

Research Article

Development of a Simple Integrative Carbon Nanomaterial Microextraction Method with GC-MS for Assessing Pesticide Residues in Apples

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The present study describes an efficient analytical method for analyzing apple pesticide residues. This novel approach employs ultrasound-assisted cleaning (UAC), solid-phase microextraction (SPME), and gas chromatography/mass spectrometry (GC-MS). A fiber coating with carbon nanomaterials (CNMs) was used for the extraction process. The methodology underwent extraction process optimization and analytical method validation. Moreover, its applicability for detecting pesticides in apples was evaluated. The validation showed satisfactory results with linearity coefficients greater than 0.99 ($R^2 > 0.99$) and linear working ranges from $0.5 \text{ ng}\cdot\text{g}^{-1}$ to $300.0 \text{ ng}\cdot\text{g}^{-1}$. The limits of detection and quantification (LODs and LOQs) ranged from $0.1 \text{ ng}\cdot\text{g}^{-1}$ to $0.3 \text{ ng}\cdot\text{g}^{-1}$ and from $0.5 \text{ ng}\cdot\text{g}^{-1}$ to $0.7 \text{ ng}\cdot\text{g}^{-1}$, respectively. The calculated recovery values ranged from 79% to 98%. The precision presented a relative standard deviation (RSD) lower than 14%. The validated method was applied to apple samples acquired from different markets and fruit stands in Belo Horizonte, Minas Gerais, Brazil. Organophosphate pesticides typically used in Brazilian apple production areas were detected in some samples. This study demonstrates the utility of UAC and CNM microextraction as an effective and highly efficient approach for analyzing pesticides in apples.

1. Introduction

Food contamination by pesticides has become a growing public health and environmental problem due to their toxicity and extensive use in agriculture. The general population, including risk groups such as the elderly and children, is primarily exposed to pesticides through its diet. Moreover, it is well known that most of these adverse effects are attributed to organochlorine pesticides, which are extremely toxic. Thus, strict regulations to control the presence of such contaminants in foods are required.

Since their introduction through organic synthesis in the last century, pesticides have been found in different matrices

and compartments such as food [1], air [2], soil [3], and water [4]. Although organic farming and other alternatives minimize pesticide application during crop growth, the constant large-scale application continues to affect human health and the environment. For example, colon [5] and epithelial ovarian cancer [6], infertility and reproductive problems [7], Alzheimer's disease [8], Hodgkin lymphoma [9], and thyroid dysfunction [10] have been associated with pesticide exposure. Additionally, some animals of vital environmental importance, such as pollinators [11] and fish species of wide biodiversity [12], are affected by the accumulation of and/or contact with pesticides. In this sense, efficient extraction, detection, and quantification methods

need to be developed to monitor these agricultural pollutants.

Determining pesticide residue concentrations in food is challenging because of the complexity of the matrix that sometimes makes it difficult to extract. Besides, the maximum contaminant levels (MCLs) allowed for consumption and export are constantly changing, being lower and more rigorous, requiring highly efficient extraction methods. Numerous protocols are followed to create a good method that complies with regulatory agencies and protects consumer health. Typically, pesticide residue determination in fresh agricultural products involves combining cleaning and extraction methods.

The clean-up steps generally utilize surfactants, ozone (O_3), ionic solvent, and chlorine treatment. However, none of these methods successfully remove pesticides without altering the physical or chemical properties of the food [13]. On the other hand, recent studies have shown an increasing interest in using the ultrasound-assisted cleaning (UAC) technique due to its environment-friendly process and effective pesticide removal [13–15].

Over the last decade, several pesticide extraction methods have been developed and applied to analyze food and water samples. The current methods have introduced improvements to avoid the high consumption of solvents and long extraction steps. Several alternatives include matrix solid-phase dispersion (MSPD) [16]; dispersive liquid-liquid microextraction based on solidification of floating organic drop (DLLME-SFO) [17, 18]; liquid-phase microextraction (LPME) [19]; microwave-assisted extraction (MAE) [20]; the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method [21]; supercritical fluid extraction (SFE) [22]; solid-phase extraction (SPE) [23]; solid-phase microextraction (SPME) [24, 25]; and stir bar sorptive extraction (SBSE) [26]. Notably, extraction technique selection depends on the characteristics of the pesticide, the matrix, and the system availability.

The SPME technique is widely utilized for extracting analytes from aqueous matrices in the laboratory. This technique employs a small-diameter fiber coated with a stationary phase placed in the headspace or directly (immersed) in the aqueous sample. The analytes migrate to the stationary phase and are thermally desorbed and separated using gas chromatography (GC). Initially, these fibers were composed of polydimethylsiloxane (PDMS), polyacrylate (PA), and polydimethylsiloxane/divinylbenzene (PDMS/DVB) polymers. However, other polymers have been evaluated for SPME methods for the analysis of pesticides; in the fruit field, the works of Li et al. [27] and Wang et al. [28] have been shown to be effective.

The number of studies discussing the usefulness of carbon nanomaterials (CNMs) as a sorbent has increased significantly in recent years. CNMs have a large specific surface area that is lightweight and highly porous. They form desirable interactions (via π - π and van der Waals interactions) with a diverse range of pesticides [29]. These properties bestow specific benefits to CNMs compared to other types of absorbent materials. Recently, our research group described an SPME device for pesticide extraction in water that employs fibers coated with CNMs [30]. The results highlighted the great absorption capacity of nanomaterials to remove contaminants. The

implementation of CNMs as passive sampling devices was also efficient when analyzing pesticides in water [31].

The present study measured pesticide concentrations in apple samples using the UAC-CNMs-SPME method coupled to GC-mass spectrometry (GC-MS). The CNMs were deposited in circular wire fibers by a chemical vapor deposition (CVD) process. Potential factors affecting the fruit cleaning and subsequent microextraction efficiency were investigated and optimized. The developed method was applied to assess trace pesticides in apples from grocery stores and fruit and vegetable stands in Belo Horizonte, Brazil.

2. Experimental

2.1. Chemicals and Materials. Four pesticides typically employed in agricultural areas such as apple tree fields in Brazil were selected for this study. In Brazil, organochlorine pesticides have been detected in the environment [32] and foods [33] despite the prohibition of these compounds [34]. Therefore, it is necessary to continue monitoring banned pesticide use, which requires greater supervision throughout the country.

The pesticide standards (>97% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The pesticides diazinon (CAS Number: 333-41-5), pirimiphos-methyl (CAS Number: 29232-93-7), chlorpyrifos (CAS Number: 2921-88-2), and trifluralin (CAS Number: 1582-09-8) were selected from among the typical pesticides employed in agricultural areas, stored products, and apple tree orchards in Brazil [35]. For the organochlorine pesticides, alpha-dichlorodiphenyldichloro ethylene (4,4-DDE, CAS Number: 72-55-9), dichlorodiphenyldichloroethane (4,4-DDD, CAS Number: 72-54-8), and methoxychlor (CAS Number: 72-43-5) were selected. Acetonitrile, methanol, and ethanol were high-purity, chromatography-grade and purchased from J. T. Baker (Xalostoc, Edomex, Mexico).

2.2. Apparatus and Chromatographic Conditions. The target pesticides were separated, detected, and quantified by a GC system (7890C, Agilent, Palo Alto, CA, USA) coupled to a mass spectrometer equipped with a quadrupole mass analyzer (5975C, Agilent). The starting oven temperature was 80°C, it was then ramped to 160°C at 20°C min⁻¹, to 255°C at 5°C min⁻¹, and to 280°C at 20°C min⁻¹ and finally held at this temperature for 1 min. The chromatographic analysis for the extract injection and desorption from the fiber was performed via injection in the splitless mode (splitless liners N° 5181-3315, Agilent) for 2 min at 250°C using a 5% phenyl, 95% dimethylpolysiloxane column (DB5MS, 30 m × 250 μm × 0.25 μm, Agilent) with a helium flow of 1.2 mL·min⁻¹. The analysis was performed in the selected ion monitoring (SIM) mode with electron ionization (EI) set at 70 eV. The injection volume was 1.5 μL, and the total analysis time was approximately 25 min. The mass to charge (m/z) ratios and retention times of the pesticides identified by GC-MS in this study are presented in Table 1.

2.3. Sample Collection. Apple samples were collected from two grocery stores and four fruit and vegetable stands in Belo Horizonte, Brazil. Three varieties of apples commonly

TABLE 1: Mass to charge (m/z) ratios and retention times of the pesticides identified by GC-MS.

^a Pesticides	Quantitation ions (m/z)	Identification ions (m/z)		Retention time
Trifluralin	306.0	264.0	289.9	8.28
Diazinon	137.0	179.1	152.0	9.81
Pirimiphos M.	290.0	276.0	305.0	12.55
Chlorpyrifos	196.8	96.9	313.9	13.14
4,4-DDE	246	317.9	176.0	16.56
4,4-DDD	235.0	165.0	198.9	18.03
Methoxychlor	227.0	152.0	212.0	21.32

^aCompounds are listed in sequence of elution.

consumed in Brazil were selected for this work: Fuji (*Malus pumila*), Gala (*Malus domestica*), and Red Delicious (*Malus domestica*). One kilogram of each apple variety was acquired from each location, packaged, labeled separately, and stored at 5°C prior to the experiment. In total, 18 apple samples were processed and analyzed.

2.4. Sample Preparation and CNM Microextraction Procedure. For the elaboration of the chromatographic method, a solution with a concentration of 100 µg·mL⁻¹ of the seven pesticides in acetonitrile was prepared. The apple sample spiked for optimization and validation processes was made from seven stock solutions as indicated in Table 2. All of the prepared stock and standard solutions were stored in the refrigerator at 5°C.

The sample preparation procedure used involved three processes: (a) ultrasound-assisted cleaning, (b) extraction and desorption, and (c) separation, detection, and quantification. The five steps of the analytical procedure are depicted in Figure 1.

In brief, (1) one kg of each apple sample variety was collected. Apples were prepared by cutting them into quarters, crushed in a food processor, and homogenized using a vortex (Ancap AT-1, São Paulo, Brazil) at 2400 rpm for 30 s. (2) Then, 0.5 g aliquots of homogenized apple samples were placed into vials along with 10.00 mL of distilled water and subjected to ultrasound for 3 min. (3) Next, salt (Na₂SO₄, 20% w/w) was added to the solution along with the CNMs (15 cm long fibers) to start the microextraction process. This process was carried out with a stirring speed of 300 rpm at room temperature (28°C) for 2 min. (4) Following microextraction, desorption was achieved by transferring the CNMs to a test tube containing 50.0 µL of acetonitrile and incubating them in a 65°C oil bath for 2 min. (5) Finally, 1.5 µL of the extract was injected into the GC-MS.

2.5. Optimization of the Ultrasound-Assisted Cleaning Process. Different apple sample amounts (0.5 g, 1.0 g, and 1.5 g) and ultrasonication times (1 min, 3 min, and 5 min) were evaluated and optimized for the ultrasound-assisted cleaning process to improve the extraction and recovery of the pesticides and the sample preparation speed.

2.6. Optimization of the CNM Microextraction Method. The optimization of the CNM microextraction procedure was performed in two stages. The first is related to the pesticide's absorption process, and the second is related to its

desorption process. Most variables were evaluated with multivariate optimization. In every case, different intervals were evaluated. The variables evaluated for the absorption process included temperature (28°C to 70°C), stirring speed (0 rpm to 600 rpm), salting-out effect (0% w/w to 20% w/w), and extraction time (2 min to 30 min). Additionally, the variables assessed for desorption included desorption time (2 min to 5 min) and temperature (50°C to 70°C). Before starting the multivariate optimization, some univariate tests were performed. Initially, the desorption modes (thermal desorption in solvent and direct desorption in the GC-MS injector) were evaluated. A CNM microextraction syringe manufactured by our research group was employed for the direct desorption test [30]. Once this parameter was defined, the solvent desorption modes (heating plate, ultrasound bath, and vortex agitation) were investigated. Desorption solvents including methanol, ethanol, and acetonitrile were also evaluated.

2.7. Method Validation. The calibration curve was constructed using spiked apple samples, thus avoiding the matrix effect and its unfavorable influence on quantitative and qualitative analyte determination. The Eurachem guide and ICH guidelines were used for method validation [36, 37], and linearity, recovery, limits of detection (LODs), limits of quantification (LOQs), and precision intraday and interday were evaluated.

Linearity was evaluated in the range from 1.0 ng·g⁻¹ to 300.0 ng·g⁻¹, with seven concentrations. The range of concentrations was selected according to the maximum residue limits (MRLs) for pesticides in food established by different national and international legislations. This parameter was evaluated through the external standard calibration curve, prepared in the sample matrix. Each concentration was analyzed in triplicate with the same sampling procedure.

Precision and recovery were calculated with three pesticide concentrations (2.0 ng·g⁻¹, 100.0 ng·g⁻¹, and 250.0 ng·g⁻¹), and six replicates were conducted using a white matrix. The precision method was represented as repeatability and intermediary precision, which were calculated by extracting and quantifying each pesticide from the real matrix on the same day (intraday) and two days later (interday). For the fiber-to-fiber repeatability, five fibers were evaluated under the same extraction conditions.

LODs and LOQs were calculated from (1) and (2) according to ICH guidelines.

TABLE 2: Analyte stock solutions and spiking volumes for optimization and validation studies.

Concentration level	Stock solution (mg·L ⁻¹)	Sample volume spiked (μL)	Apple mass (g)	Final concentration (ng·g ⁻¹)
Level 1	0.05	10.0	0.5	1.0
Level 2	0.35	10.0	0.5	7.0
Level 3	1.5	10.0	0.5	30.0
Level 4	2.5	10.0	0.5	50.0
Level 5	5.0	10.0	0.5	100.0
Level 6	10.0	10.0	0.5	200.0
Level 7	15.0	10.0	0.5	300.0

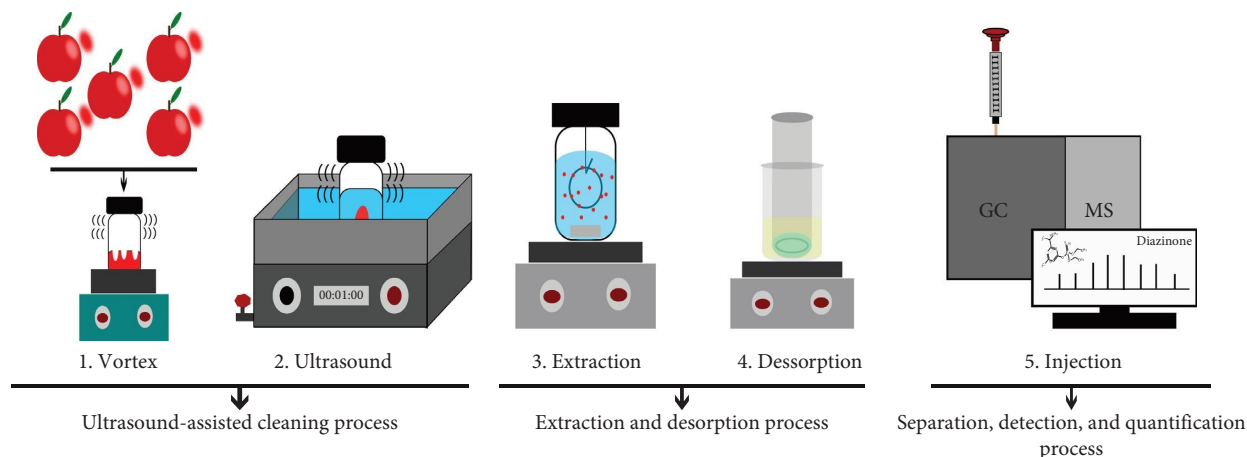


FIGURE 1: Main steps for pesticides analysis in apple samples. Ultrasound-assisted cleaning process, extraction and desorption process, and separation, detection and quantification process. (1) Homogenization of 100 g of chop unwashed apple samples; (2) Ultrasound-assisted cleaning of 0.5 g of the sample; (3) Pesticides extraction using CNMs fibers, (4) CNMs desorption with acetonitrile at 65°C; (5) Injection and analysis by GC-MS.

$$\text{LOD} = 3.3 * \frac{S_d}{b}, \quad (1)$$

$$\text{LOQ} = 10 * \frac{S_d}{b}, \quad (2)$$

where b is the slope of the calibration function and S_d is the standard deviation of residuals (S_{yx}).

2.8. Preparation and Characterization of CNM Fibers. The data related to the preparation and characterization of the CNM fibers were described previously [30, 31], providing some carbon structure information. According to Raman spectroscopy experiments (Supplementary Material; Figure S1), G and D bands (1584 cm⁻¹ and 1358 cm⁻¹, respectively) were observed in the sample spectrum. It was previously reported that the G band observed in the substrate deposited on the metal surface is related to graphitic-like structures, and the D band is related to defects in these structures [31]. This observation made it possible to determine the types of synthesized CNMs. Moreover, the scanning electron microscopy (SEM) images (Supplementary Material; Figure S2) revealed the complete covering of the rod with a rough layer on the steel after CNM growth [31].

3. Results and Discussion

3.1. Chromatographic Separation of Pesticides. Figure 2 shows the chromatographic separation of the seven studied pesticides using ultrasound-assisted cleaning and CNMs as pretreatment and extraction methods, respectively. A good chromatographic separation was achieved with perfect resolution for the pesticides, with a run time of approximately 22 min.

3.2. Optimization of the Extraction Desorption Procedure

3.2.1. Ultrasound-Assisted Cleaning Process. It has been reported that 0.5 kg to 2.0 kg of fruit or vegetable samples, homogenized by a mixer for subsequent extraction, is required to determine pesticide residues [38]. The homogenized subsamples collected for further extraction weighed between 0.5 g and 100 g [38]. Following this recommendation, our analysis used one kg of apples and subsamples ranging from 0.5 g to 1.5 g for the ultrasound-assisted cleaning process (Figure 3). Although there is no significant difference in peak area for each sample amount, it is possible to observe that increased sample quantity decreased the pesticide extraction efficiency. It is plausible that this trend is associated with the higher pesticide absorption

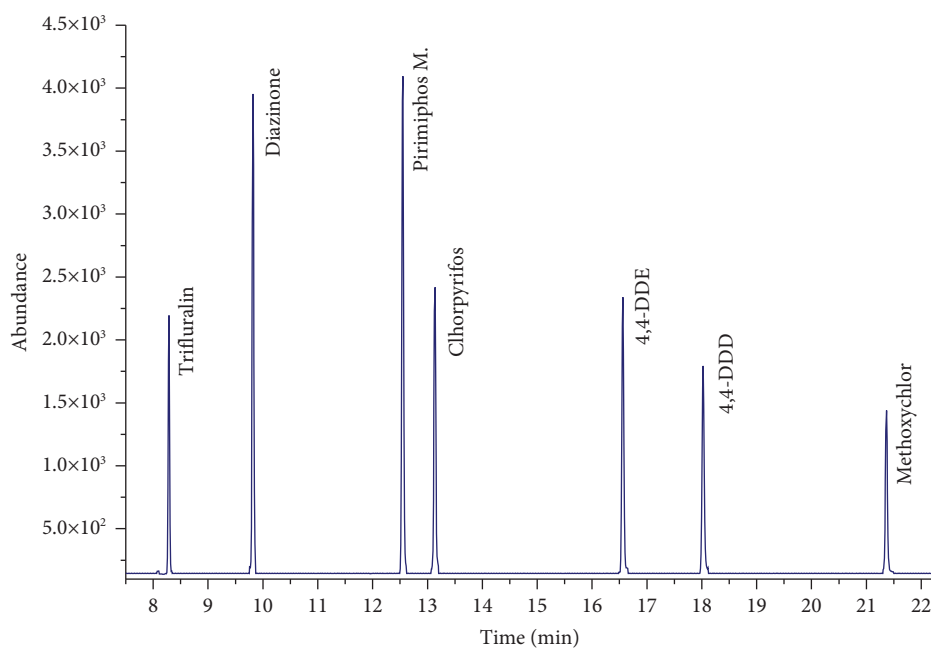


FIGURE 2: Chromatogram obtained for pesticide analysis in apple peel using carbon nanomaterial microextraction and GC-MS determination. Spiked sample at $200 \text{ ng}\cdot\text{g}^{-1}$. Chromatographic conditions are given in the Experimental section.

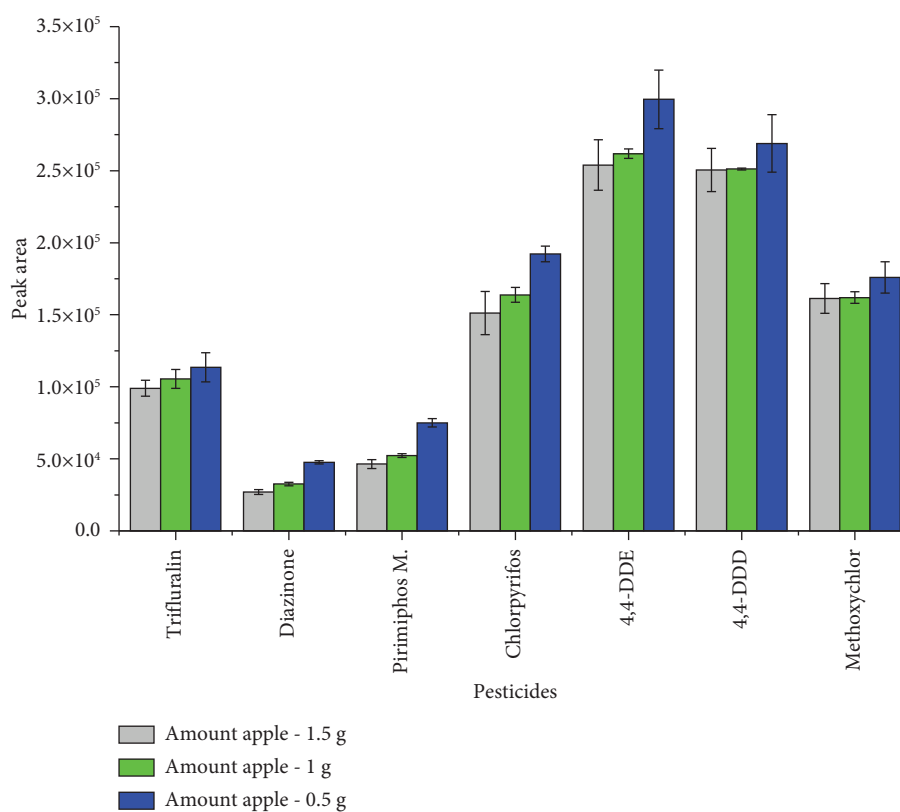


FIGURE 3: Influence of the apple sample mass on the extraction of pesticides. Amount of apple sample for pesticide extraction using ultrasound bath (5 min) and CNM microextraction. Spiked sample at $200 \text{ ng}\cdot\text{g}^{-1}$ and 15 cm long CNM fibers.

capacity that represents a larger sample area. Based on these results, it was determined that 0.5 g of subsample was the optimal amount of apple sample.

The ultrasonication time during the cleaning process was varied from 1 min to 5 min to determine the minimal sample preparation time. As shown in Figure 4, 1 min of ultrasonic

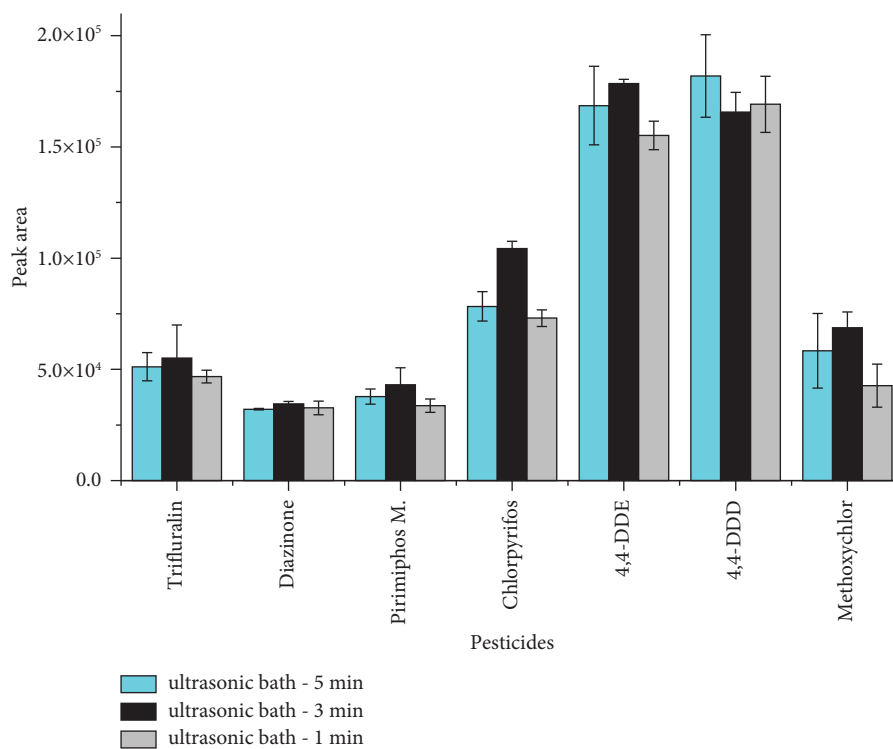


FIGURE 4: Influence of ultrasonication time on the extraction of pesticides. Ultrasound bath time to desorb the pesticides from the apple sample. Spiked sample at $200 \text{ ng}\cdot\text{g}^{-1}$ and 15 cm long CNM fibers.

extraction was sufficient to displace pesticides from apple samples into the aqueous medium. During the method validation phase, it was found that this short extraction duration yielded high recovery values (79% to 98%).

3.2.2. Absorption Condition for CNM Microextraction.

The results for extraction optimization were obtained through the full factorial study represented in individually obtained Pareto charts (Supplementary Material; Figure S3). Pareto charts represent the possible strength of the effect of optimized variables. Although these graphs do not make it possible to establish the ideal values for each variable studied, they allow us to identify which variables affect the extraction process and which level of the evaluated interval (high or low) presents better yields. The influence of the evaluated variables was the same for the seven pesticides. All Pareto plots indicate that the effects of temperature and salting out and their respective interaction (1 by 4, in Figure 5) influenced the extraction of the pesticides at a 5% significance level. The Pareto chart of the standardized effects for diazinon is presented in Figure 5 and illustrates two important conclusions. First, temperature and salting-out effects negatively and positively influenced pesticide extraction, respectively. The results indicate that low temperature and high salting-out values enhance the peak areas of analytes. A logical explanation is that low temperatures can attenuate pesticide distribution in the gas phase, preventing reduced adsorption to the extraction medium [39]. Concerning the salting-out effect, increased salt concentrations reduce the pesticides' solubility in water, fostering the interactions with CNMs and generating a better extraction.

The second conclusion was that any value within the stirring speed and extraction time intervals evaluated could be employed since changing these variables did not influence the pesticide extraction. In general, time and stirring speed are important factors in the extraction process in numerous conventional SPME pesticide isolation and detection methods employing multiwalled carbon nanotubes (MWCNTs) [39–41]. This discrepancy could be due to how the CNMs were prepared on the steel threads. For applying CNM coatings for SPME, methods such as physical deposition, chemical bonding, electrochemical sol-gel technology, and electrophoretic depositions have been reported [42]. Herein, the CNM coatings were supported directly on steel threads as a novel way to obtain the fibers through the CDV process. These coatings are less likely to detach from the supports, reducing the time needed to reach the pesticides' partition equilibrium between the fiber coating and the sample solution. In comparative terms, the optimum extraction time with the most commonly used commercially available SPME fibers (PDMS/DVB, PA, and PDMS) varies from 10 to 100 min (5 to 50 times greater than the extraction time in this research). The substantial decrease in the sample preparation time using the extraction process described herein could increase the sample throughput of laboratories that perform pesticide analyses.

3.2.3. Desorption Condition for CNM Microextraction.

The two desorption modes typically used in routine analyses are thermal desorption in the solvent and direct desorption in the GC-MS injector (Figure 6). Initially, both desorption

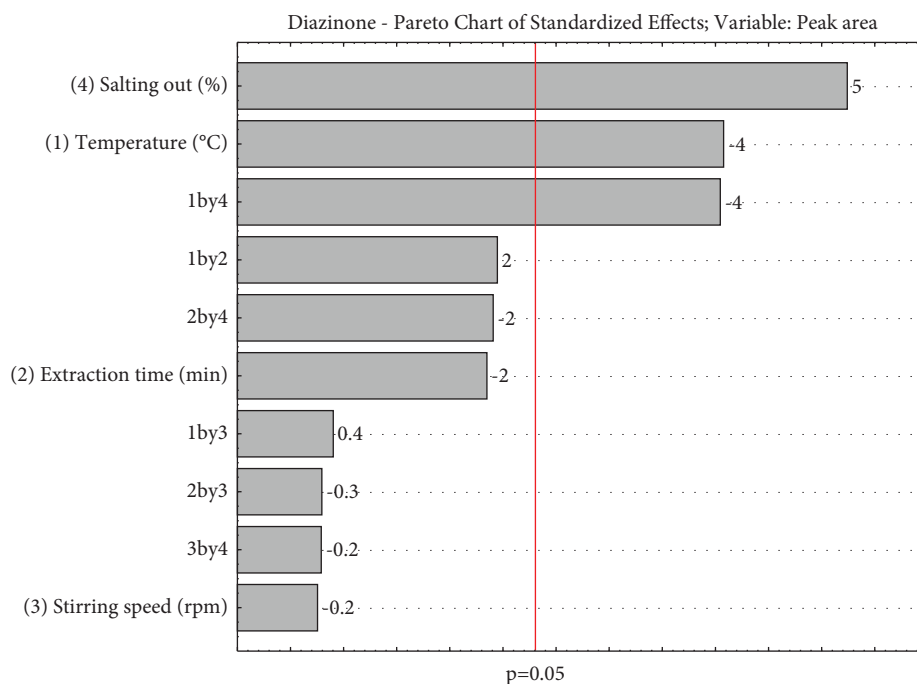


FIGURE 5: Pareto charts of standardized effects for diazinon extraction with carbon nanomaterials; variable: peak area, $2^{(4-0)}$ design. Spiked sample at $200 \text{ ng}\cdot\text{g}^{-1}$ and 15 cm long CNM fibers.

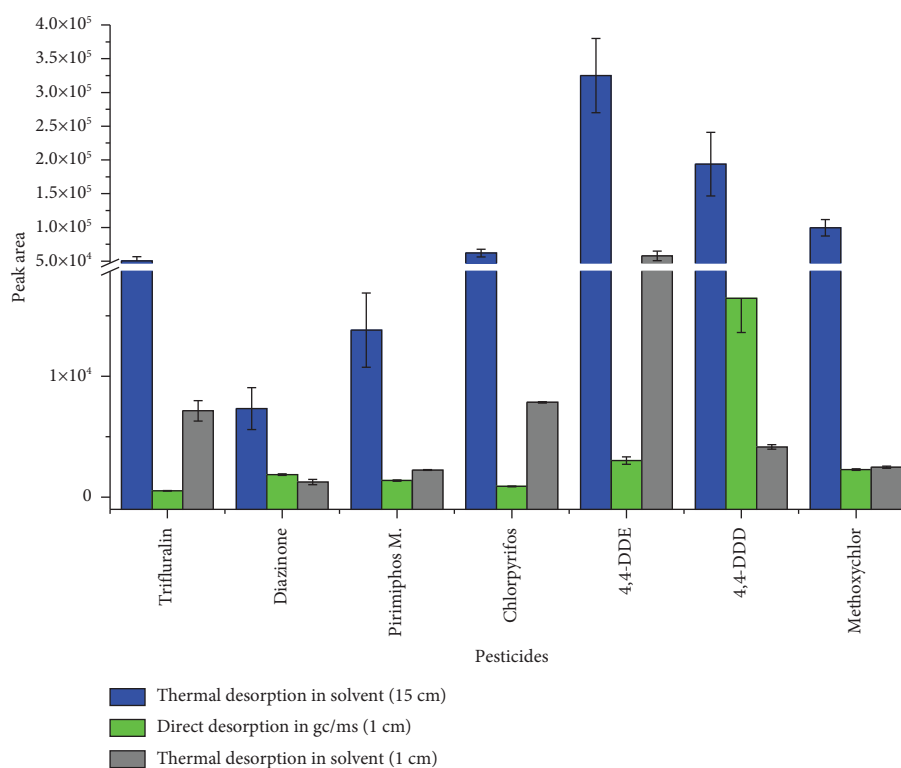


FIGURE 6: Comparison between the desorption modes, thermal desorption in solvent with 1 cm fiber or 15 cm and direct desorption in GC-MS ($n = 3$). Extraction: spiked sample at $200 \text{ ng}\cdot\text{g}^{-1}$, 10.00 mL of water, 0 w/w salt, extraction in the immersion mode, stirring of 300 rpm, room temperature, and extraction time of 2 min. Desorption: $50 \mu\text{L}$ of acetonitrile and injector temperature of 250°C .

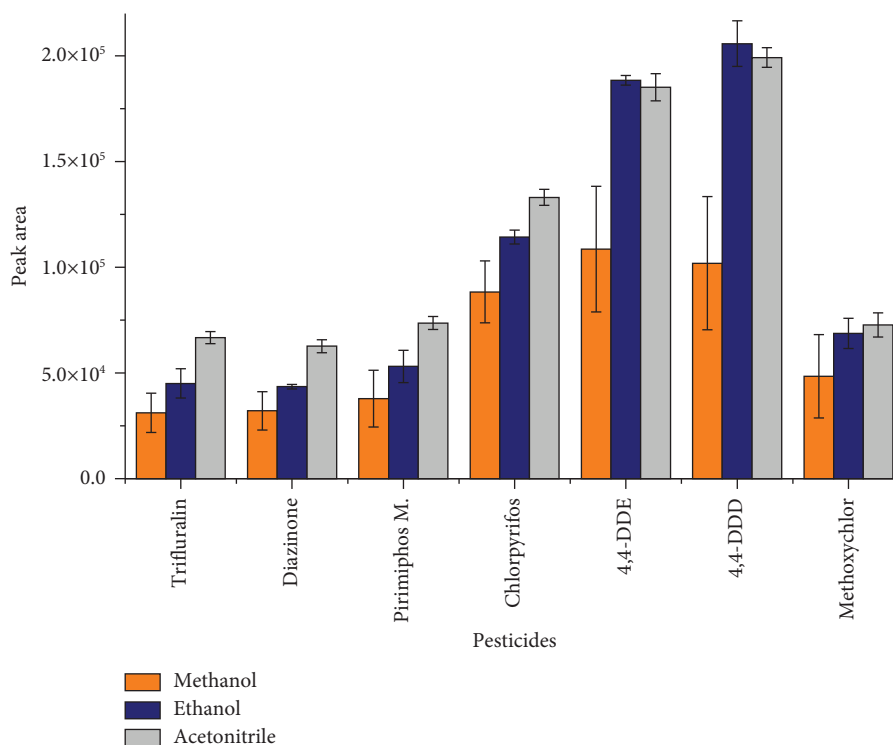


FIGURE 7: Comparison between the desorption solvents, methanol, ethanol, and acetonitrile ($n = 3$). Conditions for extraction: spiked sample at $200 \text{ ng}\cdot\text{g}^{-1}$, 10.00 mL of water, 0 w/w salt, extraction in the immersion mode, stirring of 300 rpm, room temperature, and extraction time of 2 min. Conditions for desorption: $50 \mu\text{L}$ of solvent, desorption time of 5 min, desorption temperature of 70°C , and 15 cm long CNM fibers.

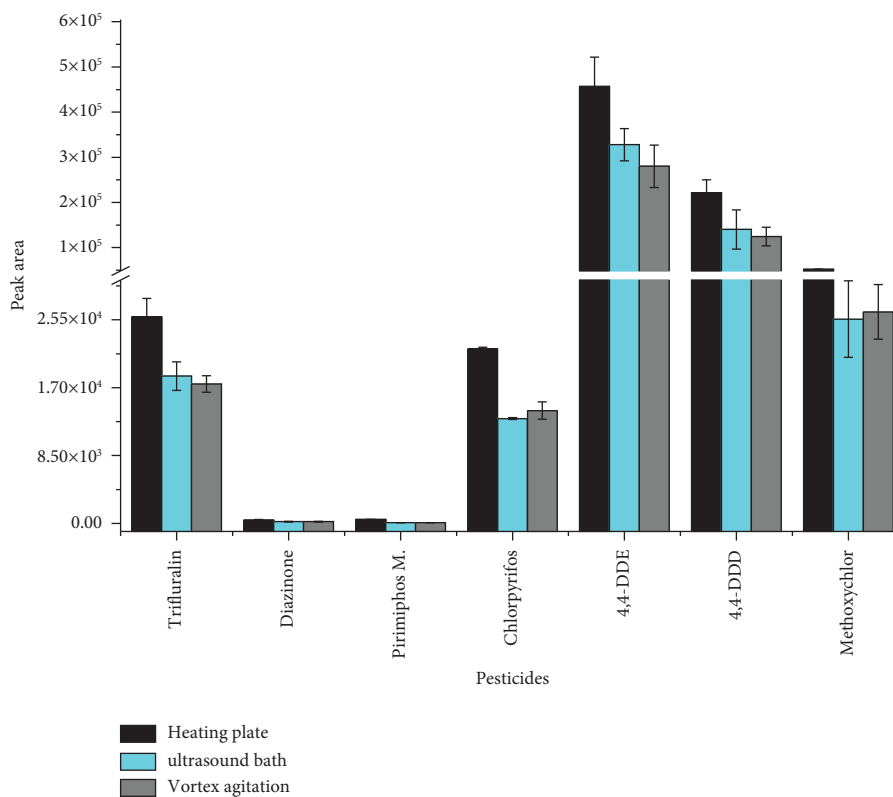


FIGURE 8: Study of solvent desorption modes (acetonitrile), heating plate (70°C), ultrasound bath, and vortex agitation ($n = 3$). Conditions for extraction: spiked sample at $200 \text{ ng}\cdot\text{g}^{-1}$, 10.00 mL of water, 0 w/w salt, extraction in the immersion mode, stirring of 300 rpm, room temperature, and extraction time of 2 min. Conditions for desorption: $50 \mu\text{L}$ of acetonitrile, desorption time of 5 min, desorption temperature of 70°C , and 15 cm long CNM fibers.

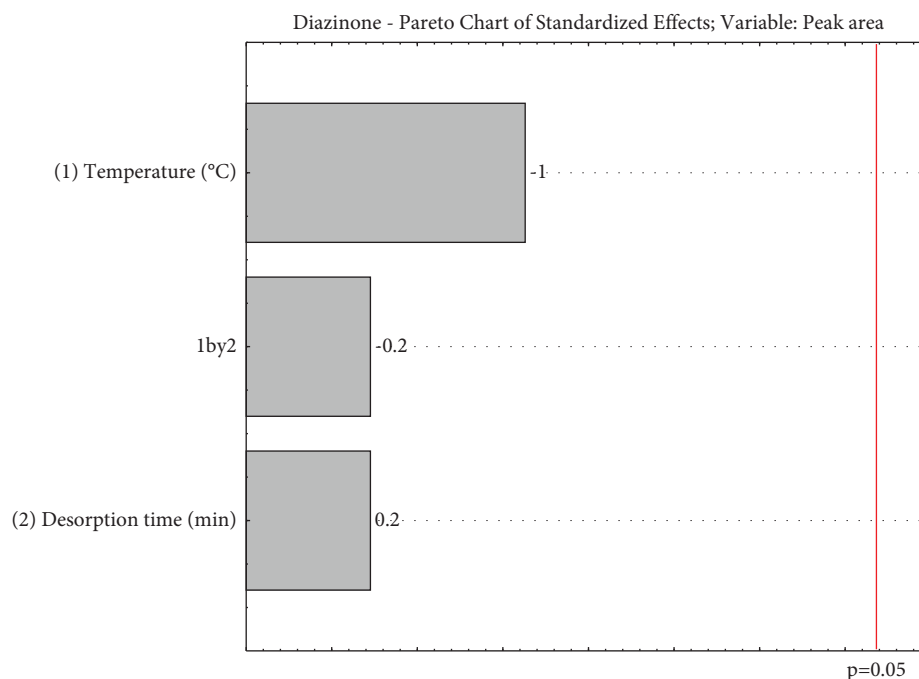


FIGURE 9: Pareto charts of standardized effects for diazinon desorption after carbon nanomaterial microextraction; variable: peak area, $2^{(2-0)}$ design. Spiked sample at $200 \text{ ng}\cdot\text{g}^{-1}$ and 15 cm long CNM fibers.

TABLE 3: Results of method optimization using ultrasound-assisted cleaning and CNMs as stationary phase during SPME for 7 pesticides in apples.

Variables	Influence	Selected value or solvent
<i>Ultrasound-assisted cleaning process</i>		
Amount of apple sample	A smaller amount of sample increases the extraction efficiency	0.5 g
Ultrasonication time	The variable did not influence the ultrasound-assisted cleaning process	1 min
<i>Pesticide absorption process</i>		
Absorption temperature	The variable negatively influenced pesticide absorption. Low values of temperature increase the peak area of the analytes.	28°C
Absorption time	The variable did not influence pesticide absorption. Any value within the intervals evaluated can be used.	2 min
Stirring speed	The variable did not influence pesticide absorption. Any value within the intervals evaluated can be used.	300 rpm
Salting-out	The variable positively influenced pesticide absorption. High values of salt increase the peak area of the analytes.	20% w/w
<i>Pesticide desorption process: heating plate</i>		
Desorption temperature	The variable did not influence pesticide desorption	50°C
Temperature desorption	The variable did not influence pesticide desorption	2 min
Desorption solvent	The variable influenced the desorption process	Acetonitrile

modes were compared using CNM fibers 1 cm in length. As expected, solvent-free direct desorption of the GC-MS yielded better chromatographic signals for most pesticides. This difference between direct desorption in GC inlet and solvent desorption is because in the direct desorption in GC inlet, almost all analytes should reach the column, while for solvent desorption, only about 3% ($1.5 \mu\text{L}/50 \mu\text{L}$) will reach the column. Although a significant increase in peak areas of analytes was observed, it did not reach the maximum contaminant levels (MCLs) of pesticides in food. It is important to highlight that the injector dimensions limit the

fiber length used in direct desorption. Thus, the application of a longer fiber (15 cm) in the solvent desorption mode was evaluated to determine if this modification could improve the sensitivity of the extraction method. Indeed, the 15 cm fiber produced better sensibility results (Figure 6). The responses increased by a factor of 11 to 19 compared to the fiber of 1 cm. Thus, our results show that this novel method can detect pesticides with MCLs in the trace (e.g., $\text{ng}\cdot\text{g}^{-1}$ to $\mu\text{g}\cdot\text{g}^{-1}$) range in food.

Acetonitrile showed better recovery and reproducibility in the desorption solvent tests than methanol (Figure 7).

TABLE 4: Main parameters evaluated in the validation process for detecting pesticides in apple samples using ultrasound bath and carbon nanomaterials.

Pesticides ^a	Working range (ng.g ⁻¹)	Recovery (%) ^b	Linearity coefficient (R ²)	Precision RSD* (%) intraday-interday	LOQ [#] (ng.g ⁻¹)	LOD ⁺ (ng.g ⁻¹)
Trifluralin	0.6–300.0	98.0 ± 9.6	0.9929	6 10	0.6	0.2
Diazinon	0.6–300.0	79.0 ± 6.8	0.9924	7 14	0.6	0.2
Pirimiphos M.	0.7–300.0	93.1 ± 5.7	0.9916	6 7	0.7	0.3
Chlorpyrifos	0.7–300.0	91.0 ± 7.6	0.9923	6 13	0.7	0.3
4,4'-DDE	0.5–300.0	94.1 ± 3.5	0.9962	6 9	0.5	0.1
4,4'-DDD	0.5–300.0	89.3 ± 4.4	0.9959	7 8	0.5	0.1
Methoxychlor	0.5–300.0	82.2 ± 11.7	0.9915	11 12	0.5	0.1

Compounds are listed in sequence of elution; ^b parameters evaluated with a mean of three concentration levels (2.0 ng.g⁻¹, 100.0 ng.g⁻¹, and 250.0 ng.g⁻¹); ^c relative standard deviation; [#] limit of quantification; ⁺ limit of detection.

TABLE 5: LODs, LOQs, mean recoveries, and linearity of pesticides in food sample using CNMs as the sorbent in different extraction techniques.

Parameter	SPME BN@MCNTs ^S [40]	SPME MMWCNTs [#] [48]	SPME MNPs/MMWCNTs ⁺ [49]	QuEChERS [50]	SPE [51]	DSPE* [52]
LOD (ng·g ⁻¹)	0.01–0.20	—	6–24	—	1.85–7.34	0.5–5.0
LOQ (ng·g ⁻¹)	0.03–0.67	100–250	20–80	0.1–2.7	—	1.5–16.7
Recovery (%)	83.7–124	73.4–110.9	72.5–99.1	73.6–97.4	73–103	78.2–113.9
Linearity (ng·g ⁻¹)	0.03–200	500–2000	80–200	2–500	—	0.5–100

^SBoron nitride modified multiwalled carbon nanotube material (fiber); [#]magnetic multiwalled carbon nanotube (powder); ⁺magnetic nanoparticles and multiwalled carbon nanotubes (powder); *dispersive solid-phase extraction.

TABLE 6: Application of the analytical method in apple samples (detected pesticides and their respective calculated concentrations).

Pesticides	Concentration found (ng·g ⁻¹)	Total positive sample/kind of apple	Regulation of pesticide residues on food (ng·g ⁻¹)	
			Regulation EC 396/2005 [53]	PARA [54]
Trifluralin	12.1	1/Fuji	10	NA
Diazinon	35.7	1/Fuji	100	500
Chlorpyrifos	ND	—	10	1000
Pirimiphos M.	4.1 and 14.8	2/Gala	10	NA
4,4-DDE	ND	—	50*	NA
4,4-DDD	ND	—	50*	NA
Methoxychlor	ND	—	10	NA

ND: not detected; NA: not authorized; *sum of p,p'-DDT, o,p'-DDT, 4,4-DDE, and 4,4-DDD.

Ethanol displayed similar recovery values as acetonitrile for organochlorine pesticides but a lower recovery for organophosphate pesticides. In addition to acetonitrile being the best desorption solvent assessed, it also solubilizes pesticides well and has a high boiling point permitting high desorption temperatures [43]. Furthermore, similar studies have utilized acetonitrile.

In Figure 8, three solvent desorption modes: heating plate, ultrasound bath, and vortex agitation, were compared. Desorption on a heating plate with an oil bath showed the best result for all pesticides. This result is likely because heating facilitates pesticide desorption from the CNMs to the solvent. Another study reported similar efficiency using ultrasound desorption and heating desorption with CNMs during pesticide extraction [43]. Notably, the authors used powdered CNMs to promote the free movement of the nanoparticles during ultrasound desorption, which improved efficiency.

Since the heating plate method produced the best results, the desorption time and desorption temperature values were evaluated to optimize the protocol. In Figure 9, the Pareto charts of the standardized effects of temperature and time of desorption for diazinon using the 2²⁻⁰ design are presented. Neither of these variables influenced pesticide desorption; hence, it is possible to use 2 min to 5 min for the desorption time and 50°C to 70°C for the desorption temperature. This behavior was observed in all pesticides analyzed individually (Supplementary Material; Figure S4).

4. Method Evaluation

The variables used for the validation process were adjusted according to the optimization results (Table 3). For this, the

values (in the univariate optimization) or levels (in the full factorial study) that showed the best results in the extraction of pesticides were selected. The calibration curves were constructed by the least-squares method weighted (LSMW) since the instrumental responses showed heteroscedasticity of variance. Results of method validation using CNM microextraction are shown in Table 4.

The working range was established using the LOQ and the upper end of the instrument working range for each pesticide validation [36]. These values were in the concentration range of 0.5 ng·g⁻¹ to 300.0 ng·g⁻¹. The determination coefficients (*R*²) ranged from 0.9915 to 0.9962. The intraday and interday precision (RSDs, *n* = 6) at three concentration levels (2.0 ng·g⁻¹, 100.0 ng·g⁻¹, and 250.0 ng·g⁻¹) ranged from 6% to 14%, respectively.

The LODs and LOQs were in the range of 0.1 ng·g⁻¹–0.3 ng·g⁻¹ and of 0.5 ng·g⁻¹–0.7 ng·g⁻¹, respectively. Notably, our approach yields low LOD and LOQ values. Other studies that applied CNMs for pesticide analyses using SPME processes have reported detection or quantification limits similar to those presented above [44], even with a green coating (cork) [45]. Moreover, the results were better than [46] or similar to [47] using other extraction techniques. In terms of recovery from samples spiked with pesticides at three concentration levels, 79% to 98% was extracted. For a better comparison and interpretation, Table 5 shows some parameters of the validation of pesticides in food samples using CNMs as the sorbent with SPME and others extraction techniques.

The fiber-to-fiber repeatability demonstrated excellent results (RSD < 4%). This RSD value is an excellent indicator not only of repeatability in extraction but also in fiber synthesis.

5. Application to Apple Samples

The results of apple sample analyses by the described method are shown in Table 6. 18 apple samples were collected from grocery stores and markets in Belo Horizonte, Brazil. Pesticides including trifluralin (dinitroaniline) and the organophosphate compounds, diazinon and pirimiphos-methyl, were detected in four samples. The PARA (*Programa de Análise de Resíduos de Agrotóxicos em Alimentos*) and PNCRC (*Plano Nacional de Controle de Resíduos e Contaminantes*) pesticide residue monitoring programs reported evidence of these specific pesticides in Brazil between 2001 and 2010 [55]. It should be pointed out that a previous study by our group detected organochlorine compounds in Brazilian environmental water [30, 31]; however, they were not detected in the present study.

In Brazil, the MRLs for all crops and pesticides can be found in the PARA of ANVISA (*Agência Nacional de Vigilância, Sanitária*), which continuously evaluates pesticide residue levels in foods of plant origin. According to these limits, none of the detected pesticides exceeded acceptable MRL values. However, the trifluralin and pirimiphos-methyl values reported herein exceeded the MRL values of other international regulatory agencies with stricter limits, such as the European Commission (regulation EC 396/2005).

6. Conclusions

Ultrasound-assisted cleaning combined with the CNM microextraction technique is an excellent alternative for detecting and quantifying pesticide residues in apples. Besides offering a fast and efficient sample preparation (approximately 6 min), this novel method displays quantification limits lower than the MRLs. During the analysis of apple samples, four of the 18 samples analyzed contained detectable concentrations of at least one of the seven pesticides studied. Although organochlorine pesticides have been detected in Brazilian water sources, these chemicals were not present at detectable levels in the analyzed apple samples.

It should be pointed out that biological materials such as microbial substances are also categorized as a contaminant in food materials. However, these potential contaminants were not observed, thus demonstrating the selectivity of the UAC-CNMs-SPME method for extracting pesticide residues.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Highlights. (1) An analytical method for 7 pesticides in apples using UAC, CNMs, and GC-MS was developed. (2) The sample preparation process used in this analytical method takes less than seven minutes. (3) The method was

shown to be accurate and precise for absorbing organochlorine pesticides, which are normally difficult to extract in water. (4) Diazinon, pirimiphos-methyl, and trifluralin were detected in apple samples in Brazil.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

This section includes Raman spectrum of the stainless-steel rod with CNMs, SEM figures of the stainless-steel rod before and after the CVD process, and part of the optimization related to Pareto charts of the different pesticides during adsorption and desorption process. (*Supplementary Materials*)

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