b} \quad (4)$$

$$F_{calc} = \frac{s^2{}_{max}}{s^2{}_{min}} \quad (5)$$

$$s^2{}_b = \frac{s^2{}_y \cdot n}{D} \quad (6)$$

$$s^2{}_y = \frac{\sum(d^2{}_i)}{n-2} \quad (7)$$

$$t_{calc,\ a} = \frac{|a-0|}{s_a} \quad (8)$$

where:

s_a = Standard deviation of the linear coefficient;
s_b = Standard deviation of the angular coefficient;
s_y = Standard deviation in the y-axis;
x_i = Individual x values;
n = Total number of points in the curve;
d_i = Vertical deviation of each point;
D = Determinant

s_{max} and s_{min} represent the highest and lowest standard deviation calculated for the replicates at each calibration concentration level, respectively. If the calculated *F*-value is greater than the tabulated *F*-value, as provided in the literature for a 95% confidence level and 3 degrees of freedom (DF = N − 2, where N = 5, representing the number of points on the curve), then the linear regression is considered significant. Conversely, if the *F* tabulated is greater than the *F* calculated, there is no linear relationship between the x and y axes, even if the *r* is near 1.00 [18].

After the curve passes the *F*-test, Student's *t*-test is applied, where it is understood that, if the *t* calculated exceeds the tabulated *t*-value at a 95% confidence level with 3 degrees of freedom, the parameter is significant and should be retained in the equation of the line regression. However, if the tabulated *t*-value is greater than the *t* calculated, the coefficient is deemed insignificant and should be excluded from the regression equation.

2.6. Determination of Matrix Effect

The matrix effect was evaluated by comparing the slopes of the calibration curves obtained in the solvent and the surface water matrix using the following equation:

$$Matrix\ effect\ (\%)\ = \frac{(b_m - b_s)}{b_s} \times 100 \quad (9)$$

where b_m represents the slope of the calibration curve prepared in the presence of the sample matrix and b_s represents the slope of the calibration curve prepared in the solvent. If the value falls outside the range −20% to 20%, it indicates the presence of a matrix effect; therefore, for an accurate quantitative analysis, the calibration curve in the matrix should be used [18].

2.7. Concentration on Real Samples

After the injection of a sample previously prepared by the adapted QuEChERS method and data processing via a calibration curve, concentration results are obtained in μg L^{-1},

which correspond to the concentrations of the extract. Thus, to obtain the estimated analyte concentration in the sample in μg L^{-1}, the following equation was used:

$$\frac{C_a}{\left[\mu g \cdot L^{-1}\right]} = \frac{\left[C_{ext}\left(\mu g \cdot L^{-1}\right) \times V_{ext}\ (L)\right]}{V_a\ (L)} \quad (10)$$

where C_a is the analyte concentration in the sample, C_{ext} is the analyte concentration in the extract (obtained from the calibration curve), V_{ext} is the volume of the extract obtained after sample preparation, and V_a is the volume of sample used in the sample preparation. This equation is used to estimate the concentration values of the analytes in the real samples, as well as the LOD and LOQ values and spiked levels.

3. Results and Discussion

3.1. Chromatographic Analysis by GC-MS

Studies have demonstrated that both GC-MS and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) are widely employed for pesticide monitoring in water, as they offer high sensitivity and selectivity [20,21]. However, when LC-MS/MS is used exclusively, polarity switching in the ionization source is required, which may restrict the number of detectable compounds in a single analysis. In this study, the objective was to utilize GC-MS as the primary analytical technique due to the thermal stability and moderate volatility of the target compound, Ametryn (AMT).

The optimized GC-MS conditions provided effective resolution for AMT, as demonstrated in Figure 1, which shows the chromatographic profile and mass spectrum for a 3 mg L^{-1} standard solution in MeOH. The compound was eluted with a retention time of 10.518 min while operating in SCAN mode.

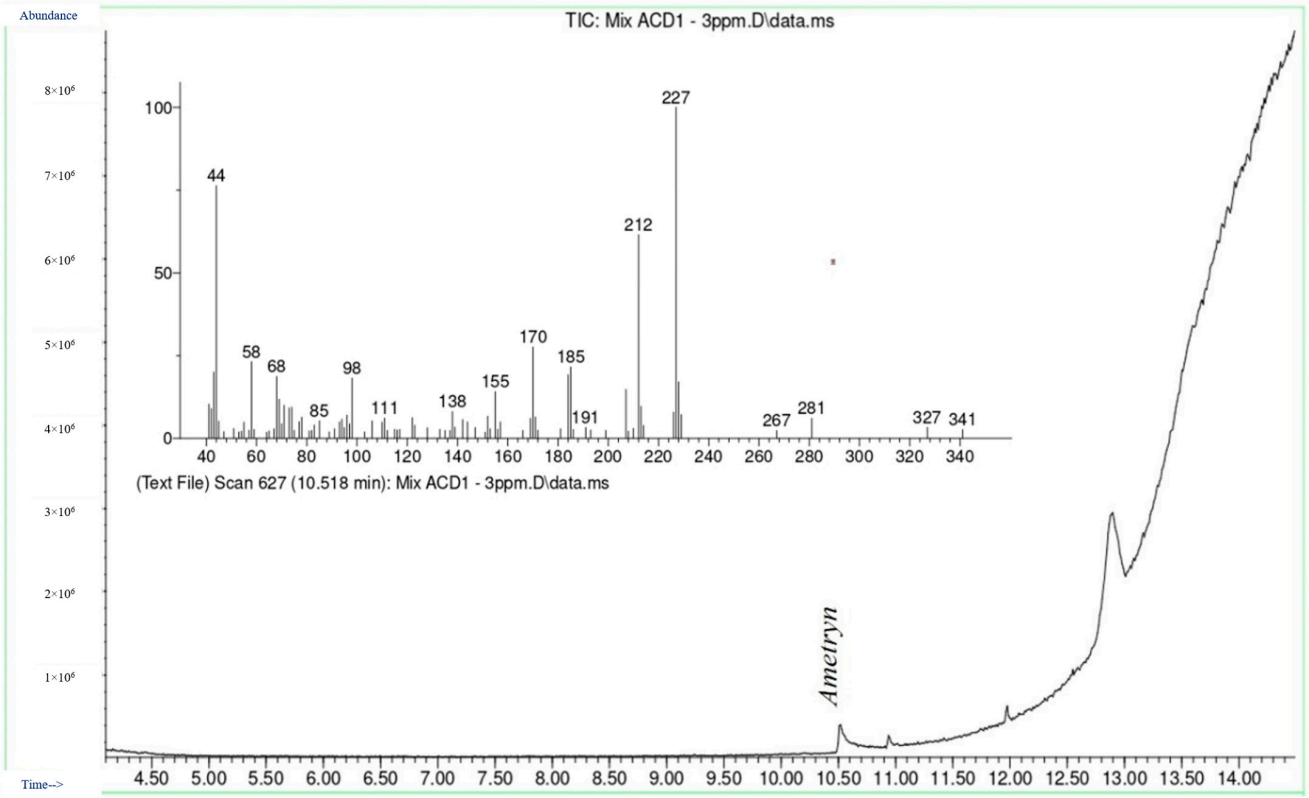


Figure 1. Chromatogram of the Ametryn standard (3 mg L^{-1} in MeOH) obtained by GC-MS in SCAN mode, along with corresponding mass spectrum.

3.2. Selectivity

The chromatographic runs were initially conducted in SCAN mode to identify the most intense m/z fragment for the analyte, followed by quantitative analysis in SIM mode. The SIM mode enhanced both the selectivity and sensitivity of the analytical method, producing simpler chromatograms and chromatographic peaks with improved signal-to-noise ratios by focusing on the specific m/z fragment of interest.

To assess the selectivity, chromatograms of the matrix without AMT (Figure 2a) were compared to those spiked with 1 mg L^{-1} of the target pesticide (Figure 2b). This comparison revealed that, during the herbicide's retention time, the target peak was masked by the 7,6-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione component from the sample matrix that eluted on the same retention time of the analyte (about 10.5 min), showing that the SCAN mode does not provide adequate selectivity, with the matrix components potentially leading to "false-positive" results.

When analyzing in SIM mode using the selected m/z fragments for quantification (m/z = 227) and confirmation (m/z = 44), the chromatogram displayed only the peak corresponding to AMT (Figure 2c), demonstrating the appropriate selectivity for the method. Therefore, this approach was used for monitoring the pesticide in the real samples.

3.3. Linearity

Figure 3 shows the overlay of the calibration curves obtained by external standardization and matrix-matched calibration, both prepared at five concentration levels (100.0, 150.0, 250.0, 500.0, and 1000.0 µg L^{-1}) in triplicate. The Pearson correlation coefficients (r) were 0.996 and 0.9994, respectively, with the linear equations being $y = 73.002x - 1972.5$ for the solvent calibration curve and $y = 205.23x - 18{,}791$ for the matrix-matched calibration curve.

The values of r indicate a linear relationship between the x and y axes for both curves. The Pearson correlation coefficient, which ranges from -1 to +1, reflects the strength and direction of the linear correlation between the two variables. A value close to ± 1 suggests a strong linear association between the variables [22].

However, r should not be the sole criterion for evaluating the linearity. In some cases, r can approach 1.00 even when the linear relationship between the x and y axes is not ideal. Therefore, linearity should be confirmed by additional statistical tests, such as Student's t-test and the F-test, using Equations (2)–(8). Table 1 summarizes the values for these parameters and the results of the statistical tests applied to the matrix-matched calibration curve.

Table 1. The t and F significance tests for the angular and linear parameters, and for the linear relationship between the x and y axes, respectively, applied to the matrix-matched calibration curve at a 95% confidence level and three degrees of freedom.

$F_{calculated}$				$F_{tabulated}$					**Test F**
2682.2				9.28					Significant
Linear coefficient					Angular coefficient				
a	S_a	t_{cal}	t_{tab}	**Teste t**	b	S_b	t_{cal}	t_{tab}	**Test t**
205.226	3.96	51.79	3.182	Significant	18,790.8	2055.22	9.14	3.182	Significant

Since $F_{calculated} > F_{tabulated}$, it is concluded that, in fact, there is a good linear fit between the x and y variables for AMT in the matrix-matched calibration. Generally, for a significant regression, the calculated F-value is high, as shown in Table 1, indicating that the variation in the y axes caused by the linear relationship exceeds the residual variation (sum of deviations or errors) [19].

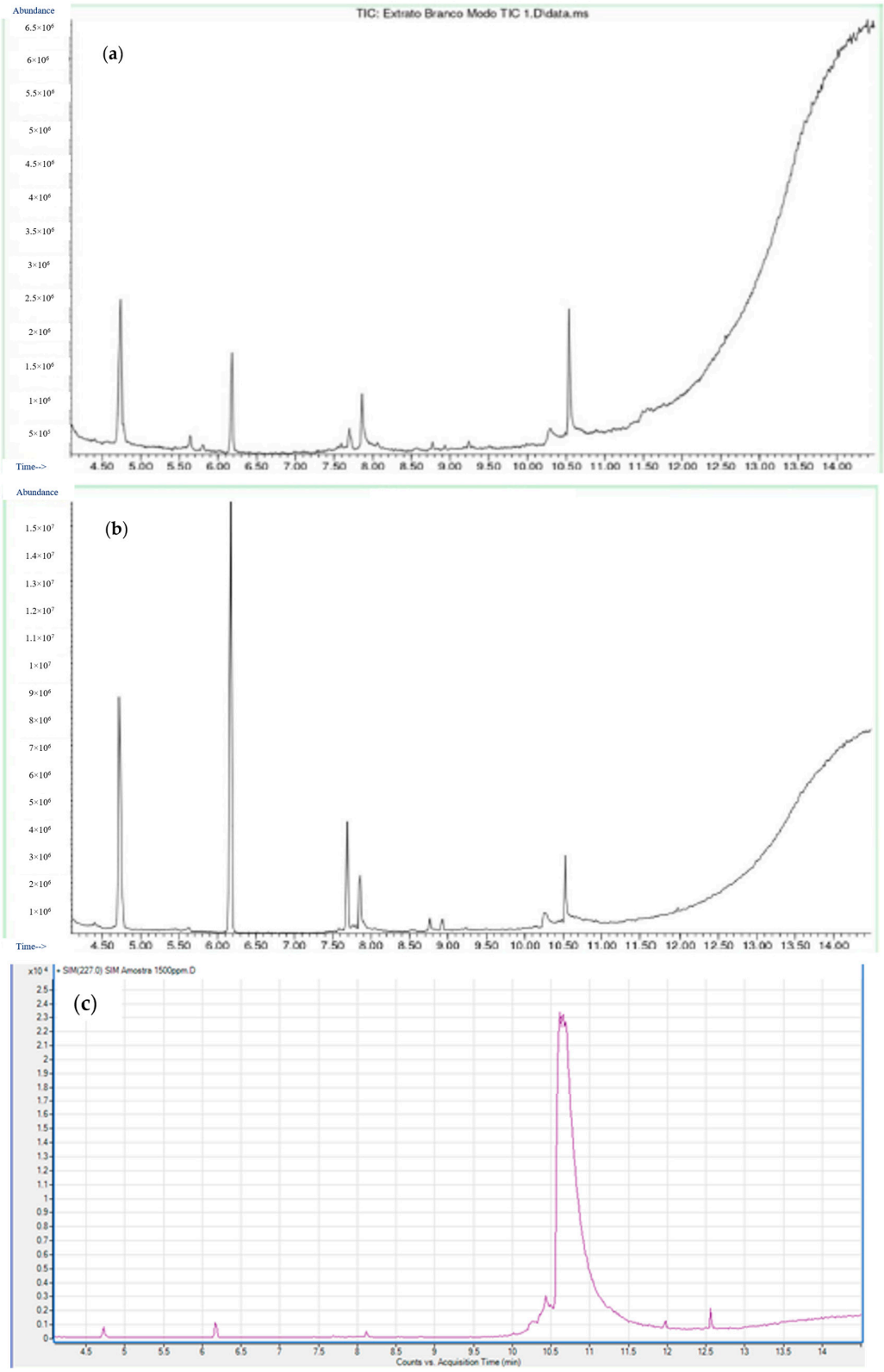


Figure 2. (**a**) Chromatogram in SCAN mode of the sample without AMT. (**b**) Chromatogram in SCAN mode of a sample spiked with 1 mg L^{-1} of AMT. (**c**) SIM mode (m/z = 227; m/z = 44) chromatogram of a sample spiked with 1 mg L^{-1} of AMT.

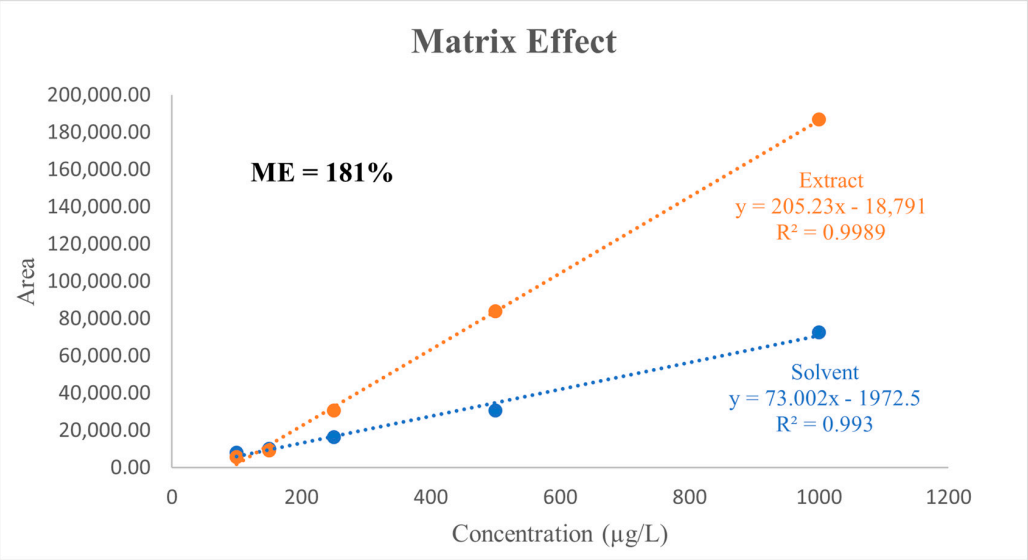


Figure 3. Calibration curves in the solvent and matrix with the corresponding determination coefficients, linear equations, and matrix effect calculation (note: ME = matrix effect).

For the statistical analysis of the parameters of the matrix-matched calibration curve, both the angular and linear coefficients were found to be significant, making them important for the analytical response. Therefore, these coefficients should remain in the linear equation. Consequently, the equation $y = 205.23x - 18{,}791$ was used for the quantitative analysis.

3.4. Matrix Effect

Figure 3 illustrates the matrix effect (ME) caused by the surface water sample, calculated according to Equation (9). The positive value of 181% indicates a strong influence of the sample components on the analytical result for AMT, implying that the matrix-matched calibration should be used for the quantitative analyses, since its value falls outside the range of −20% to 20% [19].

One explanation for such a high positive matrix effect value is the presence of active sites in the gas chromatograph itself, which interact with the analytes [23]. An example is the free silanol groups in the liner, which can promote the adsorption of pesticides or even catalyze the thermal degradation processes of certain substances [24], processes which are intensified by the high temperature of the injection system.

When the analyte is introduced into the GC system through a standard solution in the solvent, the active sites of the liner are fully available to retain this species, thus reducing the transfer of the pesticide to the column and, subsequently, to the detector [25]. However, when the analyte is introduced in the presence of matrix components, a competitive process occurs between the sample components and the target compound for the selective adsorption to the active sites of the liner. This allows for a greater amount of the analyte to be transferred to the column, thereby increasing the intensity of the analytical signal [19,25], This phenomenon may explain the increased instrumental response for the analyses conducted in the presence of the sample extract for AMT.

3.5. Sensitivity

For the curve in the solvent, the LOD and LOQ were determined to be 30 µg L^{-1} and 100 µg L^{-1}, respectively. For the curve in the matrix, the LOD and LOQ were 18 µg L^{-1} and 60 µg L^{-1}, respectively.

The method demonstrated a greater sensitivity for AMT when using the matrix-matched calibration, likely due to a positive matrix effect. Notably, the LOQ obtained is

at the same concentration level as the maximum residue limit (MRL) for AMT in surface waters, which was set at 60 μg L^{-1} according to European Union legislation (2021) [26].

3.6. Precision and Accuracy

Instrumental precision was rigorously monitored throughout the study, as all of the chromatographic injections were performed in at least three replicates. Table 2 presents the results obtained for the analytical curve of Ametryn (AMT) in the sample matrix, including the chromatographic peak areas for each concentration level and their respective relative standard deviations (RSD/%).

Table 2. Chromatographic peak area of AMT at five concentration levels with their respective relative standard deviations for the matrix-matched calibration.

Concentration Level (μg · L^{-1})	Area ± Standard Deviation (*n* = 3)	Relative Standard Deviation (RSD/%)
100	16,223.38 ± 564.87	3.48
150	19,888.14 ± 507.67	2.55
250	41,364.29 ± 1483.77	3.58
500	94,550.56 ± 1580.53	1.67
1000	197,394.94 ± 3297.95	1.67

It can be observed that the RSD% values ranged from 1.67% to 3.58%, which are well below the maximum limits established by INMETRO [15] and the European Commission Health & Consumer Protection Directorate-General [26], which permit maximum values of 15% and 20%, respectively, for trace-level analysis.

The intra-assay precision was evaluated along with the accuracy through recovery tests, as each fortification level was performed in five assay replicates. The obtained data are summarized in Table 3, with RSD% values ranging from 4.26% to 9.41% and recovery percentages from 98.63% to 109.36%. These results align with the acceptable range of 80% to 110% established by INMETRO [15], with an RSD of up to 20% for the trace analysis. These findings demonstrate that the method exhibits a high precision and accuracy for the determination of AMT in surface water samples, making it suitable for analytical applications, as per current regulations.

Table 3. Spiking levels, average recovered concentration, recovery percentage, and the Relative Standard Deviation (RSD).

Spiking Level (μg · L^{-1})	Average Concentration/(μg · L^{-1}) ± Standard Deviation (*n* = 5)	Recovery/%	RSD/%
100	109.39 ± 4.66	109.36	4.26
500	493.16 ± 46.43	98.63	9.41
1000	1034.60 ± 66.44	103.46	6.42

3.7. Qualitative and Quantitative Analysis of Water Samples

The validated method was applied to three real water samples, corresponding to the spring of Ribeirão Grotão Lake (S1), the stream (S2), and the waters of the lake itself (S3), located in the Bielândia district of the city of Filadélfia, state of Tocantins, Brazil.

In the analyses conducted in SIM mode, no pesticide residues were detected in the samples. However, when performing the analysis in SCAN mode for the qualitative investigation of possible contaminants, a consistent peak was identified at the retention time of 7.872 min in all three samples. The comparison of the mass spectrum obtained with the NIST library revealed similarities of 27.7% (S1), 34.6% (S2), and 49.6% (S3) with

2,4-Di-tert-butylphenol. These results are illustrated in Figure 4, using the spring point (S1) as an example.

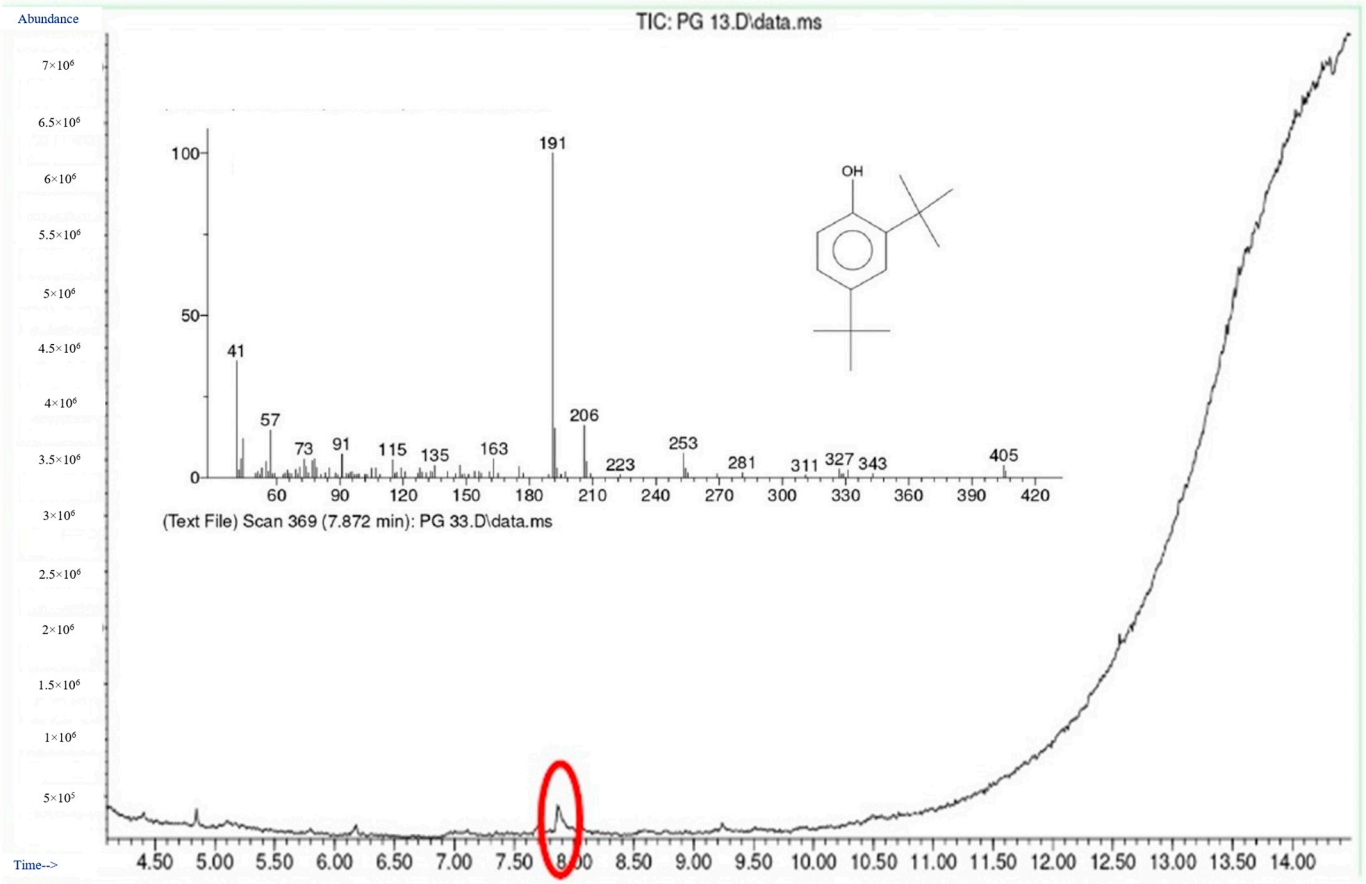


Figure 4. Chromatogram in SCAN mode of sample S1 highlighting (red circle) the 2,4-Di-tert-butylphenol peak with the corresponding mass spectrum.

The identified compound is one of the byproducts of the biodegradation of the organochlorine herbicide pentachlorophenol (PCP), which is used by the communities living around Ribeirão Grotão Lake. Chlorophenols, such as PCP, are known to be highly toxic and environmental pollutants. According to Leontievsky et al. [27], PCP has been proven to be biodegradable, with one of its degradation products being 2,4,6-Trichlorophenol (TCP). According to Moraes [28], 2,4-Di-tert-butylphenol may be a byproduct of TCP degradation, which justifies the presence of this compound in the analyzed samples.

4. Conclusions

The QuEChERS sample preparation method, adapted and combined with GC-MS quantification, demonstrated effective analytical performance for the determination of the pesticide AMT in groundwater and surface water samples. This method proved to be suitable for routine analyses, especially in areas with known contamination by this substance. It is noteworthy that the matrix-matched calibration curve was more suitable for the quantification of AMT in this type of sample than external standard calibration.

The validated method was applied to three real water samples (spring, stream, and lake), which did not show any residues of the target pesticide. However, the presence of 2,4-Di-tert-butylphenol, a degradation byproduct of the organochlorine pesticide pentachlorophenol, was detected.

It is important to note that organochlorine compounds, such as pentachlorophenol, are not authorized in Brazil. According to ANVISA, pentachlorophenol and its salts are prohibited due to their toxicity to animals and humans, as well as their persistence in the environment.

Author Contributions: Conceptualization, L.F.L.G. and D.B.A.; methodology, L.F.L.G. and D.B.A.; software, L.F.L.G. and D.B.A.; validation L.F.L.G., formal analysis, L.F.L.G. and M.Z.F.d.S., investigation, L.F.L.G.; resources, R.F.d.N.; data curation, L.F.L.G.; writing—original draft preparation, L.F.L.G. and M.Z.F.d.S.; writing—review and editing, L.F.L.G. and D.B.A.; visualization, L.F.L.G., M.Z.F.d.S., R.F.d.N. and D.B.A.; supervision, D.B.A.; project administration, L.F.L.G. and D.B.A.; funding acquisition, D.B.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by PROPESQ/UFNT, Call for Proposals No. 010/2024. CNPq Call No. 09/2022—Research Productivity Fellowships (PQ) of Ronaldo Ferreira do Nascimento.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author at daniel.alcantara@ufnt.edu.br.

Acknowledgments: The authors thank the Chromatography Laboratory (LabCrom) of the Federal University of Northern Tocantins (UFNT) for providing the infrastructure necessary to carry out the research. We also thank the Trace Analysis Laboratory (LAT) of the Federal University of Ceará (UFC) for donating some reagents for the QuEChERS method and the analytical standard of Ametryn. We also thank the Instituto Natureza do Tocantins–Naturatins for allowing access to the collection of samples in the conservation unit Monumento Nacional das Árvores Fossilizadas do Tocantins (MNAFTO) and for providing the brigade team that supported the sampling efforts.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

References

1. Shattar, S.F.A.; Zakaria, N.A.; Foo, K.Y. Utilization of montmorillonite as a refining solution for the treatment of ametryn, a second generation of pesticide. *J. Environ. Chem. Eng.* **2017**, *5*, 3235–3242. [CrossRef]
2. Szewczyk, R.; Kuśmierska, A.; Bernat, P. Ametryn removal by *Metarhizium brunneum*: Biodegradation pathway proposal and metabolic background revealed. *Chemosphere* **2018**, *190*, 174–183. [CrossRef] [PubMed]
3. Agência Nacional de Vigilância Sanitária. Participação Social. Available online: https://antigo.anvisa.gov.br/documents/10181/6243353/CONSULTA+P%C3%9ABLICA+N+1027+GGTOX.pdf/42006f98-70c0-47c6-991a-1bc2f78391e4 (accessed on 9 April 2024).
4. Martins, F.C.; Souza, D. New Approaches in Electroanalytical Determination of Triazines-Based Pesticides in Natural Waters. *Analytica* **2023**, *4*, 84–101. [CrossRef]
5. Boussouga, Y.A.; Sacher, F.; Schäfer, A.I. Water quality of The Gambia River: A prospective drinking water supply. *Sci. Total Environ.* **2023**, *878*, 162794. [CrossRef]
6. Tsiantas, P.; Bempelou, E.; Dougla, M.; Karasali, H. Validation and Simultaneous Monitoring of 311 Pesticide Residues in Loamy Sand Agricultural Soils by LC-MS/MS and GC-MS/MS, Combined with QuEChERS-Based Extraction. *Molecules* **2023**, *28*, 4268. [CrossRef]
7. Padhye, L.P.; Srivastava, P.; Jasemizad, T.; Bolan, S.; Hou, D.; Shaheen, S.M.; Rinklebe, J.; Connor, D.O.; Lamb, D.; Wang, H.; et al. Contaminant containment for sustainable remediation of persistent contaminants in soil and groundwater. *J. Hazard. Mater.* **2023**, *455*, 131575. [CrossRef]
8. Vara, M.G.; Postigo, C.; Palma, P.; Bleda, M.J.; Alda, M.L. QuEChERS-based analytical methods developed for LC-MS/MS multiresidue determination of pesticides in representative crop fatty matrices: Olives and sunflower seeds. *Food Chem.* **2022**, *386*, 132558. [CrossRef]
9. Kim, L.; Lee, D.; Cho, H.K.; Choi, S.D. Review of the QuEChERS method for the analysis of organic pollutants: Persistent organic pollutants, polycyclic aromatic hydrocarbons, and pharmaceuticals. *Trends Environ. Anal. Chem.* **2019**, *22*, e00063. [CrossRef]
10. Anastassiades, M.; Lehotay, S.J.; Stajnbaher, D.; Schenck, F.J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412–431. [CrossRef]

11. Curbelo, M.A.G.; Rodrígues, B.S.; Herrera, A.V.H.; Sálamo, J.G.; Borges, J.H.; Delgado, M.A.R. Evolution and applications of the QuEChERS method. *TrAC Trends Anal. Chem.* **2015**, *71*, 169–185. [CrossRef]
12. SBrondi, H.G.; Macedo, A.N.; Vicente, G.H.L.; Nogueira, A.R.A. Evaluation of the QuEChERS method and gas chromatography-mass spectrometry for the analysis pesticide residues in water and sediment. *Bull. Environ. Contam. Toxicol.* **2011**, *86*, 18–22. [CrossRef]
13. Ribani, M.; Bottoli, C.B.G.; Collins, C.H.; Jardim, I.C.S.F.; Melo, L.F.C. Validation in chromatographic and electrophoretic methods. *New Chem.* **2004**, *27*, 771–780. [CrossRef]
14. Agência Nacional de Vigilância Sanitária. Normas Regulátorias da ANVISA. Available online: https://antigo.anvisa.gov.br/documents/10181/2721567/RDC_166_2017_COMP.pdf/d5fb92b3-6c6b-4130-8670-4e3263763401 (accessed on 9 April 2024).
15. Instituto Nacional de Metrologia, Normalização e Qualidade Industrial. Orientação sobre validação de métodos analíticos DOC-CGCRE-008. Available online: https://app.sogi.com.br/Manager/texto/arquivo/exibir/arquivo?eyJ0eXAiOiJKV1QiLCJhbGciOiJIUzI1NiJ9AFFIjAvMTM4ODM3NS9TR19SZXF1aXNpdG9fTGVnYWxfVGV4dG8vMC8wL0RPUS1DZ2NyZS04XzA5LnBkZi8wLzAiAFFBcMYdNmecpDn0m0Dj4vzJmvMJZMAYtW6mtkIlj0C7fk (accessed on 9 April 2024).
16. Alcântara, D.B.; Fernandes, T.S.M.; Nascimento, H.O.; Lopes, A.F.; Marília, M.G.G.; Lima, A.C.A.; Carvalho, T.V.; Grinberg, P.; Milhome, M.A.L.; Oliveira, A.H.B.; et al. Diagnostic detection systems and QuEChERS methods for multiclass pesticide analyses in different types of fruits: An overview from the last decade. *Food Chem.* **2019**, *298*, 124958. [CrossRef]
17. Alcântara, D.B.; Riceli, P.; Almeida, A.D.S.; Luz, L.R.; Nascimento, H.O.; Fernandes, T.S.M.; Dionísio, A.P.; Castro, A.C.R.; Nascimento, R.F.; Lopes, G.S.; et al. Development, Optimization, and Validation of an Ultrasound-Assisted Liquid-Liquid Microextraction (UALLME) for Selenomethionine Analyses in Cashew Nut (*Anacardium occidentale*) by Ultra-Performance Liquid Chromatography Coupled to Electrospray Ionization/Single Quadrupole Mass Spectrometer (UPLC-ESI/QDa). *Food Anal. Methods* **2022**, *15*, 3196–3208. [CrossRef]
18. Harris, D.C. *Quantitative Chemical Analysis*, 9th ed.; W. H. Freeman and Company: New York, NY, USA, 2017; pp. 127–141.
19. Alcântara, D.B.; Paz, M.S.O.; Rodrigues, T.H.S.; Fernandes, T.S.M.; Barbosa, P.G.A.; Loiola, A.R.; Grinberg, P.; Zocolo, G.J.; Brito, E.S.; Nascimento, R.F. Organophosphorus pesticide in sapodilla (*Manilkara zapota*) fruit. *J. Braz. Chem. Soc.* **2018**, *29*, 2180–2188. [CrossRef]
20. Tóth, E.; Tölgyesi, Á.; Simon, A.; Bálint, M.; Ma, X.; Sharma, V.K. An Alternative Strategy for Screening and Confirmation of 330 Pesticides in Ground- and Surface Water Using Liquid Chromatography Tandem Mass Spectrometry. *Molecules* **2022**, *27*, 1872. [CrossRef]
21. Tölgyesi, Á.; Korozs, G.; Tóth, E.; Bálint, M.; Ma, X.; Sharma, V.K. Automation in quantifying phenoxy herbicides and bentazon in surface water and groundwater using novel solid phase extraction and liquid chromatography tandem mass spectrometry. *Chemosphere* **2022**, *286*, 131927. [CrossRef]
22. Schober, P.; Boer, C.; Schwarte, L.A. Correlation Coefficients: Appropriate Use and Interpretation. *Anesth. Analg.* **2018**, *126*, 1763–1768. [CrossRef]
23. Schenck, F.J.; Lehotay, S.J. Does further clean-up reduce matrix enhancement effect in gas chromatography analysis of pesticide residue in food? *J. Chromatogr. A* **2000**, *868*, 51–61. [CrossRef]
24. Lazzari, E.; Silva, E.A.S.; Bjek, T.R.; Schneider, J.K.; Caramão, E.B. Evaluation of the matrix effect in the quantitative bio-oil analysis by gas chromatography. *Fuel* **2021**, *290*, 119866. [CrossRef]
25. Pinho, G.P.; Neves, A.A.; Queiroz, M.E.L.R.; Silvério, F.O. Matrix effect in the quantification of pesticides by gas chromatographyç. *New Chem.* **2009**, *31*, 987–995. [CrossRef]
26. Europan Commission. Guidelines—Maximum Residue Levels. Available online: https://food.ec.europa.eu/plants/pesticides/maximum-residue-levels/guidelines-maximum-residue-levels_en (accessed on 9 April 2024).
27. Leontievsky, A.A.; Myasoedova, N.M.; Baskunov, B.P.; Golovleva, L.A.; Bucke, C.; Evans, C.S. Transformation of 2,4,6-trichlorophenol by free and immobilized fungal laccase. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 85–91. [CrossRef]
28. Moraes, M.A.R. Bacteria Associated with the Sponge *Hymeniacidon heliophila*: Physiological Role and Application in Biotechnology. Master's Thesis, Biosciences Institute of the University of São Paulo, São Paulo, Brazil, 2022. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.