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Research Article

Coprecipitation-assisted coacervative extraction coupled to high-performance liquid chromatography: An approach for determining organophosphorus pesticides in water samples

An analytical methodology based on coprecipitation-assisted coacervative extraction coupled to HPLC-UV was developed for determination of five organophosphorus pesticides (OPPs), including fenitrothion, guthion, parathion, methidathion, and chlorpyrifos, in water samples. It involves a green technique leading to an efficient and simple analytical methodology suitable for high-throughput analysis. Relevant physicochemical variables were studied and optimized on the analytical response of each OPP. Under optimized conditions, the resulting methodology was as follows: an aliquot of 9 mL of water sample was placed into a centrifuge tube and 0.5 mL sodium citrate 0.1 M, pH 4; 0.08 mL $Al_2(SO_4)_3$ 0.1 M; and 0.7 mL SDS 0.1 M were added and homogenized. After centrifugation the supernatant was discarded. A 700 μL aliquot of the coacervate-rich phase obtained was dissolved with 300 μL of methanol and 20 μL of the resulting solution was analyzed by HPLC-UV. The resulting LODs ranged within 0.7–2.5 ng/mL and the achieved RSD and recovery values were <8% ($n = 3$) and >81%, respectively. The proposed analytical methodology was successfully applied for the analysis of five OPPs in water samples for human consumption of different locations of Mendoza.

Keywords:
Coprecipitation-assisted coacervative extraction / Green chemistry / HPLC / Organophosphorus pesticides / Water samples DOI 10.1002/elps.201600335

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1 Introduction

Organophosphorus pesticides (OPPs) are the most common class of pesticides used to pest control in agriculture and households [1]. They have been used increasingly since 1970s, when the persistent organochlorine pesticides were banned [1]. Stock piles are still available, due to the free and open publication of their synthetic methods [2]. Physicochemical properties of OPPs condition their distribution, bioaccumulation, and biomagnification in environment, as well as in wildlife [3]. The OPP bioaccumulation capability in the environment could be estimated from their octanol/water partition coefficient K_{ow} [4]. Supporting Information Table S1 summarizes the relevant physicochemical properties of the target OPPs. The studied OPPs show log K_{ow} within the range of 2.2 to 4.7, methidathion being the OPP with the lowest log K_{ow} value [1]. The persistence of the target OPPs is considered low to moderated [5]; guthion being the most labile (5–23 days of half-lives) and parathion the most persistent (100–200 days). Once released to the environment, they can easily reach the aquatic systems (surface or groundwater basins) and can be transported by mechanisms such as one-point source pollution, ground water discharge, or atmospheric deposition [6]. Drinking water is one of the main exposure routes to OPPs for humans. They exhibit toxicity by inhibition of acetylcholinesterase enzyme, which leads to accumulation of the neurotransmitter acetylcholine in synapses and overstimulates the postsynaptic cholinergic receptors, resulting in

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Abbreviations: **CAE**, coacervative extraction; **Cop-CAE**, coprecipitation-assisted coacervative extraction; **CPE**, cloud-point extraction; **OPPs**, organophosphorus pesticides

muscarinic and nicotinic symptoms and signs [7]. There are current reports about water samples with OPPs' concentration up to 100 ng/mL [8,9]. Due to their toxicity and potential risk for human health [10], sensitive and selective analytical methodologies are required for analyzing OPPs in water samples for human consumption. The instrumental techniques generally reported for determination of OPPs include LC with DAD as well as MS [11] and, to a lesser extent, GC with nitrogen-phosphorus, electron capture, and flame photometric detectors [11]. It is well known that the sample preparation step plays an important role in the analytical methodology for achieving the required analytical performance for determining OPPs at trace levels. Most of the methodologies report the use of conventional techniques, such as liquid–liquid extraction (LLE) and SPE [2], for extraction and isolation of analytes from the matrices. Watanabe et al. [12] developed a cloud-point extraction (CPE) technique, which is based on the use of nonionic surfactant as a micellar extraction medium. This extraction technique reduces the use of toxic solvents by using non- or less-toxic nonionic surfactants in accordance with the proposed principles of green chemistry [13]. Coacervative extraction (CAE) technique is a variant of CPE technique based on the use of ionic surfactants for extraction of the analytes from aqueous samples [12]. There are reports about the combination of CAE with coprecipitation agents that leads to a new analytical approach, named coprecipitation-assisted coacervative extraction (cop-CAE) technique [14], which has been recently investigated and applied in analytical chemistry [15]. This technique is based on the formation of aggregated structures called "admicelles or hemimicelles," by means of electrostatic interactions between monomers of ionic surfactant and complex species oppositely charged (discrete and soluble) [15].

Hydrophobic character of the admicelles in cop-CAE is different to the micelles in CPE. This aspect is relevant when considering that the extraction efficiency of the technique is conditioned by the affinity of the analytes toward the coacervate medium, and this is conditioned by its hydrophobicity. [16]. Cop-CAE technique was applied for determination of different analytes, such as estrogens [17], crystal violet dye [14], and PAHs [16] by HPLC. However, it was not applied for determination of OPPs in water samples.

The aim of the present work was to study and apply the cop-CAE technique for extraction and preconcentration of the most used OPPs in Argentina (guthion, fenitrothion, parathion, methidathion, and chlorpyrifos) for their determination in tap, well, and river water samples by HPLC-UV. Tap water from Mendoza City is taken from Tunuyán and Mendoza Rivers and is provided to the population after physical and chemical potabilization treatment through a network. Well water considered in this work from El Carrizal location is taken from the source and consumed without a chemical treatment. The methodology was carried out in compliance with USEPA guidelines [18] and validated in terms of LOD, reproducibility, recovery (%), and linear working range.

2 Materials and methods

2.1 Reagents

The OPP standards were purchased from Chem Service West Chester, Pennsylvania, USA, and consisted of guthion (gut, 99% purity), fenitrothion (fen, 98% purity), parathion (par, 99% purity), methidathion (met, 99% purity), and chlorpyrifos (chlor, 99.9% purity), which were stored at –20°C. Further dilutions were prepared weekly in methanol (MeOH) at concentration levels of 50 mg/L and stored in brown bottles at –20°C. HPLC-grade MeOH was purchased from Sintorgan, Argentine. Pure hydrochloric acid was purchased from Dalton, Argentine. Ultrapure water (18 MΩ cm) was obtained from a milli-Q water purification system (Millipore, Paris, France). Citric acid (98% purity) and sodium hydroxide (98% purity) were purchased from Mallinckrodt Chemical Works, USA ,and Anedra, Argentine, respectively, and used for the preparation of buffer solution. SDS (99% purity) was purchased from Promega, USA. Aluminum sulfate (99.76% purity) was purchased from Carlo Erba, France. Aluminum sulfate solution (0.1 mol/L) was prepared in HCl 0.01 mol/L. An SDS solution (0.1 mol/L) was prepared in ultrapure water. Sodium citrate buffer 0.1 mol/L, pH 4, was prepared with ultrapure water. All reagents were of analytical grade or above.

2.2 Instrumental analysis

HPLC-UV analyses were carried out on a Perkin Elmer series 200 liquid chromatograph coupled to a Perkin Elmer series 200 UV/Vis detector (Shelton, CT, USA) and operated by TotalChrom Data System (6.3.1. software version). The HPLC column used was a Zorbax Sb-Aq (150 × 4.6 mm id, 5 μm particle size; Agilent Technologies, USA). The oven column was operated at 25°C. The mobile phase was MeOH (A) and ultrapure water (B) at a flow rate of 0.8 mL/min. The gradient program was set as follows: 0–5 min, 50% A; 5–8 min, 50–60% A; 8–17 min, 60% A; 17–30 min, 60–77% A; and then 30–31 min, 77–95% A; and 220 nm was used as working wavelength. Peak identification in samples was carried out by comparing retention times with reference standards.

2.3 Sampling and sample preparation

The procedure was applied for the determination of five OPPs in water samples from two locations of Mendoza Province, Argentina: Mendoza city and El Carrizal, and the two main River basins: Mendoza River and Tunuyán River. Mendoza Province is located in the Andean piedmont (32°53′00″S 68°49′00″W) and characterized by arid climatic conditions, with a regime of summer rainfalls of 250 mm per year. Due to the desert conditions of the region, water is scarce; therefore, it is considered a priority its care and distribution.

The tap and well water samples were collected after letting it run for about 20 min. Water samples from Mendoza

River and Tunuyán River were collected at 10 cm depth from the surface. All samples were collected free of air bubbles and preserved in brown bottles at –20°C. Samples were filtered through 0.22 μm pore size membrane filters and analyzed within 24 h after collection.

2.4 Coprecipitation-assisted coacervative extraction

A 9 mL aliquot of water sample was added into a 15 mL centrifuge tube. Aliquots of 0.5 mL sodium citrate buffer 0.1 M, pH 4; 0.08 mL aluminum sulfate 0.1 M, and 0.7 mL SDS 0.1 M were then added to the tube and homogenized using a vortex stirrer (at 8 × *g*) for 8 min. The resulting cloudy solution was kept at 25°C for 5 min before centrifuging at 1500 rpm (232 × *g*) for 10 min to accelerate the separation of phases. The resulting aqueous upper phase was removed and discarded while keeping the coacervate-rich phase for further analysis. A 700 μL aliquot of the coacervate rich-phase obtained was dissolved with 300 μL of MeOH and stirred for homogenization. An aliquot of 20 μL of the resulting solution was analyzed by HPLC-UV. Along the optimization of variables, ultrapure water, originally free of OPPs, was spiked with OPP standard-mix (50 ng/mL met, gut, par, fen, and chlor in MeOH). All experiments were carried out in triplicate.

3 Results and discussion

As most of the techniques used for sample preparation, the efficiency of the cop-CAE can be conditioned by physicochemical variables of the extraction system, as well as by the matrix of the sample; conditioning thus, the sensitivity of the methodology [14]. Experimental variables that might condition the analytical responses of the OPPs were evaluated on synthetic aqueous samples; including pH of solution, $Al_2(SO_4)_3$ and SDS concentration, stirring time, extraction temperature and time, and centrifugation. These studies were carried out by modifying one variable at the time while keeping the remaining constant. The chromatographic peak area was used to evaluate the impact of modified experimental conditions on the analytical signal of the target OPPs. To optimize each variable, the relative response (RR (%)) for each analyte was considered. The relative response for each analyte was calculated as follows: RR (%) = Aij/Aijmax × 100, where Aijmax is maximum analytical signal (area of the chromatogram) of a target analyte (i) obtained in a specific assay (j) and Aij is analytical signal (area of the chromatogram) of a target analyte (i) obtained in the specific assay (j) at different levels of the studied variable.

3.1 Precursor of the coprecipitation agent and pH

There are reports about the use of diverse coprecipitation agents for cop-CAE technique, including species of aluminum [19], magnesium [16], and iron [20], among others. These species were proposed to be used in combination with different anionic surfactants, including SDS [16, 19], dioctyl sodium sulfosuccinate [21], and sodium oleate [19], SDS being the surfactant mostly used. Aluminum compounds, including aluminum chloride and aluminum sulfate, were mainly reported as precursors of the coprecipitation agent because of the aluminum species occurring within the wide pH range of 3.5 ∼ 12.5 [22]. On the other hand, it is necessary to consider that the pH of the extraction medium also affects the electrostatic interactions within hemimicellar system, conditioning thus its hydrophobicity [15] and, thus the extraction of the OPPs. Additionally, it is worth to consider that most of the OPPs might degrade under strong alkaline conditions (>9, Table 1 of ESI) [8]. These aspects might also condition the extraction efficiency of the cop-CAE technique.

Based on these considerations, $Al_2(SO_4)_3$ was used as precursor of coprecipitation agent, due to its p*K*a values (3.3–3.6) and to avoid OPPs degradation along the study. Assays for evaluating the effect of pH on the extraction efficiency, and thus, on the analytical response of the OPPs, were carried out at $Al_2(SO_4)_3$ 2 mM within the pH range 4–9. The cop-CAE methodology was carried out as described in Section 2.4. Figure 1A shows the RR of each OPP at different assayed extraction pH. It was observed that the highest RR for the OPPs was achieved at pH 4. At this pH, the predominant aluminum specie is the hepta-charged aluminum complex ($[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{+7}$) [22], which is relevant for the hemimicelles formation. The low pH maximizes the positive surface charges of aluminum species, which in turn, enhanced the interaction of the aluminum species with the anionic surfactant, SDS. Sodium citrate (5 mM, pH 4) was used in further experiments to adjust the pH of the extraction medium at pH 4.

3.2 $Al_2(SO_4)_3$ and SDS: Concentration and addition order

$Al_2(SO_4)_3$ and SDS concentration effect and their addition order are variables that condition the extraction efficiency of the cop-CAE technique, and thus, the analytical response of OPPs. The addition of $Al_2(SO_4)_3$ leads to the formation of the aluminum species, hepta-charged complex, required as the hemimicellar support. The hemimicelles formation would be conditioned by the surface area of the aluminum hepta-charged complex, which depends on its concentration [14]. In CAE technique, surfactant monomers self-aggregate above a surfactant concentration known as "critical micellar concentration" (CMC), leading to micelles formation that then constitute the coacervate phase. The CMC for SDS is 8.1 mM. The hemimicellar concentration in cop-CAE technique is analogous to CMC in CAE [14]. The preexistence of SDS monomers when $Al_2(SO_4)_3$ is added can form SDS complexes [14], which could affect the dynamic formation of the proper aluminum species (Section 3.1) required for supporting the

Table 1. Analytical figures of merit of the proposed methodology and recovery for OPPs determination in water samples for human consumption

	Met					Gut					Fen					Par					Chlor				
LOD (ng/mL)	1.7					1.1					2.5					1.1					0.7				
Linear range (ng/mL)[a)]	5.7–500					3.5–500					8.3–400					3.5–500					2.2–500				
Correlation coefficient	0.997					0.996					0.999					0.997					0.992				
Added (ng/mL)	250	100	50.0			250	100	50.0			250	100	50.0			250	100	50.0			250	100	50.0		
Intra-day precision																									
Found (ng/mL)[b)]	233 ± 5	90.1 ± 3	45.5 ± 1			215 ± 6	87.4 ± 3	42.5 ± 1			205 ± 4	80.1 ± 2	39.0 ± 1			203 ± 5	80.2 ± 1	39.5 ± 1			233 ± 3	92.1 ± 2	46.5 ± 1		
Inter-day precision																									
Found (ng/mL)[b)]	230 ± 8	91.3 ± 4	45.9 ± 1			218 ± 8	88.2 ± 5	43.5 ± 1			198 ± 8	80.2 ± 4	40.3 ± 1			198 ± 8	80.3 ± 2	40.1 ± 1			233 ± 8	94.4 ± 4	46.9 ± 1		
Recovery studies for OPPs determination in water samples[c)]																									
Added (ng/mL)	0	10.0	50.0	200	0	10.0	50.0	200	0	10.0	50.0	200	0	10.0	50.0	200	0	10.0	50.0	200					
Mendoza City (tap water)																									
Found (ng/mL)[b)]	n.d.	9.2 ± 0.2	45.5 ± 0.6	184 ± 4.0	n.d.	8.8 ± 0.2	42.5 ± 0.9	176 ± 4.0	n.d.	8.2 ± 1.3	39.3 ± 1.2	190 ± 7.0	n.d.	8.1 ± 1.3	39.5 ± 1.2	190 ± 7.0	n.d.	9.7 ± 0.1	49.7 ± 0.4	194 ± 2.0					
El Carrizal (well water)																									
Found (ng/mL)[b)]	7.5 ± 0.2	16.6 ± 0.2	49.7 ± 0.4	193 ± 4.0	n.d.	8.9 ± 0.2	44.0 ± 0.9	178 ± 4.0	n.d.	8.3 ± 1.2	40.4 ± 1.1	192 ± 6.0	n.d.	8.3 ± 1.2	40.4 ± 1.1	189 ± 8.0	10 ± 0.1	12.6 ± 0.1	51.3 ± 0.3	200 ± 2.0					
Mendoza River																									
Found (ng/mL)[b)]	n.d.	9.3 ± 0.2	45.9 ± 0.8	186 ± 3.0	n.d.	8.9 ± 0.2	46.5 ± 0.4	177 ± 4.0	n.d.	8.3 ± 1.2	40.4 ± 1.1	174 ± 8.0	n.d.	8.2 ± 1.3	40.5 ± 1.1	189 ± 8.0	n.d.	9.6 ± 0.1	46.5 ± 0.9	192 ± 2.0					
Tunuyán River																									
Found (ng/mL)[b)]	n.d.	9.4 ± 0.2	45.7 ± 0.5	187 ± 3.0	n.d.	8.8 ± 0.2	43.5 ± 0.8	176 ± 4.0	n.d.	8.4 ± 1.1	44.0 ± 0.8	174 ± 8.0	n.d.	8.2 ± 0.3	41.3 ± 1.0	189 ± 8.0	n.d.	9.6 ± 0.1	46.3 ± 0.6	192 ± 2.0					

a) 95% confidence interval; $n = 3$.
b) Results expressed as $\overline{X} \pm t\,\mathrm{SD}/\sqrt{n}$; $n = 2$; 95% confidence interval.
c) Recovery (%) = [(Found-base)/added] × 100.
Extraction conditions as described in Section 2.4.
RSD (%) = $s/\overline{X} \times 100$, 95% confidence interval; $n = 3$, where, s is standard deviation and $\overline{X}$ is the sample mean.
n.d., not detectable.

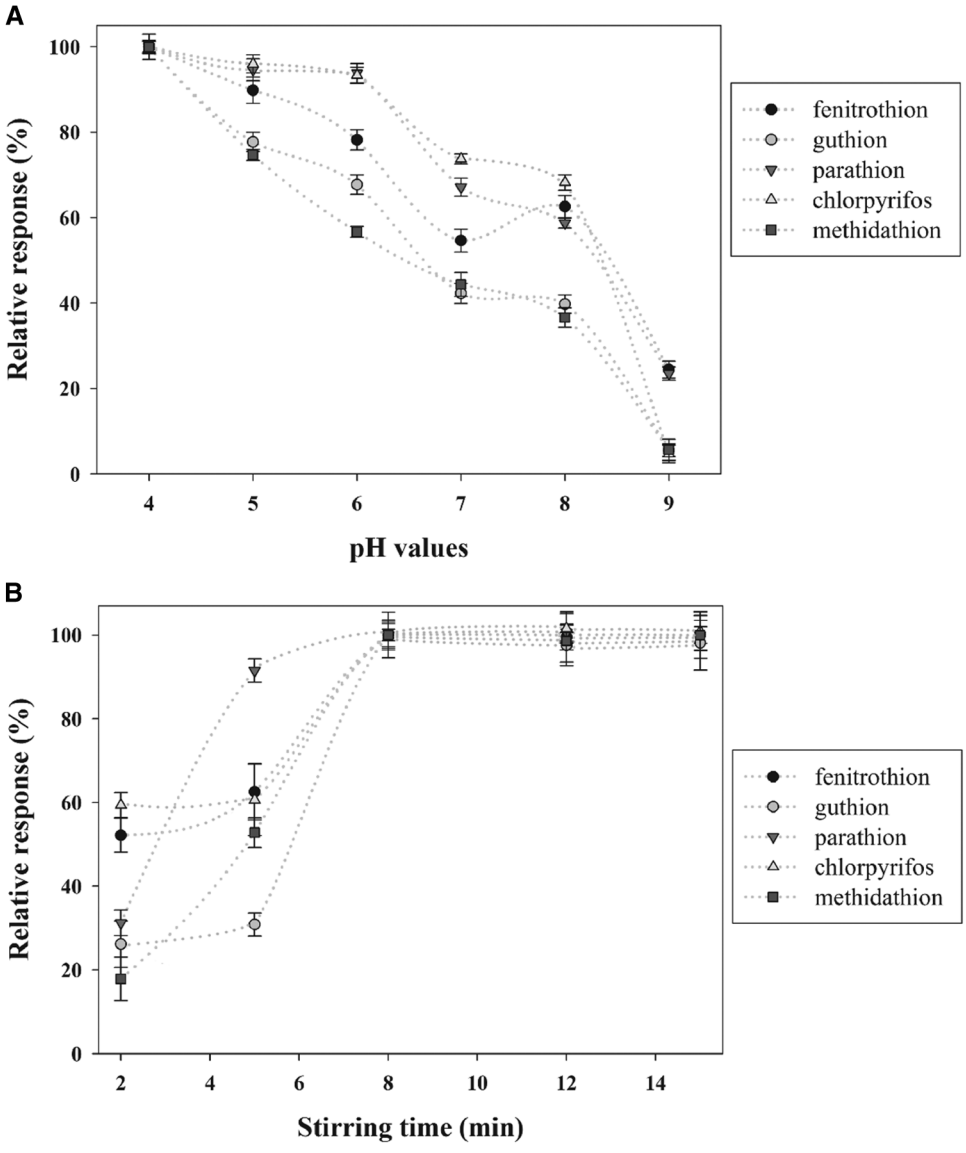

Figure 1. Effects of experimental conditions on the relative response of OPPs: (A) pH—extraction conditions: $Al_2(SO_4)_3$ and SDS concentration, 2 and 4 mM, respectively; stirring time, 5 min; extraction temperature and time, 25°C and 5 min; and centrifugation, 232 × *g*, 10 min; (B) stirring time—extraction conditions: solution pH, 4; $Al_2(SO_4)_3$ and SDS concentration, 0.8 and 7 mM, respectively; extraction temperature and time, 25°C and 5 min; and centrifugation, 232 × *g*, 10 min.

hemimicelles. Therefore, the addition order of the reagents also plays an important role on the hemimicelle formation, since it could then affect the extraction efficiency of the cop-CAE technique, and thus, the analytical response of the OPPs analyzed.

Based on these considerations, the optimization of $Al_2(SO_4)_3$ concentration was first carried out, and then SDS concentration. Aluminum sulfate solution (0.1 mol/L) was prepared in HCl 0.01 mol/L instead of in H_2SO_4 to avoid common ion effect, which would have implied a diminishing in the solubility of $Al_2(SO_4)_3$ and a potential undesirable precipitation of the precursor within the assayed working conditions. Two assays were carried out to evaluate the significance of the $Al_2(SO_4)_3$ and SDS and the role of each of them on the extraction efficiency of the technique, and thus, its impact on the analytical response of the target OPPs: (i) assay of different concentrations of $Al_2(SO_4)_3$ 0.5 to 5 mM and SDS 4 to 8 mM and (ii) assay of addition order of $Al_2(SO_4)_3$ and SDS (a) $Al_2(SO_4)_3$ without preexistence SDS; and (b) $Al_2(SO_4)_3$ with preexistence of SDS. The cop-CAE coupled to HPLC methodology was carried out as described in Section 2.4. The highest RR values of the target analytes were obtained for 0.8 mM $Al_2(SO_4)_3$ (Fig. 2A) and 7 mM SDS (Fig. 2B), respectively. The results evidenced that neither the surfactant nor coprecipitation agent was able to efficiently extract the target OPPs from the sample bulk, requiring from their combination to achieve successful extraction efficiency of the technique. The RR values of the OPPs in the addition order assay were slightly higher (1–4%) when $Al_2(SO_4)_3$ was added without the preexistence of SDS. The results achieved at SDS concentrations lower than 7 mM could be attributed to an insufficiency of available SDS monomers for covering the surface of the aluminum core, leading thus to low density hemimicelles of adsorbed monomers. This fact might

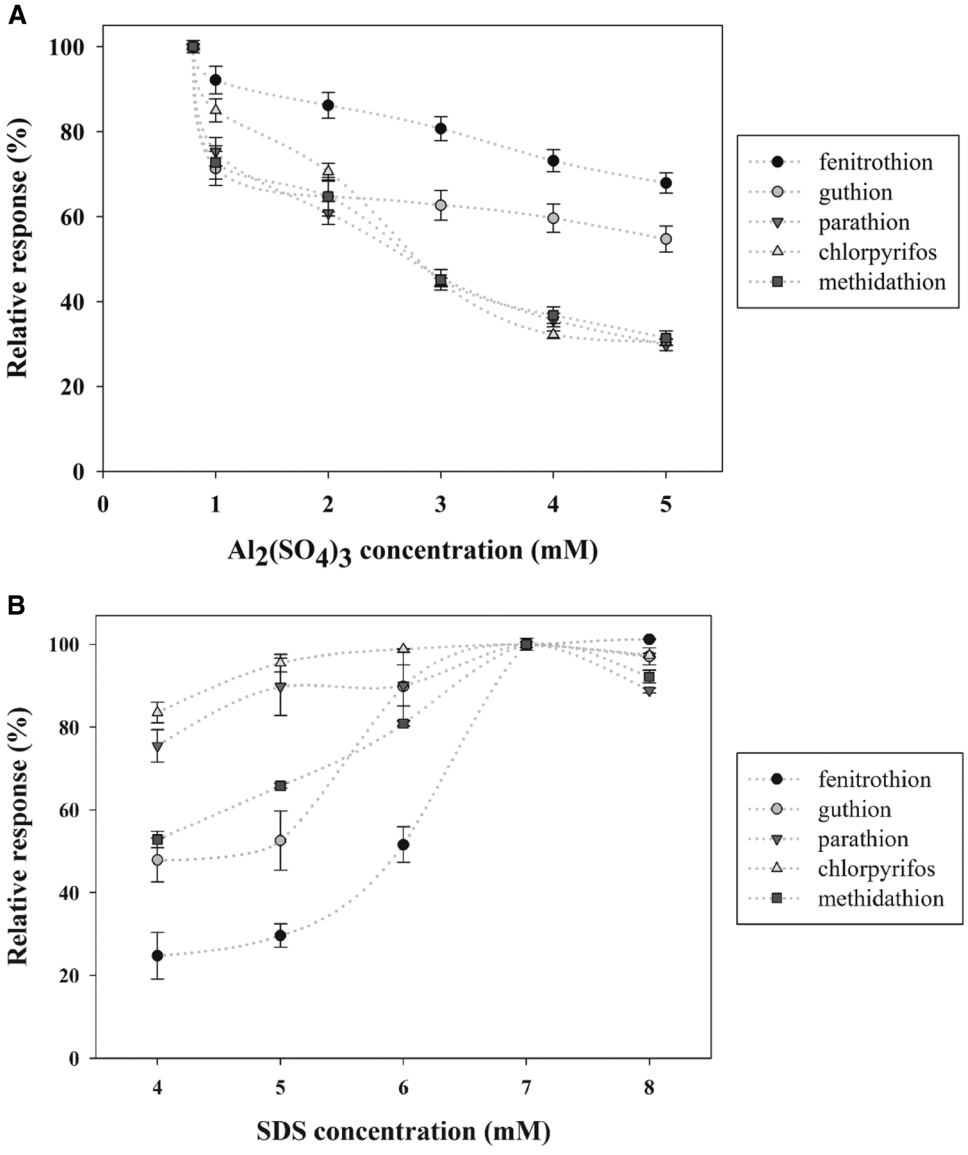

Figure 2. Effects of experimental conditions on the relative response of OPPs: (A) $Al_2(SO_4)_3$ concentration—extraction conditions: solution pH, 4; SDS concentration, 4 mM; stirring time, 5 min; extraction temperature and time, 25°C and 5 min; and centrifugation time, 232 × *g*, 10 min; (B) SDS concentration—extraction conditions: solution pH, 4; $Al_2(SO_4)_3$ concentration, 0.8 mM; stirring time, 5 min; extraction temperature and time, 25°C and 5 min; and centrifugation time, 232 × *g*, 10 min.

affect the hydrophobic characteristics of the hemimicelles, conditioning thus, the affinity of the target OPPs. As the SDS concentration increased, the density of SDS monomers onto the aluminum core increased, until the SDS coverage reached an equilibrium of saturation [23]. Under these conditions, the cop-CAE showed the highest RR for the target OPPs, no observing improvements at higher SDS concentration. Concentrations of $Al_2(SO_4)_3$ lower than 0.8 mM were disregarded, because the coacervate phase was not formed, turning the technique nonfeasible. For concentrations higher than 0.8 mM, aluminum species tend to condensate and thus, the hemimicellar support has lower surface area [17]. As a consequence, the density of SDS monomers on the aluminum cores decreases, affecting thus the hydrophobicity of the hemimicelles, and consequently the extraction efficiency of the cop-CAE. These facts affect the analytical response of the target OPPs, as it is observed on Fig. 2A. Based on these considerations and results, $Al_2(SO_4)_3$ 0.8 mM (added without SDS preexistence) and SDS 7 mM were chosen for further assays.

3.3 Stirring time effect

Agitation of the solution during extraction process is essential to facilitate mass transference of the analytes from the matrix bulk to the hemimicellar phase [9] and to accelerate the hemimicelles formation [19]. Based on these considerations, a stirring vortex was used at 1500 rpm (8 × *g*) and different stirring times were assayed (2–15 min) to evaluate its impact on the extraction efficiency of the target analytes (Fig. 1B). The cop-CAE coupled to HPLC methodology was carried out as described in Section 2.4. The highest RR for the target OPPs was observed by stirring

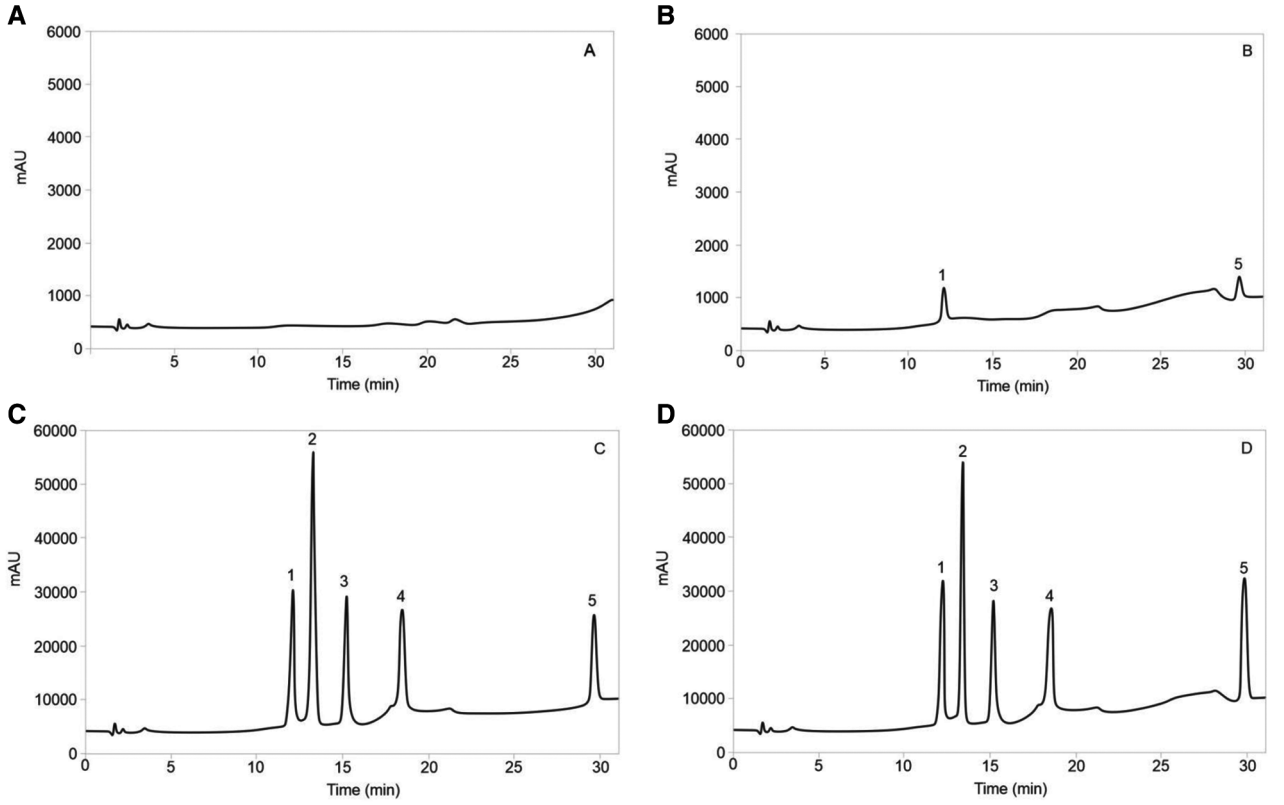

Figure 3. Chromatograms of water samples: (A) and (C) tap water from Mendoza city; (B) and (D) well water from El Carrizal. Samples C and D were spiked at 50 ng/mL with each OPP. Methodological conditions were described in Section 2.4. Peak identification: (1) methidathion, (2) guthion, (3) fenitrothion, (4) parathion, and (5) chlorpyrifos.

the extraction system for 8 min. Stirring time higher than 8 min did not show improvement on the analytical response of the target analytes. Thus, agitation using a vortex for 8 min was selected as the optimum stirring time for further assays.

3.4 Complementary variables: Extraction temperature and time, and centrifugation

In CAE and cop-CAE techniques, both extraction temperature and time play important roles [14]. These variables affect the mass transference process that governs the analyte partition between aqueous bulk and the hemimicelles, as well as they rule the micelles and hemimicelles dehydration process, which condition the volume of the resulting coacervate-phase [14]. Centrifugation is necessary to favor the partition of the analytes from the aqueous bulk to the coacervate phase [24]. These variables condition the relative response on the analytical signal of the target analytes, additionally to the extraction efficiency, thus affecting the sensitivity of the resulting analytical methodology. In this sense, three different studies were carried out in order to evaluate the effect of these variables on the analytical response of the target OPPs. The cop-CAE coupled to HPLC methodology was carried out as described in Section 2.4. The extraction time was kept at 5 min, while the extraction temperature was assayed at 0, 25, 35, and 50°C. Within these results, no significant changes on the RR on the analytical signal were observed. Therefore, a working extraction temperature of 25°C was chosen for further studies. Afterward, different extraction times, ranging from 3 to 20 min, were studied while keeping the extraction temperature at 25°C. The highest analytical response was achieved for 5 min of extraction time at 25°C, which remained invariant for higher assayed extraction times. Therefore, 5 min was selected as the working extraction time. The centrifugation time assayed at 1500 rpm (232 × *g*) ranged from 8 to 20 min. The highest relative response was observed for a centrifugation time of 10 min, or longer. Therefore, a centrifugation time of 10 min was adopted for further assays. Supporting Information Figures S1–S3 for the five OPPs were included. These results are in agreement with those reported previously for a comparable extraction system [17].

3.5 Coacervate-phase conditioning for HPLC analysis

Since the coprecipitated coacervate phase after centrifugation resulted viscous, it could not be injected as such in the HPLC, avoiding a sure clogging of the analytical instrument. Different dilution agents were considered for proper conditioning of the coacervate-phase before HPLC injection. Among the

Table 2. Comparison of cop-CAE-HPLC/UV with other methodologies for the determination of OPPs in water samples

Instrumental technique	Extraction technique	Extraction time (min)	Sample volume (mL)	Extraction solvent volume (mL)	Analytes	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	RSD (%)	Recovery (%)	Ref.
HPLC-DAD	IPA-LLE	20	5	1.5	Chlor	4.8–300	1.4	5	73–105	[28]
					Diazinon	8.0–450	2.4	4		
					Met	5.5–450	2.5	4		
					Fen	7.1–450	2.1	9		
HPLC-DAD	HF-MMSLPE	30	15	0.7	Isocarbophos	1–200	0.1	5	85–101	[29]
					Phosmet	1–200	0.1	5		
					Par-methyl	1–200	0.1	6		
					Triazophos	1–200	0.3	6		
					Phonophos	1–200	0.1	5		
					Phoxim	1–200	0.2	7		
HPLC-DAD	UASEME	3	5	0.15	Isocarbophos	1–200	0.1	3	86–106	[8]
					Phosmet	1–200	0.1	5		
					Par-methyl	1–200	0.1	4		
					Fen	1–200	0.3	4		
					Par	1–200	0.1	4		
					Phonophos	1–200	0.3	6		
					Phoxim	1–200	0.3	4		
HPLC-UV	Cop-CAE	5	10	–	Met	5.7–500	1.7	6	81–97	Current work
					Gut	3.5–500	1.2	8		
					Fen	8.3–400	2.5	2		
					Par	3.5–500	1.1	2		
					Chlor	2.2–500	0.7	6		

IPA-LLE, ion-pair assisted liquid–liquid extraction; HF-MMSLPE, hollow-fiber microporous membrane solid–liquid phase microextraction; UASEME, ultrasound-assisted surfactant-enhanced emulsification microextraction.

reported dilution agents, the use of MeOH [25], a MeOH:H_2O 1:1 mixture [17], as well as concentrated HCl and HCl solutions [26] was reported. The assays were carried out on the obtained coacervate -phase (700 μL) taking it to a final volume of 1 mL with the above dilution agents. The extraction procedure was carried out as described in Section 2.4. Among the studied dissolution agents, 300 μL of MeOH showed the best performance, achieving a homogeneous solution after stirring with vortex. Aqueous solutions (MeOH:H_2O 1:1 and HCl 0.02 mM) led to a turbid solution and no homogeneous solution was achieved. Concentrated HCl led to a homogeneous solution after adding 300 μL to the coacervate-phase; however, the pH of the resulting solution was not compatible with the chromatographic column, and was also discarded. Based on these results, 300 μL of MeOH was used for dissolving the resulting coacervate phase for HPLC analysis.

3.6 Analytical performance and application to real samples

The proposed analytical methodology, cop-CAE-HPLC-UV, was validated in terms of linearity, intra- and interday precision, sensitivity, and recovery. The analytical figures of merit are summarized in Table 1. Each analytical sequence includes a blank, which was used to monitor background levels and possible carryover between samples. The determination of the target OPPs was accomplished by using a calibration curve build with synthetic samples spiked with OPPs standard-mix at different concentration levels before carrying out the extraction procedure. The calibration data was fitted using linear equation and showed a satisfactory linearity within concentration ranges of 5.7–500, 3.5–500, 8.3–400, 3.5–500, and 2.2–500 ng/mL for met, gut, fen, par, and chlor, respectively, which are the recommended range for water analysis [18]. Calibration data were fitted by using a $1/x$ curve resulting in correlation coefficients (r) > 0.99 for all analytes. The precision of the methodology was evaluated over three replicates, leading to RSD values <8%. Precision was measured in repetitive conditions (same operator, same instrument) according to Association of Analytical Communities (AOAC) International guidelines [27]. In turn, these repetitive conditions were divided in intra- and inter-day precision. These assays were performed on water samples to levels of 50.0, 100, and 250 ng/mL with OPPs standard mix. The LOD was calculated as three times the SD of the blank and the LOQ as ten times the SD of the blank for individual measures, according to AOAC International. In order to validate the analytical methodology, a recovery study of OPPs was carried out over water samples of different types. Samples were spiked with OPPs mix-standard achieving to OPP concentrations of 10, 50, and 200 ng/mL (Table 1). Chlorpyrifos and methidathion were detected in well water samples from El Carrizal, at concentration levels of 10 and 7.5 ng/mL, respectively. In Fig. 3, chromatograms for two of the analyzed samples together with a blank were shown.

3.7 Comparison with other analytical methodologies

The proposed methodology, cop-CAE-HPLC/UV, developed for determining multiple OPPs in water samples presents several characteristics that makes it suitable for routine analysis. Table 2 summarizes analytical methodologies reported in open literature for determining OPPs in water samples by using HPLC. The analytical figures of merits of the proposed methodology, cop-CAE-HPLC/UV, were comparable to those previously reported. The proposed sample preparation technique, cop-CAE, is suitable for batch preparation by using simple equipment available in most laboratories. A batch of 20 samples can be prepared for injection in 23 min, including an extraction stage of 5 min. This sample preparation methodology neither includes solvent evaporation steps nor requires toxic solvent such as those regularly used for liquid–liquid extraction and SPE.

4 Concluding remarks

This is the first time that cop-CAE-HPLC/UV methodology is proposed for the determination of OPPs in water samples. The univariant method used to optimize the methodology allowed to study individually the pertinent variables and better understand the physicochemical principles that govern the extraction technique. The proposed analytical methodology based on cop-CAE was demonstrated to be an effective approach for sample preparation of water samples for OPPs determination by HPLC/UV. The cop-CAE technique offers a convenient analytical alternative for determining OPPs in different water samples with high-throughput sample, simplicity, and effectiveness figures. Additionally, this analytical technique has green chemistry characteristics considering that it uses small volume of nontoxic solvents and produced minimum waste. The proposed methodology could be easily coupled to other analytical instrumentation, if required.

Under optimized working conditions, LODs were in the order of nanograms per millilitre, suitable for real-world applications with an acceptable precision at trace levels according to international regulations, such as EPA. The developed methodology was successfully applied for analyzing water samples from the two main River of Mendoza province and two locations from Mendoza province. Two of the analyzed OPPs (met and chlor) were determined in well water samples. Considering their low half-life, it could be inferred that these OPPs would be recently used close to the sampled region. Due to the scarcity of water resources in the region, the finding reported here is a significant contribution, since a simple, low-cost methodology is proposed for monitoring the natural water sources for human and agricultural uses.

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