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Original article

Optimization of the methodology for the extraction of organophosphate pesticides by HS–SPME–GC–NPD

Optimización de la metodología de extracción de plaguicidas organofosforados por HS-SPME-GC-NPD

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Abstract

Organophosphate compounds are widely present in the environment and are known to exert harmful effects on human health by inhibiting acetylcholinesterase (AChE), a key enzyme of the nervous system. This study aimed to optimize a solvent-free extraction methodology to determine five organophosphate residues —chlorpyrifos, methamidophos, fenitrothion, dichlorvos, and dimethoate— in water samples using solid-phase microextraction (SPME) combined with gas chromatography and nitrogen–phosphorus detection (GC–NPD). We applied an experimental design to evaluate five critical variables: fiber type, extraction time, temperature, stirring speed, and salt concentration. The best extraction performance was achieved by using a DVB/CAR/PDMS fiber for 20 minutes at 50°C, 500 rpm, and 1% NaCl. Under these conditions, the method had excellent linearity (r^2 up to 0.9892), detection limits between 1.088 and 3.114 µg/L, quantification limits between 3.264 and 9.342 µg/L, and precision with %RSD values ranging from 0.998 to 3.599. These results confirmed that the proposed method is fast, simple, sensitive, and robust. Its optimization contributes to the development of green analytical strategies for monitoring pesticide contamination in aqueous environments.

Keywords: Environmental analysis; Green methodology; GC–NPD; SPME; Organophosphorus compounds.

Resumen

Los compuestos organofosforados se encuentran ampliamente en el medio ambiente y se sabe que ejercen efectos nocivos en la salud humana al inhibir la acetilcolinesterasa (AChE), una enzima clave del sistema nervioso. El objetivo de nuestro estudio fue optimizar una metodología de extracción sin disolventes para la determinación de cinco residuos organofosforados, clorpirifos, metamidofós, fenitrotión, diclorvos y dimetoato, en muestras de agua mediante microextracción en fase sólida (SPME) combinada con cromatografía de gases y detección de nitrógeno-fósforo (GC-NPD). Se aplicó un diseño experimental para evaluar cinco variables críticas: tipo de fibra, tiempo de extracción, temperatura, velocidad de agitación y concentración de sal. El mejor rendimiento en la extracción se obtuvo utilizando una fibra DVB/CAR/PDMS durante 20 minutos a 50 °C, 500 rpm y 1% de NaCl. Bajo estas condiciones el método demostró una excelente linealidad (r^2 hasta 0,9892), límites de detección entre 1,088 y 3,114 µg/L, límites de cuantificación entre 3,264 y 9,342 µg/L, y una precisión con valores de %RSD que oscilaron entre 0,998 y 3,599. Estos resultados confirmaron que el método propuesto es rápido, sencillo, sensible y sólido. Su optimización contribuye al desarrollo de estrategias analíticas ecológicas para la monitorización de la contaminación por pesticidas en ambientes acuosos.

Palabras clave: Cromatografía; GC-NPD; Organofosforados; Plaguicidas.

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Introduction

Organophosphate pesticides are a group of organic compounds containing a phosphorus atom typically bonded to three or four oxygen atoms and one sulfur atom (**Fernández-de Miguel *et al.***, 2009; **Wu *et al.***, 2024). A wide variety of compounds belonging to this family are classified and used in industrial, medical, military, and agricultural sectors. Their extensive application, particularly in agriculture, has led to increasingly frequent reports of pesticide poisoning, especially among farmers who handle these substances without appropriate protective measures **(Gervilla-Caño *et al.***, 2007; **Bravo *et al.***, 2022).

Organophosphates replaced organochlorine pesticides, which were banned or restricted due to their severe environmental and health effects. However, organophosphates themselves also pose significant risks to ecosystems and human health, although their use remains legal in many countries (**Fernández *et al.***, 2010; **Mali *et al.***, 2023). The primary toxicological mechanism of organophosphates involves the irreversible inhibition of acetylcholinesterase (AChE), a key enzyme in the transmission of nerve impulses. This inhibition can result in neurotoxicity, muscle paralysis, and, in severe cases, death. Additional effects include damage to vital organs such as the liver and heart (**Toro-Osorio *et al.***, 2017; **Ciriello *et al.***, 2018).

Exposure routes to organophosphates include direct contact during pesticide application, ingestion of contaminated food, and consumption of water polluted by agricultural runoff. In Colombia, for example, surface and groundwater sources are frequently contaminated by agrochemical residues, particularly in regions with intensive monoculture practices **(Philippat *et al.***, 2018). The severity of poisoning depends on the exposure level and route, ranging from mild symptoms to severe intoxication and death (**Castro *et al.***, 2004; **Murcia & Stashenko**, 2008). Given their chemical stability and environmental persistence, residues of these pesticides are commonly found in fruits, vegetables, and water, demanding continuous monitoring and regulation (**Wani *et al.***, 2019). The International Union of Pure and Applied Chemistry (IUPAC) agrochemical commission has emphasized the importance of monitoring the full life-cycle of these compounds, from their application on crops to the final residue present in consumable products (**Guerrero**, 2003).

In Colombia, the use of pesticides such as methamidophos, parathion, and endosulfan is still permitted in agriculture (**Betancourt-Arango *et al.***, 2025), although some, including fenitrothion, malathion, and glyphosate, are under surveillance due to rising concerns about their toxicity and environmental impact (**Jaramillo-Colorado**, 2016). However, updated national data show increasing detection of organophosphate residues in water bodies and food products, especially in high-production agricultural zones like Valle del Cauca and Antioquia, underscoring the urgent need for more effective monitoring techniques.

For the identification and quantification of organophosphates in complex matrices, highly sensitive and selective analytical techniques are essential. Gas chromatography (GC) coupled with selective detectors such as the nitrogen phosphorus detector (NPD) (**Betancourt-Arango *et al.***, 2021), or with mass spectrometry (GC–MS) is widely recognized for its capability to detect trace levels of these compounds (**Quintero *et al.***, 2013; **Gavidia *et al.***, 2017). The NPD offers increased sensitivity for analytes containing nitrogen and phosphorus atoms, which makes it especially suitable for organophosphates. However, the success of any chromatographic method depends heavily on the sample preparation step.

In recent years, there has been a strong push toward "green" analytical chemistry practice. In this context, solid-phase microextraction (SPME) has emerged as a powerful solvent-free extraction technique that significantly reduces environmental impact while maintaining high analytical performance **(Fabjanowicz *et al.***, 2018). SPME enables efficient pre-concentration of analytes with minimal sample handling and no need for toxic solvents, which is ideal for environmental applications, particularly in low-resource settings or routine analysis scenarios where simplicity and reproducibility are essential (**Anjos & Andrade**, 2014; **Mu *et al.***, 2018; **Cao *et al.***, 2020; **Vievard *et al.***, 2022).

Despite prior studies on the use of SPME for organophosphate analysis (**Tsoukali *et al.***, 2005; **Milhome *et al.***, 2011), few have optimized multiple extraction parameters simultaneously under GC-NPD conditions, particularly in the context of water analysis in Colombia. Some of these studies report the use of headspace SPME (HS-SPME), while others rely on direct immersion (DI-SPME), and often do not provide clear methodological distinctions. Our main objective, therefore, was to develop and validate a robust, sensitive, and environmentally sustainable method for the detection of five organophosphate pesticides, chlorpyrifos, methamidophos, fenitrothion, dichlorvos, and dimethoate, in aqueous samples using SPME coupled with GC-NPD. Extraction conditions were optimized through a factorial experimental design evaluating five critical variables: fiber type, extraction time, extraction temperature, stirring speed, and NaCl concentration, each at three levels. The novelty of the study lies in its comprehensive optimization approach, its use of an accessible detection system (GC-NPD), and its potential applicability in real environmental monitoring scenarios in Colombia and other similar contexts.

Materials and methods

Reagents

We evaluated the following organophosphate pesticides: methamidophos (CAS No. 10265-92-6), chlorpyrifos (CAS No. 2921-88-2), dichlorvos (CAS No. 62-73-7), fenitrothion (CAS No. 122-14-5), and dimethoate (CAS No. 60-51-5). All pesticide standards were of ≥98% purity and obtained from Sigma-Aldrich® (USA). Ethyl acetate (≥98% purity) was purchased from PanReac AppliChem® (Spain), the solid-phase microextraction (SPME) fibers of various coatings were obtained from Supelco® (USA), and ultrapure water (Type 1) was produced using a Milli-Q purification system.

Standards preparation

Stock solutions of each pesticide (1000 mg/L) were prepared individually in ethyl acetate and stored at −20°C in amber glass vials to prevent photodegradation. Working solutions were prepared by diluting the stock solutions with ethyl acetate to concentrations of 1 and 5 mg/L for each pesticide. These working solutions were mixed in 10 mL volumes and used to determine the retention times of each compound, and to establish the limits of detection (LOD) and quantification (LOQ) for the analytical method.

Extraction conditions

The extraction of organophosphate pesticides was performed using headspace solid-phase microextraction (HS-SPME). We used a volume of 10 mL of ultrapure water (Type 1) previously spiked with a mixture of the five target pesticides at a concentration of 50 µg/L as the extraction matrix. Each 10 mL aliquot was placed in a 20 mL glass vial equipped with a PTFE/silicone septum and sealed with an aluminum cap. Before fiber exposure, 1% NaCl (w/v) was added directly to the aqueous sample to enhance ionic strength and facilitate the partitioning of analytes into the vapor phase. The solution was then stirred using a magnetic stir bar and a temperature-controlled stir plate. No organic solvent was used during the extraction phase; ethyl acetate was applied exclusively in the preparation of calibration standards. Once the salt was fully dissolved, the fiber was manually introduced through the septum and exposed to the headspace of the vial under the specific extraction conditions of each experimental run, as defined by the experimental design (**Table 1**). These parameters included extraction time (10, 20, 30 min), temperature (50, 60, 70°C), and stirring speed (300, 500, 700 rpm). The fiber remained suspended in the headspace while the sample was stirred throughout the extraction.

At the end of the extraction time, the fiber was retracted into its needle housing and immediately inserted into the GC–NPD injector port for thermal desorption. Desorption was carried out at 250 °C in splitless mode for 10 minutes, allowing the adsorbed analytes to be released directly into the column. The fiber was then removed and conditioned before reuse, following the manufacturer's recommendations.

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Table 1. Experimental design of type N + (N – 1) for organophosphate pesticide analysis by GC–NPD

Factors	Fiber type			NaCl concentration (%)			Extraction time (min)			Stirring speed (rpm)			Extraction temperature (°C)		
Levels	DVB/CAR	PDMS	DVB/CAR/PDMS	1	5	10	20	30	40	300	500	700	50	60	70
1	x				x			x			x			x	
2		x			x			x			x			x	
3			x		x			x			x			x	
4			x	x				x			x			x	
5			x		x			x			x			x	
6			x			x		x			x			x	
7			x		x		x				x			x	
8			x		x				x					x	
9			x		x			x		x				x	
10			x		x			x				x			
11			x		x			x			x		x		
12			x		x			x			x				x

The matrix design used "X" to indicate the variation of each experimental factor across the runs. Fiber types are abbreviated as follows: DVB/CAR = divinylbenzene/carboxen, PDMS = polydimethylsiloxane, DVB/CAR/PDMS = divinylbenzene/carboxen/polydimethylsiloxane. Each experimental run was performed in triplicate.

Chromatographic conditions

The chromatographic analysis was carried out using a Thermo Scientific Trace 1310 gas chromatograph (USA) equipped with a nitrogen phosphorus detector (NPD). Separation of the organophosphate compounds was achieved using a ZB-5 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness), purchased through Outsourcing Comercial SAS (Manizales, Caldas, Colombia). The injector was operated in splitless mode at a temperature of 250°C to ensure optimal sample transfer, and the detector temperature was maintained at 300 °C. The oven temperature program was as follows: initial temperature of 90 °C held for 1 minute, followed by a ramp of 10°C/min to 300°C, where it was held for 1 minute for a total run time of 16 minutes. Nitrogen was used as the carrier gas at a constant flow rate of 1.0 mL/min. For the NPD, hydrogen was supplied at 2.3 mL/min and air at 60 mL/min, according to manufacturer specifications. The SPME fiber was thermally desorbed in the injector port for 10 minutes to ensure complete analyte release. The chromatographic data for the organophosphate pesticides were processed using Chromeleon™ Chromatography Data System (CDS) software (Thermo Fisher Scientific, USA).

Linearity, limit of detection (LOD), and limit of quantification (LOQ)

To validate the fundamental analytical parameters of the method, we evaluated the following variables: calibration curve, linearity, limit of detection (LOD), and limit of quantification (LOQ). Linearity was assessed by constructing a calibration curve using standard solutions at ten different concentrations, ranging from 50 µg/L to 500 µg/L. Each concentration level was analyzed in triplicate. The calibration curve was generated by plotting the analyte concentration against the corresponding peak area obtained from the GC–NPD analysis. Linear regression analysis was used to determine the correlation coefficient (r^2) for each pesticide. The LOD and LOQ were determined using the signal-to-noise (S/N) ratio method. Analytical solutions containing a mixture of the five pesticides were prepared and analyzed in decreasing concentrations until the peak signal reached

approximately three times the noise level (S/N = 3) for LOD determination, and ten times the noise level (S/N = 10) for LOQ. Each level was analyzed in triplicate. The calculated relative standard deviation (RSD) values, used to evaluate method precision, were based on peak area measurements for each analyte at different concentration levels.

Results and discussion

The optimized solid-phase microextraction (SPME) method applied through headspace (HS-SPME) was successfully coupled with gas chromatography using a nitrogen-phosphorus detector (GC–NPD) to determine the selected organophosphate pesticides. **Figure 1** shows a representative chromatogram of the extracted analytes under the optimized conditions. All five target compounds were successfully detected within a retention time window of 6 to 13 minutes using the previously described chromatographic program. By order of elution, the more polar and lower molecular weight organophosphates, i.e., methamidophos and dichlorvos, appeared first. These compounds are characterized by their high polarity and the presence of electron-rich atoms, which result in weak interactions with the non-polar stationary phase of the column. Dimethoate, which has a moderately higher molecular weight and slightly lower polarity, eluted next. Finally, fenitrothion and chlorpyrifos, the largest and least polar molecules among the set, exhibited longer retention times due to their greater affinity with the non-polar stationary phase. These retention behaviors are consistent with previous studies, such as that of **Huba *et al.*** (2018), who compared different fiber coatings (DVB/PDMS, CarbonWR/PDMS, and polyacrylate) for the extraction of a mixture of pesticides and other emerging contaminants. Their findings reinforce the importance of understanding the physicochemical interactions between analytes, fiber coatings, and chromatographic phases to achieve optimal separation and detection efficiency.

The evaluation of pesticide extraction using different fiber types showed that the PDMS fiber extracted only two of the five compounds, while the PDMS/DVB fiber extracted three. In contrast, the DVB/CAR/PDMS fiber enabled the extraction of all five pesticides, although with noticeable differences in peak areas between compounds. This disparity suggests that certain analytes exhibit higher affinity for this fiber, likely due to a better match in polarity and molecular size, while the others are less efficiently retained, as shown in **Figure 2**.

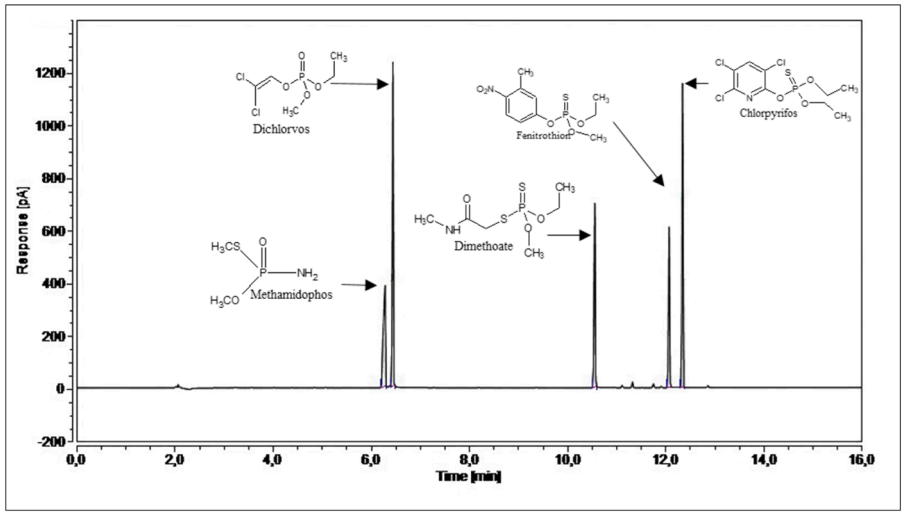

Figure 1. Chromatogram obtained by GC – NPD of the pesticide mixture extracted using the HS-SPME technique. Extraction conditions: 20 min, 50°C, 1% NaCl, DVB/CAR/PDMS fiber, and 500 rpm stirring speed

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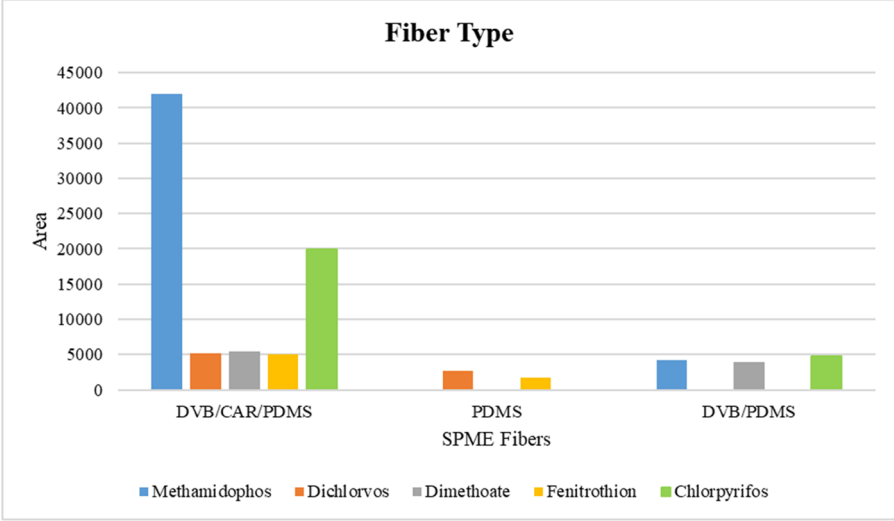

Figure 2. Pesticide extraction area using different SPME fibers (DVB/CAR/PDMS, PDMS/DVB, and PDMS) under fixed extraction conditions: 20 min, 50°C, 1% NaCl, and 500 rpm stirring speed

These results support the selection of DVB/CAR/PDMS as the most versatile and efficient fiber for multiresidue organophosphate analysis under the conditions tested. Similar findings were reported by **Liang *et al.*** (2019). The authors demonstrated that different SPME coatings exhibit significant variations in their selectivity and efficiency when applied to wastewater samples containing organophosphates. **Tankiewicz *et al.*** (2013) used a PDMS/polyacrylate (PA) fiber to detect 16 pesticides with varying polarities, achieving detection limits of around 10 µg/L. Their methodology proved to be selective, sensitive, and precise for the simultaneous determination of pesticides from various chemical groups, including organophosphates, in environmental water samples. Moreover, the concentrations detected in real water samples exceeded the limits established by the European Union legislation, indicating a potential environmental risk. The authors emphasized that the procedure was simpler, more economical, and less labor-intensive than conventional methods such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE), positioning SPME as a viable green alternative for multiresidue pesticide analysis using GC systems.

Harwood *et al.* (2013) compared different methods for estimating the bioavailable fraction of pyrethroid pesticides in contaminated sediments, including field-deployed and lab-exposed SPME fibers, as well as 24-hour Tenax extraction. Their findings showed that the Tenax method was generally more sensitive and better correlated with benthic invertebrate mortality than SPME fibers, indicating SPME limitations in detecting toxicologically relevant fractions under certain conditions. These results highlight that while SPME is a promising tool for passive sampling, its performance can vary depending on the analyte type and matrix, which reinforces the importance of careful fiber selection (**Tankiewicz *et al.***, 2013; **Harwood *et al.***, 2013; **Liang *et al.***, 2019).

After identifying the optimal fiber, the experimental design allowed for the evaluation of other critical extraction parameters. Regarding stirring speed, we observed that excessively high agitation (700 rpm) reduced extraction efficiency, likely due to turbulence exceeding the analytes' adsorption rate onto the fiber surface. Conversely, insufficient agitation (300 rpm) resulted in poor mass transfer. A moderate speed of 500 rpm was found to be optimal, providing sufficient convection without disrupting analyte-fiber interactions. Similarly, **Silva *et al.*** (2013) observed that moderate agitation enhances extraction by reducing the boundary layer around the fiber, though excessive stirring may risk damaging the coating.

Table 2 shows a comparison of the areas obtained for fenitrothion and chlorpyrifos, which showed the best response in terms of intensity of the chromatographic area against the evaluation of the different treatment levels in the agitation speed.

In terms of the extraction time, balance was reached at approximately 20 minutes, beyond which no significant signal increase was observed. Longer times even resulted in a slight decline in peak areas, possibly due to analyte desorption or degradation. This behavior is consistent with the observations by **Filho *et al.*** (2010), who reported that extraction times beyond 30 minutes do not yield statistically significant gains for organophosphate and organochlorine compounds. **Table 3** shows the different working levels of the pesticides fenitrothion and chlorpyrifos and the effect of the extraction time.

Temperature also played a critical role. Increasing the extraction temperature improved analyte transfer to the headspace/fiber due to enhanced diffusion and volatility. However, beyond 50°C, certain analytes began to degrade or volatilize excessively, reducing signal intensity, for which 50°C was selected as the optimal temperature. **Chen *et al.*** (2017) and **Li *et al.*** (2016) also noted that high temperatures (≥60 °C) can compromise analyte stability during SPME. A comparison of the effect of extraction temperature on the working levels for fenitrothion and chlorpyrifos compounds is shown in **Table 4**.

The addition of salt (NaCl) had a limited but measurable effect. Increasing the ionic strength via salt addition facilitated analyte transfer to the fiber through the salting-out effect. However, concentrations above 1% did not produce significant improvements and, in some cases, reduced repeatability, possibly due to salt precipitation, for which 1% NaCl was chosen as the optimal concentration. This result is supported by **Hassan *et al.*** (2020) and **Filho *et al.*** (2010), who found that excessive salt can destabilize certain analytes or impair extraction reproducibility. **Table 5** shows a comparison between the different treatment levels used for the evaluation of the salt effect on fenitrothion and chlorpyrifos compounds.

Table 2. Comparison of stirring speed for fenitrothion and chlorpyrifos compounds treated at different working levels

Variables	Levels		
Stirring speed (rpm)	**700**	**500**	**300**
Fenitrothion (pA)	0.9725	0.5548	1.4409
Chlorpyrifos (pA)	4.7602	6.1951	9.9481

Table 3. Comparison of the extraction time for fenitrothion and chlorpyrifos compounds treated at different working levels

Variables	Levels		
Extraction time (min)	**20**	**30**	**40**
Fenitrothion (pA)	0.8723	0.5548	0.6273
Chlorpyrifos (pA)	9.0316	6.1951	3.2729

Table 4. Comparison of the extraction temperature for fenitrothion and chlorpyrifos compounds treated at different working levels

Variables	Levels		
Extraction temperature (°C)	**70**	**60**	**50**
Fenitrothion (pA)	0.8217	0.9178	1.9178
Chlorpyrifos (pA)	4.6386	12.386	11.0265

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Table 5. Comparison of the salt effect for fenitrothion and chlorpyrifos compounds treated at different working levels

Variables	Levels		
Saline effect (%)	**1**	**5**	**10**
Fenitrothion (pA)	1.0593	1.9178	1.2295
Chlorpyrifos (pA)	11.8032	11.0265	11.9772

The method's performance was confirmed through analytical validation. Calibration curves for each pesticide exhibited good linearity in the 50–500 µg/L range, with correlation coefficients (r^2) of up to 0.9892. The calculated limits of detection (LOD) ranged from 1.088 to 3.114 µg/L, while limits of quantification (LOQ) ranged from 3.264 to 9.342 µg/L, indicating high sensitivity and suitability for trace-level detection. These values are notably lower than those reported by **Wang *et al.*** (2018), who achieved detection in the mg/L range using conventional methods. Comparable performance was reported by **Musshoff *et al.*** (2002) using SPME-GC for organophosphate detection in blood samples.

Precision was also satisfactory: relative standard deviation (RSD) values ranged from 0.998% to 3.599%, based on peak area measurements, which is well below the generally accepted threshold of 5% for environmental analyses, confirming the method's repeatability. **Pellicer-Castell *et al.*** (2018) reported similar results using alternative sorbent-based extraction methods, with RSDs under 12%. These findings validate the efficiency and reliability of the optimized SPME–GC–NPD method for simultaneous extraction and detection of multiple organophosphate pesticides. Detailed performance metrics are summarized in **Table 6**.

Table 6. Analytical validation values of organophosphates. Extraction conditions: 20 min, 50°C, 1% NaCl, DVB/CAR/PDMS fiber, and 500 rpm stirring speed

Number	Pesticide	RT	% RSD	LOD (ppb)	LOQ (ppb)	Equation of the line
1	Methamidophos	6.280	3.599	3.114	9.342	Y= 0.204x + 0.253
2	Dichlorvos	6.437	2.653	1.198	3.594	Y= 0.285x + 0.149
3	Dimethoate	10.552	1.356	2.755	8.265	Y= 0.189 + 2.128
4	Fenitrothion	12.065	0.998	1.088	3.264	Y= 0.162 + 2.029
5	Chlorpyrifos	12.337	1.371	1.110	3.330	Y= 0.271 + 0.851

Conclusions

Our study successfully developed and optimized a chromatographic method for the extraction and detection of five organophosphate pesticides (chlorpyrifos, dichlorvos, dimethoate, methamidophos, and fenitrothion) in water samples using solid-phase microextraction (SPME) coupled with gas chromatography and nitrogen-phosphorus detection (GC–NPD). The method was validated by establishing essential analytical parameters, including linearity, detection and quantification limits, and precision.

Among the tested SPME fibers, DVB/CAR/PDMS demonstrated a better performance, enabling the extraction of all five target compounds with high peak resolution. Under optimized conditions (20 min extraction at 50°C, 1% NaCl, 500 rpm stirring), the method achieved detection limits ranging from 1.088 to 3.114 µg/L and quantification limits from 3.264 to 9.342 µg/L. Relative standard deviation (RSD) values remained between 0.998% and 3.599%, confirming the precision and repeatability of the method.

These results are consistent with international performance criteria for trace-level pesticide detection in environmental samples. Although our study was conducted using spiked ultrapure water, the optimized methodology demonstrates strong potential for future application in environmental monitoring and regulatory compliance, particularly in agricultural regions where organophosphate use still prevails. Further work is recommended to evaluate the method's performance in real environmental matrices (e.g., surface or wastewater) and to assess matrix effects under field conditions, which would increase its robustness and practical relevance.

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Author contributions

JAFL: Document writing, research and data analysis; **GTO:** Conceptualization document and project management; **JPBA:** Data analysis and document writing

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Conflicts of interest

The authors declare there are no conflicts of interest related to the content of this article.

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