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Research Article

Linearity and Matrix Effect Verification of the Pesticide Ametryn in Corn (*Zea mays L.*) Using the QuEChERS-GC-MS Method

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Abstract:

Agriculture's reliance on pesticides has intensified with global population growth, industrial expansion, and urbanization, particularly since the "Green Revolution" of the 1950s. Brazil, a major pesticide consumer, saw a 14% increase in pesticide registrations in 2021, marking the highest number since 2000. Despite their benefits in enhancing productivity, excessive pesticide use poses severe environmental risks. This study focuses on the herbicide ametryn, approved for corn cultivation by Brazil's Legislation (ANVISA), analyzing its presence in corn samples, collected in the city of Araguaína, Tocantins, Brazil, using the QuEChERS method coupled with Gas Chromatography-Mass Spectrometry (GC-MS). The method was validated for selectivity, linearity, precision, and limits of detection and quantification. Although ametryn was not detected, the analysis uncovered other organic contaminants such as dibutyl phthalate and oxymetazoline in certain samples. The study highlights the challenges in detecting pesticide residues in complex matrices and underscores the need for further research with larger samples.

Keywords: herbicide; method validation; corn; QuEChERS; GC-MS

Introduction:

Agriculture is an economic practice that involves the production of food, which throughout human history has cultivated fertile lands in river valleys and optimized techniques to enhance soil productivity. With the growing global population, the rise of industries, and the expansion of cities, agriculture has increasingly relied on innovations in agricultural inputs, establishing interconnections with other sectors.[1] To ensure food security and meet societal demands, the use of pesticides became necessary, beginning in the United States in the 1950s during the "Green Revolution." This movement marked the large-scale use of agrochemicals to modernize and boost agricultural productivity. A decade later, Brazil adopted similar practices, and today it is one of the world's largest consumers of pesticides.[2-4]

In 2021, Brazil saw the highest number of pesticide registrations since the historical series began in 2000, with a 14% increase compared to 2020, totaling 562 pesticides, of which 33 were new releases.[3] Despite the benefits and increased productivity, excessive and improper pesticide use leads to environmental issues, causing irreversible damage to both the biotic and abiotic systems, including soil, water bodies, air, animals, and humans.[2,3]
In Brazil, three main agencies regulate, monitor, and set maximum residue limits (MRLs) for agrochemicals: the National Health Surveillance Agency (ANVISA), the Ministry of Agriculture, Livestock, and Food Supply (MAPA), and the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA).

Corn is one of the key commodities in Brazilian agriculture, playing a crucial role not only in human diets but also as a major component of animal feed, particularly for cattle and poultry.[5] The National Supply Company (CONAB)[6] reported that corn exports in October 2023 reached 8.45 million tons, compared to 8.76 million tons in the previous month and 6.79 million tons in the same period last year. From January to October 2023, exports totaled 42.4 million tons, up 36.8% from 31 million tons in the same period of the previous year. This growth was driven by increased demand for animal protein and the rise of corn-based ethanol. By mid-November, 45.8% of the first corn crop had been planted, compared to 53.9% in the same period the previous year.
Given the increasing use of pesticides in Brazil and the significance of corn, this study aimed to investigate validation parameters for the herbicide ametryn, as its use is approved by ANVISA for corn cultivation. Additionally, real samples collected from markets of the city of Araguaína, in the state of Tocantins, were analyzed using the QuEChERS method for sample preparation and Gas Chromatography-Mass Spectrometry (GC-MS) for detection and quantification.

Materials And Methods

Chemicals and Reagents

Acetonitrile (99.9%, v/v), methanol (99.97%, v/v), and n-hexane (99.97%, v/v) were obtained from Vetec (Brazil). Anhydrous magnesium sulfate P.A., sodium chloride P.A., and trisodium citrate dihydrate P.A. were also sourced from Vetec. Sodium hydrogen citrate sesquihydrate P.A. came from Sigma-Aldrich (Brazil), while primary secondary amine (PSA) and graphitized carbon black (GCB) were purchased from Supelco (USA). Water used in the study was purified using a Milli-Q system (Millipore, Academic, USA), with a resistivity of 18.2 MΩ cm.

The powder analytical standard of ametryn (99.0%, w/w), provided by Merck (Brazil), was used to prepare a stock solution at a concentration of 1000 mg L^{-1} in methanol. From this stock solution, working solutions were prepared at concentrations of 10 mg L^{-1} and 1 mg L^{-1} using chromatographic-grade methanol.
These working solutions were subsequently diluted to create lower concentration solutions (100, 150, 250, 500, and 1000 µg L^{-1}) for constructing the analytical curve (concentration vs. area) using the external standard method. In parallel, a matrix-matched calibration curve was prepared by diluting the working solutions with sample extracts to achieve the same concentration levels.

Sampling and QuEChERS Sample Preparation

For the quantitative and qualitative analysis of the herbicide ametryn in corn (Zea mays L.), corn ears were collected from four locations: the municipal market (Sample 01) and a supermarket (Sample 02) located in Araguaína, Tocantins, Brazil; in a city in the state of Goiás, Brazil (Sample 03), and a farm near Araguaína (Sample 04).

In the laboratory, 10 g of pre-treated corn sample were weighed into a 50 mL Falcon tube. To this, 10 mL of acetonitrile was added. The mixture was vortexed for 1 minute. Next, a salt mixture was added, consisting of 4.0 g of anhydrous magnesium sulfate, 1 g of sodium chloride, 1 g of sodium citrate tribasic, and 0.5 g of sodium hydrogen citrate sesquihydrate. The mixture was vortexed for an additional 1 minute and then centrifuged at 3000 rpm for 10 minutes to obtain the primary extract.

For cleanup, a 1 mL aliquot of the supernatant was transferred to a 15 mL Falcon tube containing cleanup salts: 150 mg of anhydrous magnesium sulfate, 150 mg of primary secondary amine (PSA), and 50 mg of graphitized carbon black (GCB). The mixture was vortexed for 1 minute to disperse the adsorbents and then centrifuged at 3000 rpm for 10 minutes to yield the post-cleanup extract, which was transferred to a vial for chromatographic analysis.

GC-MS Analytical Conditions

The chromatography was performed using the Agilent Technologies GC-MS system (models 7890B and 5977A), with helium (99.999%) as the carrier gas at 1.2 mL/min. A HP5-mS capillary column from Agilent (30 m x 0.25 mm, 0.25 µm stationary phase thickness) was used. The injector operated in splitless mode.

A 1.0 µL sample was injected with the injector temperature set at 250 ºC. The temperature program was as follows: initial temperature of 50 ºC for 0 minutes, heating at 20 ºC/min to 250 ºC (1 minute), then heating to 300 ºC at 20 ºC/min and holding at 300 ºC for 1 minute, resulting in a total run time of 14.5 minutes, as described by Alcântara et al.[6]

The mass spectrometer was configured with electron ionization (EI) mode at 70 eV. The ion source was set to 230 ºC and the transfer line temperature was maintained at 250 ºC. The MS operated in both Full Scan (SCAN) and Select Ion Monitoring (SIM) modes. In SCAN mode, it covered the *m/z* range of 40–400, while in SIM mode, it specifically targeted *m/z* 227 and 44 for the quantification and confirmation of ametryn, respectively.

Matrix Effect

The evaluation of the matrix effect in the corn extract was conducted by comparing the slope coefficients obtained from the analytical curves generated by external standard calibration and matrix-matched calibration, according to Equation 1:
Where:

$$ME/\% = \frac{(b_m - b_s)}{b_s} \times 100 \qquad \text{(I)}$$

- b_m: slope coefficient of the curve in the matrix;
- b_s: slope coefficient of the curve in the solvent;
- $ME/\%$: matrix effect expressed as a percentage.

If the matrix effect value falls outside the range of -20% to 20%, it indicates the presence of a significant matrix effect.[6,7] In such cases, to ensure accurate quantitative analysis, it is essential to use the calibration curve in the matrix.

Method Validation

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The method validation was conducted using sample 1, following the guidelines established in the MAPA Analytical Validation and Quality Control Guide (2011).[8]

Selectivity was ensured by using the mass spectrum fragments obtained in SCAN mode for the quantification ion (m/z = 227) and confirmation ion (m/z = 44) of the analyte. The similarity of the analyte's mass spectra with those in the NIST library was confirmed. Subsequent analyses were performed using Selective Ion Monitoring (SIM) mode for quantitative analysis.

Linearity was assessed by preparing analytical curves in both the solvent and matrix extract. A linear fit was considered adequate when the coefficient of determination (R^2) approached 1.

Precision was determined using the repeatability method, calculating the relative standard deviation (RSD/%) of the areas obtained from three injections of analytical standards for both calibrations (external standard and matrix matched).

Detection limits (LOD) and quantification limits (LOQ) were estimated through consecutive injections of standard solutions, starting with the lowest concentration and increasing until achieving a signal-to-noise ratio of 3 for LOD and 10 for LOQ, respectively.

Results and Discussions

An example of the chromatogram for ametryn in SIM mode, at a concentration of 1500 μg L⁻¹ prepared with matrix extract, is shown in Figure 01. This chromatogram was obtained by monitoring the mass spectrum fragments corresponding to the quantification ion (m/z = 227) and confirmation ion (m/z = 44). The mass spectrum of ametryn was obtained using the SCAN mode with the 3 μg L⁻¹ analytical standard in solvent (Figure 2). Calibration curves, both in solvent and matrix, were generated using the SIM mode for all concentration levels.

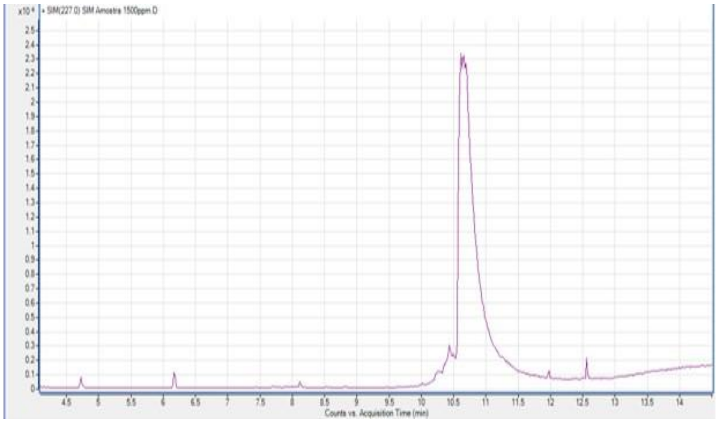


Figure 1: Chromatogram of the Ametryn Standard at 1500 μg L⁻¹ in Methanol Acquired by GC-MS in SIM Mode for m/z 227 and 44.

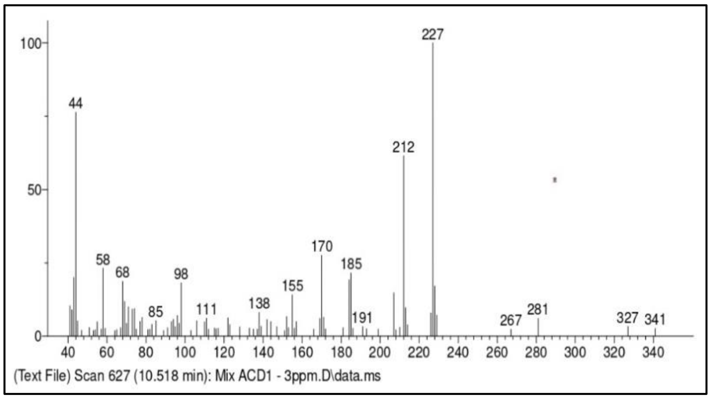
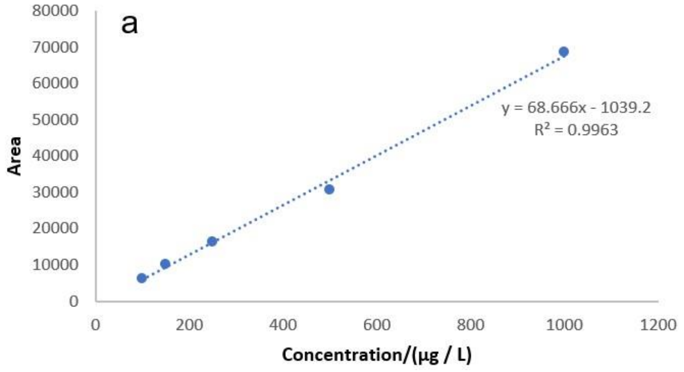


Figure 2: Mass Spectrum of Ametryn from the 3 μg L⁻¹ Standard Solution in Methanol Analyzed in SCAN Mode.

Figure 3a shows the calibration curve using the solvent, covering the range of 100 to 1000 μg L⁻¹. This range presented a good linear correlation between the x and y axes, resulting in five concentration levels, as recommended by regulations, starting from the limit of quantification (LOQ) of 100 μg L⁻¹. However, when the concentration exceeds 1000 μg L⁻¹, the curve begins to lose linearity, with progressively lower values for the coefficient of determination (R^2). The limit of detection (LOD) was around 30 μg L⁻¹. The R^2 for the curve obtained via the external standard method was 0.9963, while the correlation coefficient (r) was 0.9981, both meeting the regulatory requirements.

For the calibration curve using the matrix (Figure 3b), the linear range was from 100 to 1500 μg L⁻¹, with an estimated LOQ of 100 μg L⁻¹ and an LOD of 30 μg L⁻¹. The coefficient of determination (R^2) remained at 0.9886, and the correlation coefficient (r) at 0.9943.

Using Equation I, it was observed that the matrix effect exceeded 400%, far surpassing the recommended range (-20% to 20%). Therefore, the use of the matrix-matched calibration curve for quantitative analyses is necessary to avoid potential measurement errors.[9] This overestimated value is due to the complexity of the sample, as food matrices typically generate higher analytical signals due to the presence of co-extracted compounds. According to Alcantara et al.[6], complex samples often contain significant levels of co-extractives. In chromatographic analyses coupled with mass spectrometry, the substance undergoes various steps from injection to detection, which can result in variations in the measured analytical signal.



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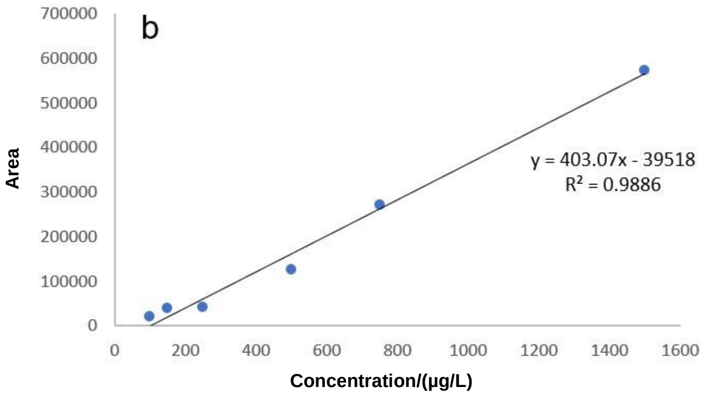

Figure 3. (a) Analytical Calibration Curve for Ametryn Using External Standardization. (b) Analytical Calibration Curve for Ametryn by Matrix-Matched Calibration.

The positive matrix effect can also be explained by interactions between the analyte and sample components within the liner. After injection, the sample is vaporized in the heated injector, which is highly susceptible to the matrix effect. At high temperatures, the active sites in the liner, formed by free silanol groups and trace metals, can adsorb analytes or even catalyze the thermal degradation of pesticides.[6,10]

When standard solutions are injected in pure solvent, the liner's active sites are prone to retaining the analytes, resulting in a smaller amount of pesticides being transported to the chromatographic column and subsequently detected. However, when standard solutions are prepared in matrix extract, there is competition for active sites between the analytes and matrix components, allowing a greater amount of pesticide to be transferred to the column and subsequently detected.

Fernandes et al.[11] also notes that, during the initial analyses on a new chromatographic column, little or no matrix effect is observed. However, with repeated injections, the column becomes progressively contaminated, leading to an increasingly pronounced matrix effect.

Qualitative Detection of Organic Contaminants in Corn Samples

The herbicide ametryn was not detected in any of the analyzed samples. However, during a qualitative analysis to check for the presence of other organic contaminants, Dibutyl Phthalate was identified in Sample 01. According to Muscogiuri and Colao[12], this compound is an organic contaminant commonly derived from plastics and can be found in various products such as paints, toys, cosmetics, and even food.

Although the chromatogram and mass spectrum indicated the possible presence of Dibutyl Phthalate, the low percentage of similarity between the sample spectrum and the NIST library spectrum prevents a definitive confirmation, requiring further investigation. Figures 4 show the chromatogram and mass spectrum corresponding to Dibutyl Phthalate identified in the sample.

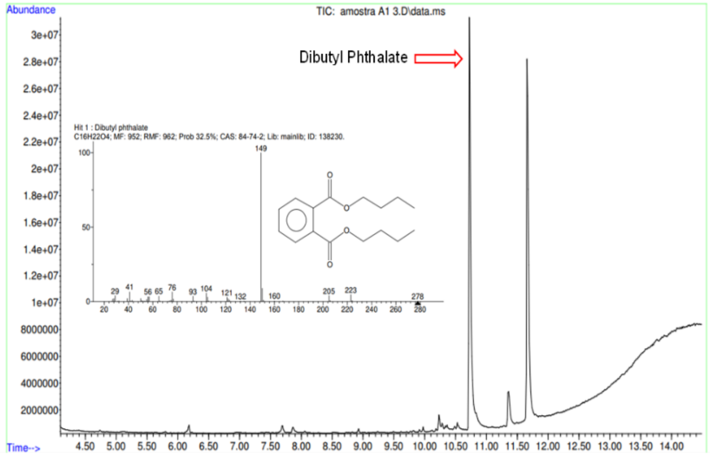

Figure 4. Chromatogram of Sample 1 Extract Obtained in SCAN Mode by GC-MS, with Mass Spectrum of Dibutyl Phthalate.

In Sample 02, a compound identified by the NIST library as Oxymetazoline, a nasal decongestant, was detected. However, as in the previous case, the low percentage similarity between the sample spectrum and the library prevents accurate confirmation of this compound's presence, necessitating more detailed studies for verification. Figures 5 display the total ion and extracted ion chromatograms, along with the mass spectrum.

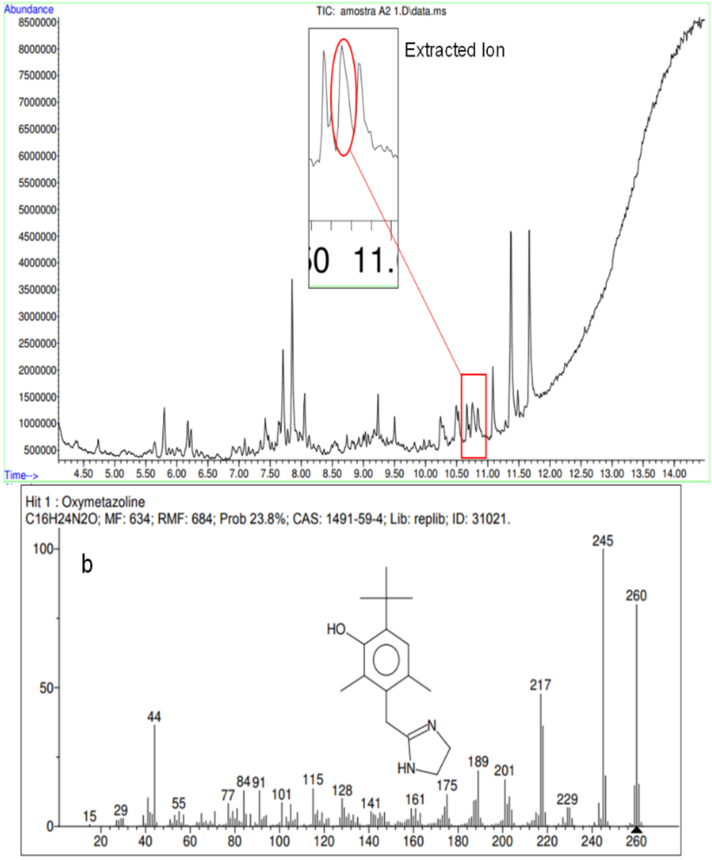

Figure 5. (a) Total Ion Chromatogram (TIC) of Sample 2 Obtained in SCAN Mode and Extracted Ion Chromatogram of Oxymetazoline. (b) Mass Spectrum of Oxymetazoline Obtained from the Sample Analysis.

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In Sample 03, no organic contaminants were observed, except for components intrinsic to the sample matrix. In Sample 04, the herbicide ROUNDUP, whose active ingredient is glyphosate, had been applied by the farmer. However, glyphosate was also not detected in the qualitative analysis. According to the product label, if it rains within four hours of application, the herbicide loses its effectiveness, despite being systemic (absorbed by the plant and transported through the xylem and phloem). The farmer reported that it rained a few hours after application, which may have influenced the lack of detection. Another relevant factor is that the corn cobs from this sample were collected from a higher area, and the rain on the day of application could have caused the herbicide to run off into lower areas.

Although the number of samples analyzed may be considered limited for a study of this nature, the application of the method to real samples coincided with the corn off-season, which ends in October, while the real sample collection took place in November, during the beginning of soybean cultivation. This timing directly impacted the availability of material for analysis.

Conclusion

In this study, an efficient method for the determination of ametryn in corn samples using the GC-MS technique was developed and validated, yielding satisfactory results in terms of selectivity, linearity, precision, and detection and quantification limits. Although the herbicide ametryn was not detected in the analyzed samples, the research revealed the presence of other organic contaminants, such as dibutyl phthalate, suggesting the need for a broader evaluation of potential contamination sources. The challenges encountered during the analysis, such as variations in environmental conditions and the limited number of samples, highlight the importance of future studies with more controlled experiments and a more representative sample size to ensure the robustness of the conclusions. Overall, the method proved to be promising for analyses in complex matrices, such as food, and can be applied in future investigations of pesticide residues in agricultural crops.

Conflict of interest

The authors declare that they have no conflict of interest.

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As a corresponding author, I confirm that all authors of this manuscript have been named in this document without exception. I also confirm that the order of the authors in the manuscript is the consensus of all.

References

1. Lima, A.; Silva, E.; Iwata, B. Agriculturas e agriculturas familiar no Brasil: uma revisão de literatura. *Retratos de Assentamentos* **2019**, *22* (1), 65-84.
2. Alcântara, D. B.; Dionísio, A. P.; Artur, A. G.; Silveira, B. K. S.; Lopes, A. F.; Guedes, J. A. C.; Luz, L. R.; Nascimento, R. F.; Lopes, G. S.; Hermsdorff, H. H. M.; Zocolo, G. J. Selenium in Brazil nuts: An overview of agronomical aspects, recent trends in analytical chemistry, and health outcomes. *Food Chem. 372* (**2022**a).
3. Alcântara, D. B.; Fernandes, T. S. M.; Nascimento, H. O.; Lopes, A. F.; Menezes, M. G. G.; Lima, A. C. A.; Carvalho, T. V.; Grinberg, P.; Milhome, M. A. L.; Oliveira, A. H. B.; Becker, H.; Zocolo, G. J.; Nascimento, R. F. Diagnostic detection systems and QuEChERS methods for multiclass pesticide analyses in different types of fruits: An overview from the last decade. *Food Chem. 298* (**2019**).
4. Silva, R. Aspectos técnicos e científicos da cromatografia líquida de alta eficiência (HPLC). Ph.D. Dissertation, Universidade Federal do Maranhão, **2021**. Available at: https://ufma.br. Accessed: Dec 6, 2023.
5. Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). Expansão Potencial do plantio de 2ª safra do milho no Brasil no sistema de rotação soja-milho considerando o zoneamento de risco climático. Available at: https://embrapa.br. Accessed: Feb 24, **2023**.
6. Alcântara, D.; et al. Organophosphorus pesticide in sapodilla (Manilkara zapota) fruit. *JBCS*, *29* (10), 2180-2188, **2018**.
7. Alcântara, D. B.; Nascimento, R. F.; Lopes, G. S.; Grinberg, P. Evaluation of different strategies for determination of selenomethionine (SeMet) in selenized yeast by asymmetrical flow field flow fractionation coupled to inductively coupled plasma mass spectrometry (AF4-ICP-MS). *Analytical Methods*, (**2020**).
8. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Guia de validação e controle de qualidade analítica. Brasília, **2011**.
9. 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.; Zocolo, G. J. 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 Analytical Methods*, (**2022**b).
10. Sodré, F. F.; Freire, D. J. S.; Alcântara, D. B.; Maldaner, A. O. Understanding illicit drug use trends during the Carnival holiday in the Brazilian capital through wastewater analysis. *Frontiers in Analytical Science, 2* (**2022**).
11. Fernandes, T. S. M.; Alcântara, D. B.; Gordiano, P. G. A.; Paz, M. S. O.; Zocolo, G. J.; Nascimento, R. F. Matrix effect evaluation and method validation of organophosphorus pesticide residues in bell peppers (Capsicum annuum L.) by GC-MS determination. *International Journal of Environmental Analytical Chemistry 99*, 1–16 (**2019**).
12. Muscogiuri, G.; Colao, A. Phthalates: new cardiovascular

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health disruptors?. *Arch. Toxicol*. **2017**, *91*, 1513-1517.

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