

ORIGINAL RESEARCH ARTICLE

Quantification of chlorpyrifos in aqueous samples by liquid-liquid microextraction and gas chromatography using a μ -ecd detector

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ABSTRACT

The evaluation of contaminants present in the environment is a recurring theme at the international level, which is why it is imperative to develop new methodologies for the extraction of analytes from matrices such as surface water, with a low impact on the environment. Chlorpyrifos (CP) is an organophosphate compound widely used for pest control, toxic to living organisms, which can be mobilized from soils to different water sources. Dispersive liquid-liquid microextraction (DLLME) can be considered as a methodology with green analytical chemistry characteristics, due to its low solvent and time consumption. The objective of this work was to optimize DLLME for the analysis of CP in water samples. For the extraction, methanol was used as dispersing agent and CS₂ as extracting agent, and five extraction times were evaluated: 5, 10, 15, 15, 20 and 25 min. The extracts were analyzed by gas chromatography with electron microcapture detector (GC- μ ECD). The results of the shaking time evaluation showed that, for the shaking times of 10 and 15 min, a CP recovery percentage of 108% and 88% was obtained, respectively, with a detection and quantification limit of 18.3 and 22.4 μ g/L, respectively, and a precision expressed as relative standard deviation of less than 14.2%, which is acceptable for this type of extraction. It can be concluded that the DLLME methodology associated with GC- μ ECD is a suitable alternative for the quantification of CP in aqueous matrices with a low environmental impact.

Keywords: chlorpyrifos; gas chromatography; liquid-liquid microextraction; green analytical chemistry

1. Introduction

Organophosphorus compounds are among the most widely used pesticides worldwide, due to their low cost and high effectiveness, being detected in groundwater, surface water, drinking water, soil and plant samples^[1,2]. Among them, chlorpyrifos [O,O-diethyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate] (CP) stands out, whose toxicity can increase in the presence of its metabolites, such as chlorpyrifos oxon (CPO) (**Figure 1**), one of its metabolites resulting from its oxidation.

CP is an insecticide widely used in the home and in agriculture where it is applied directly to crops. This compound is degraded by the action of sunlight, bacteria or other chemical processes^[3]. It has been reported that its persistence in soil ranges from a few days to 4 years and in vegetables from 3 to 60 days, approximately, depending on the physicochemical characteristics of the soil; the content of organic matter present being

ARTICLE INFO

Received: 08 September 2023 | Accepted: 30 October 2023 | Available online: 15 November 2023

CITATION

Reyes TG, Escobar NC, Crisosto JM. Quantification of chlorpyrifos in aqueous samples by liquid-liquid microextraction and gas chromatography using a μ -ecd detector. *Advances in Analytic Science* 2023; 4(2): 1980. doi: 10.54517/aas.v4i2.1980

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relevant^[4].

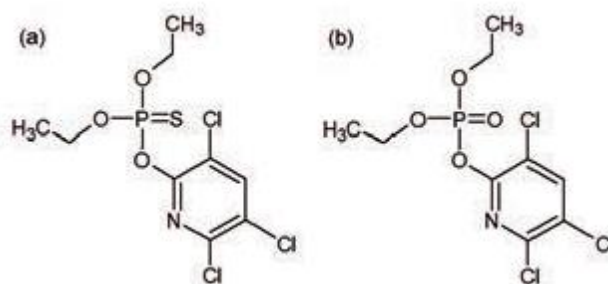


Figure 1. (a) chlorpyrifos; (b) chlorpyrifos oxon.

It has been described that CP is distributed 49.6% in soil, 46.3% in sediments and 2.3% in water, however, these ranges vary according to application rate, ecosystem type and environmental factors^[5]. CP as a contaminant is capable of mobilizing through the soil profile and reaching groundwater, or being transported to surface water. At the toxicity level, CP is highly toxic to both fish and invertebrate organisms^[6] and for human life it classifies as a class II pesticide^[7].

For the analysis of contaminants in environmental matrices, the extraction of the analyte of interest from the matrix constitutes one of the critical steps in the process. In this sense, it is necessary to evaluate new extraction methodologies under the concept of green analytical chemistry, which involves the development of analytical methodologies with an environmental concern, eliminating or decreasing the sources of contaminants with the intention that products and processes do not endanger any form of life. Green analytical chemistry encourages the use of direct analysis, as well as miniaturization, automation and savings in both energy and consumables, thus reducing waste generated and ensuring operator safety^[8].

Classical liquid-liquid extraction requires long periods of time, large volumes of solvents and multiple stages, where problems such as sample emulsification hinder the extraction of the analyte from the matrix^[9]; to improve this process and reduce the amount of solvents used, dispersive liquid-liquid micro-extraction (DLLME) can be an excellent alternative. This methodology was developed to pre-concentrate organic analytes from aqueous matrices and has been applied to food matrices, biological fluids and solid samples^[10,11]. DLLME integrates in one step the extraction and concentration of analytes, using two immiscible liquid phases: an aqueous phase (where the sample is located) and an organic phase (organic solvent)^[12], to which the analyte of interest is transferred for subsequent quantification. Among the most commonly used techniques for the quantification of CP is gas chromatography with electron microcapture detector (GC- μ ECD).

According to what has been described, it is of great importance to have environmentally friendly extraction methodologies with adequate figures of merit that allow the determination of contaminants such as CP in water samples and to evaluate their mobility in the environment, which is the objective of this research.

2. Materials and methods

A calibration curve was prepared between 25 and 400 μ g/L for CP with the addition of internal standard PCB 103 (SI) at concentration 150 μ g/L, using n-hexane as solvent. The analyses were performed on a gas chromatograph with micro electron capture detector, 7890A, Agilent Technologies, using chromatographic conditions optimized for this analysis.

An extractant mixture consisting of 20 mL of methanol (dispersing agent) and 1 mL of CS₂ (extracting agent) was used to carry out DLLME. A blank and an aqueous solution containing CP at a concentration of

250 µg/L were considered for the analyses. For the extraction process, glass tubes with lids were used, into which 5 mL of the CP solution was added. Then 1 mL of the extractant solution was added. These mixtures were then agitated, where the agitation time was optimized, considering 5 times between 5 and 30 min (quadruplicate analysis). After this, the mixtures were centrifuged at 2000 rpm for 3 min. The drop formed was extracted with a Pasteur pipette and deposited in an amber-colored vial, and then taken to dryness with a current of N₂ at 40 °C in a dry bath. Finally, the samples were reconstituted with 0.5 mL of n-hexane with addition of SI, to be analyzed by GC-µECD. The analyses were performed in triplicate.

The limit of detection (LD) and limit of quantification (LC) were determined by injecting ten blanks. For LD the formula $YLD = YB + 3\sigma B$ was used, where σB is the standard deviation of the areas obtained for the blank and YB is the average area of the blanks. For the calculation of LC the same procedure is done, but the standard deviation is multiplied by ten ($YLC = YB + 10\sigma B$). Subsequently these values (YLD and YLC) were interpolated in the calibration curve, obtaining the corresponding concentration for LD and LC. Precision in this study was evaluated by repeatability and accuracy was evaluated by calculations of percent recovery of the analyte.

3. Results

The results obtained from the GC-µECD analysis showed an average retention time for CP of 8.80 minutes, while for SI it was 9.196 min. The chromatographic conditions used are presented in **Table 1**.

A temperature program was used for the analysis (**Table 2**) which considers three ramps, from 100 to 280 °C, with a total time of 15 min.

Table 1. Chromatographic conditions used for GC-µECD analysis of CP.

Injection temperature	Detector	Gas	Make up N ₂	Flow	Injection volume	Injection mode	Column
250 °C	300 °C	N ₂	20 mL/min	1 mL/min	10 µL	Splitless	HP-5: 325 °C: 30 m × 320 µm × 0.25 µm

Table 2. Temperature program used for GC-µECD analysis of CP.

Points	Reason [°C/min]	Temp [°C]	Weather [min]	Total time [min]
Initial	-		1	1
Ramp 1			0	1.5
Ramp 2			0	3.6
Ramp 3			0	

Regarding the calibration curve of CP with addition of SI (**Figure 2**), the results showed a good correlation based on the linearity of the curve ($Y = 0.7712X - 0.0792$, $r = 0.997$).

The L_D and L_C obtained were 3.2 and 6.8 µg/L, respectively, with a precision expressed as the relative standard deviation (RSD) of 16.2%.

Blank extracts obtained from DLLME were analyzed, where it was determined that there are no matrix signals hindering the quantification of the analytes at the retention times determined by GC-µECD (**Figure 3**).

The results show a precision expressed as relative standard deviation of less than 6% for times less than 20 min. Regarding the droplet collection, the volumes used allowed obtaining a droplet of adequate size for its separation from the extraction solution and its subsequent quantification.

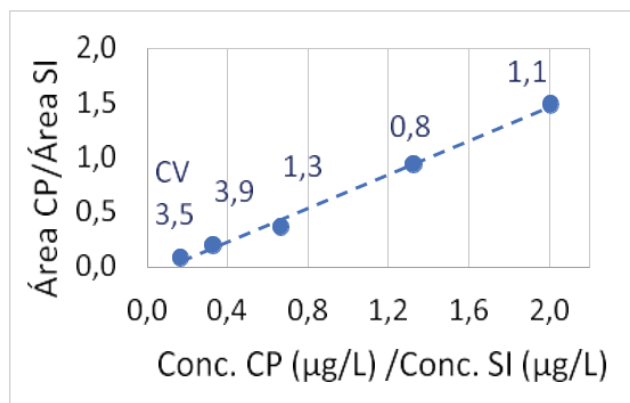


Figure 2. Calibration curve for CP ($Y = \text{AreaCP}/\text{AreaSI}$; $X = [\text{CP}]/[\text{SI}]$).

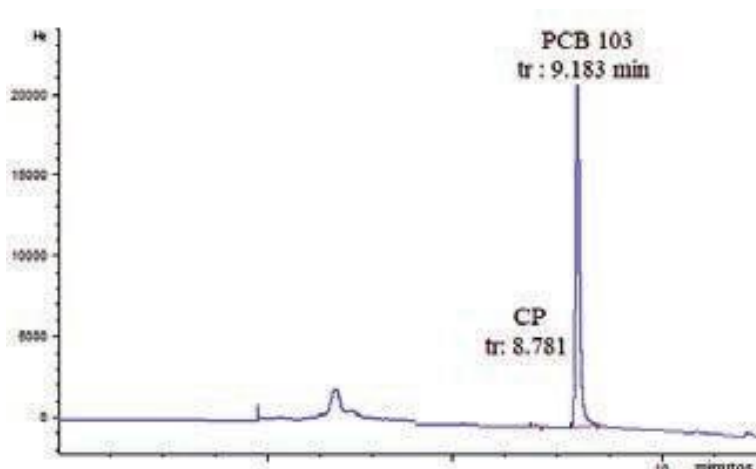


Figure 3. Optimized chromatogram of the blank sample.

The results of the DLLME tests considering different shaking times, for the extraction of CP in aqueous sample ($250 \mu\text{g/L}$) are shown in **Figure 4**, where it can be observed that the highest percentage of CP recovery is obtained for the shaking times 10 and 15 min with 108% and 88%, respectively.

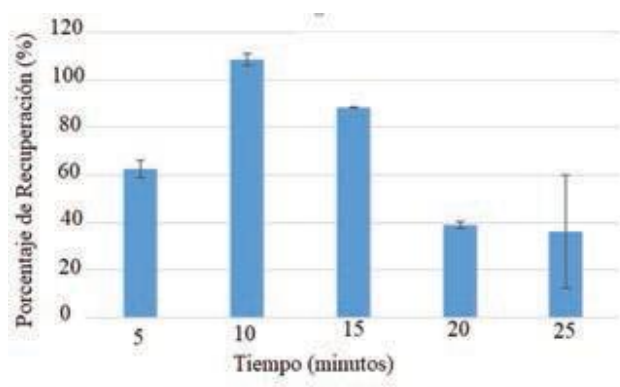


Figure 4. Relationship between percentage of CP recovery and agitation time used in DLLME.

4. Discussion

When comparing the L_D and L_C obtained with those reported in the literature, it is evident that the DLLME methodology with GC- μECD has a high sensitivity, with L_D and L_C lower than those reported in the literature for this extraction methodology (**Table 3**), this is due to the fact that the electron microcapture detector has a higher sensitivity, added to the fact that the DLLME methodology obtained adequate recovery percentages for

the analysis of this type of contaminant in an aqueous matrix. When comparing the L_C and L_D obtained by DLLME and SPE of the analyte from aqueous matrix, the latter methodology allows quantifying the analyte at a concentration twice lower than for DLLME. The discrepancy between the results may be due to the differences in the procedures of the methodologies used, since SPE drags a smaller amount of possible interferents, thus allowing to have less noise by matrix effect and therefore discriminate at a lower concentration the signal of the analyte^[9,13]. The DLLME presented a precision expressed as the relative standard deviation of less than 14.2%, and an accuracy expressed as the recovery for a shaking time of 15 min of 88%.

There is a concern for the development of new extraction methodologies, which not only allow the proper determination of contaminants of interest from environmental matrices, but also conform to what is known as green and sustainable analytical chemistry, which implies low environmental impact, low harmful effects on operators and affordability^[14].

Table 3. Comparison of LOD and LC obtained for CP determination using SPE and DLLME.

	SPE	DLLME	DLLME
IMQ/U	[13]	East, work	[9]
L_D		18.3	
L_C		22.4	318

DLLME is a methodology with low solvent consumption, with few process steps, which reduces analysis times, unlike SPE where larger volumes of solvent are used, and which considers more than one step, such as the conditioning of the extraction column, sample loading, washing, and subsequent elution of the analyte. Given this scenario, the DLLME methodology optimized in this work is an alternative that fits the concept of green analytical chemistry, with figures of merit suitable for the analysis of environmental samples.

5. Conclusion

The DLLME methodology using as extractant solution methanol: CS₂ in a 20:1 ratio, stirring time of 15 minutes, allowed extracting the analyte from the aqueous matrix. A LOD and LC of 18.3 and 22.4 µg/L, respectively, a precision expressed as relative standard deviation of less than 14.2%, and a recovery rate of 88% were determined for DLLME. Thus, it can be concluded that DLLME associated with GC-µECD is an alternative with adequate figures of merit for the quantification of CP from aqueous matrix, with the advantage of being a methodology with less environmental impact than SPE.

Funding

This work was funded by Fondecyt, project No. 11110223.

Conflict of interest

The authors declare no conflict of interest.

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