Journal of Chromatography A, 1217 (2010) 6334–6341

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Coacervative microextraction ultrasound-assisted back-extraction technique for determination of organophosphates pesticides in honey samples by gas chromatography–mass spectrometry

Ariel R. Fontana [a], Alejandra B. Camargo [b,c,d], Jorgelina C. Altamirano [a,b,*]

[a] Grupo de Investigación y Desarrollo en Química Analítica (QUIANID) (LISAMEN, CCT CONICET-Mendoza), P.O. Box 131, 5500 Mendoza, Argentina
[b] Departamento de Química, Instituto de Ciencias Básicas, Universidad Nacional de Cuyo, Mendoza, Argentina
[c] Laboratorio de Análisis de Residuos Tóxicos, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Alte. Brown 500, 5505 Chacras de Coria, Mendoza, Argentina
[d] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Mendoza, Argentina

ARTICLE INFO

Article history:
Received 8 June 2010
Received in revised form 29 July 2010
Accepted 6 August 2010
Available online 13 August 2010

Keywords:
Coacervative microextraction
Ultrasound-assisted back-extraction
Organophosphates pesticides
Honey analysis
Gas chromatography–mass spectrometry

ABSTRACT

Coacervative microextraction ultrasound-assisted back-extraction technique (CME-UABE) is proposed for the first time for extracting and preconcentrating organophosphates pesticides (OPPs) from honey samples prior to gas chromatography–mass spectrometry (GC–MS) analysis. The extraction/preconcentration technique is supported on the micellar organized medium based on non-ionic surfactant. To enable coupling the proposed technique with GC, it was required to back extract the analytes into hexane. Several variables including, surfactant type and concentration, equilibration temperature and time, matrix modifiers, pH and buffers nature were studied and optimized over the relative response of the analytes. The best working conditions were as follows: an aliquot of 10 mL 50 g L^{-1} honey blend solution was conditioned by adding 100 μL 0.1 mol L^{-1} hydrochloric acid (pH 2) and finally extracted with 100 μL Triton X-114 100 g L^{-1} at 85 °C for 5 min using CME technique. Under optimal experimental conditions, the enrichment factor (EF) was 167 and limits of detection (LODs), calculated as three times the signal-to-noise ratio ($S/N = 3$), ranged between 0.03 and 0.47 ng g^{-1}. The method precision was evaluated over five replicates at 1 ng g^{-1} with RSDs ≤9.5%. The calibration graphs were linear within the concentration range of 0.3–1000 ng g^{-1} for chlorpirifos; and 1–1000 ng g^{-1} for fenitrothion, parathion and methidathion, respectively. The coefficients of correlation were ≥0.9992. Validation of the methodology was performed by standard addition method at two concentration levels (2 and 20 ng g^{-1}). The recoveries were ≥90%, indicating satisfactory robustness of the methodology, which could be successfully applied for determination of OPPs in honey samples of different Argentinean regions. Two of the analyzed samples showed levels of methidathion ranged between 1.2 and 2.3 ng g^{-1}.

1. Introduction

Organophosphates pesticides (OPPs) are widely used in agricultural practices for pests and diseases control. Slow degradation of pesticides in the environment and extensive or inappropriate use by farmers can lead to environmental contamination [1]. The widespread distribution of pesticides caused several problems to apiculture industry including residues in hive products (honey, wax and propolis, etc.) [1]. Honey bees are greatly affected by pesticides and transport them to the colony as contaminated nectar which ends as a contaminated honey. These residues finally get to the consumers [2,3]. This constitutes a potential risk for human health, because of their sub acute and chronic toxicity [4]. Argentina is one of the main honey producers and exporters in the world, occupying the second place after China, with an exportation rate of 73,159 tons [5]. Therefore, its quality is of great concern for internal and external market. One of the quality control parameters in honey is the pesticides residues content. The European Union (EU) regulations establish a maximum permissible concentration of pesticide residues for honey, expressed as the Maximum Residues Limits (MRLs), of 10 ng g^{-1} [6].

As a consequence of the control of pesticides in honey, there is a growing interest in developing analytical methodologies specifically designed for this type of analysis. Sample preparation is an important stage in the determination of OPPs because of the complexity of honey matrix and the low concentration at which the OPPs are permitted by legislation (≤10 ng g^{-1}). Current methods for the extraction of pesticides in honey typically involve

* Corresponding author at: Grupo de Investigación y Desarrollo en Química Analítica (QUIANID) (LISAMEN, CCT CONICET-Mendoza), P.O. Box 131, 5500 Mendoza, Argentina. Tel.: +54 261 5244064; fax: +54 261 5244001.
E-mail address: jaltamirano@mendoza-conicet.gob.ar (J.C. Altamirano).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.08.021

several sample preparation steps such as extraction, clean-up and concentration before instrumental analysis. The extraction of pesticides from honey samples has been carried out using conventional extraction techniques such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE) [7–10]. These extraction techniques are laborious, time-consuming and require large volumes of organic solvents. Moreover, due to the low concentration of analytes in the samples, large sample volumes are typically required to ensure detectability. Solid-phase microextraction (SPME) has been proposed for the extraction of OPPs from honey samples [11]. Solid-phase microextraction is a fast, simple and solvent-free extraction technique [12]. The main drawbacks of this technique are the fragility and cost of the fibers, in addition to possible sample carry-over effects between runs [13,14]. In recent years, with the developing interest in miniaturization in analytical chemistry for solvent and sample savings, some newer miniaturized approaches to LLE have been reported. Several different types of liquid-phase microextraction (LPME) have been developed including, single drop microextraction (SDME) [15], dispersive liquid–liquid microextraction (DLLME) [16] and ultrasound-assisted emulsification microextraction (USAEME) [17,18]. An alternative to the microextraction with traditional organic solvents is the coacervative microextraction technique (CME) [19]. It has been successfully applied for extraction of analytes prior to liquid chromatography (LC) analysis. The coacervative phenomenon is based on the aggregation of surfactants monomers under specific physicochemical conditions. It depends on the nature of the amphiphile and its concentration. Once the monomers' aggregation has started to form micelles, it is observed a diminishing of the micelles' water solubility and a sharp increment in the micelle aggregation number leading to two isotropic phases: coacervate and aqueous bulk [20–22]. The micelles provide different regions with diverse polarities that enhance its potential for solubilizing solutes in a wide range of polarities. The solutes affinity varies with the nature of the solubilized species and the surfactant structure. Hydrophobic solutes are solubilized in the inner micellar core, polar/charged analytes believed to be solubilized in the polar region through a number of interactions (e.g. electrostatic, π-cation, hydrogen bonds, etc.), and amphiphilic solutes are incorporated to the micelles through both hydrophobic and polar interactions, forming mixed aggregates [22]. Thus, the analytes can be *in situ* extracted to the coacervate phase and selectively separated from the aqueous bulk. Considering the extraction efficiency of the CME and the notorious differences between the coacervate phase volume (≤100 μL) and the aqueous bulk volume (10–20 mL) leads to a convenient alternative as a preconcentration technique. Additionally, CME is low cost, simple to operate and environmentally friendly because uses alternative solvents such as surfactants, lowering the organic solvent consumption. The potential of coacervates as extractants have been explored prior to several separative and non-separative techniques [20,23]. However, the use of coacervates prior to GC analysis has not been widely developed due to the nature of surfactants, which are characterized by their high viscosity and low volatility. Furthermore, the direct introduction of the coacervate phase could clog the injector or column. Additionally, they can absorbed onto the stationary phase altering the analyte's interaction with the stationary phase of the GC column [24]. To overcome these disadvantages, different approaches have been proposed including ultrasound-assisted back-extraction, microwave-assisted back-extraction, columns with silica gel, florisil or cation exchangers as absorbents and post-extraction surfactant derivatization step [24–30].

The goal of this work was to develop and validate a methodology for the determination of OPPs in honey using coacervative microextraction-ultrasound-assisted back-extraction-gas chromatography–mass spectrometry (CME-UABE-GC–MS). It is the first time that CME is applied for extraction and preconcentration of OPPs or other analytes from honey or other matrices prior to GC–MS analysis. Several factors, including surfactant type and concentration, equilibration temperature and time, matrix modifiers, pH, UABE solvent and UABE volume were studied and optimized over the relative responses of the target OPPs. The analytical performance of the proposed methodology was evaluated in terms of enrichment factor (EF), limits of detection (LODs), repeatability and linear working range. Moreover, the procedure was applied for the determination of OPPs in honey samples of different regions of Argentina and its robustness was evaluated in terms of recovery factors (RF%).

2. Experimental

2.1. Reagents

The standards of OPPs were purchased from Chem Service Inc. (West Chester, PA, USA) and consisted of: fenitrothion (98% purity), chlorpyrifos (99.9% purity), parathion (99% purity) and methidathion (99% purity). The internal standard (IS) 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) was purchased from Accustandard (New Haven, CT, USA). The OPPs standards were stored at −20 °C. Stock solutions of OPPs were prepared in methanol at concentration levels of 5 g L^{-1}. Further dilutions were prepared weekly in methanol and stored in brown bottles at −20 °C.

Methanol, ethanol, hexane, ethyl acetate and isooctane were purchased from Merck (Darmstadt, Germany). 1-Propanol and 1-butanol were purchased from Sigma–Aldrich (Steinheim, Germany). Triton X-114 and Triton X-100 were purchased from Sigma–Aldrich and used without further purification. 100 g L^{-1} aqueous stock solution of each non-ionic surfactant was prepared. Sodium chloride, hydrochloric acid, potassium chloride, sodium hydroxide and potassium hydrogen phthalate were all purchased from Merck. An aqueous stock solution of sodium chloride was prepared at 6.15 mol L^{-1}. Hydrochloric acid and sodium hydroxide were prepared with ultrapure water and the final concentration was 0.1 mol L^{-1}. The buffer solution was prepared with ultrapure water and the final concentration was as follows: potassium phthalate (0.05 mol L^{-1}, pH 2.2). Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). All reagents were of analytical grade or above.

2.2. Equipment and working conditions

A 40 kHz and 600 W US-bath (Test Lab, Buenos Aires, Argentina) was used for assisting the back-extraction process. The volume of coacervate phase and UABE phase volume was measured using a 100 μL Hamilton glass syringe (Reno, NV, USA). Injections into the GC–MS were made using a 5 μL Hamilton glass syringe. GC–MS analyses were performed on a Varian 3900 GC equipped with an ion trap mass detector Varian Saturn 2000 (Varian Inc., Walnut Creek, CA, USA). The system was operated by Saturn GC–MS Work Station v6.4.1 software. The GC column used was VF-5MS (25 m × 0.25 mm, 0.25 μm film thickness; Varian, Lake Forest, CA, USA). The temperature program was: 80 °C – held for 2 min; increased at the rate of 10 °C min^{-1} to 220 °C – held for 2 min, rating 20 °C min^{-1} to a final temperature of 300 °C and held for 2 min. Helium (purity 99.999%) was used as a carrier gas at a flow rate of 1.0 mL min^{-1}. The injector temperature was set at 250 °C and the injections were performed in the splitless mode. The mass spectrometer was operated in electron impact ionization mode at −70 eV. The trap, manifold and transfer line temperatures were set at 220, 120 and 280 °C, respectively. Samples were analyzed in the full scan EI mode. The peak identifi-

cation was based on the retention time, base peak and confirmation ions of the OPPs. Quantification was carried out using m/z 277, 314, 291, 145; and m/z 125, 258, 109 and 157 were used as confirmation ions for fenitrothion, chlorpirifos, parathion and methidathion, respectively.

2.3. Sampling and sample preparation

For method optimization, a honey blend was prepared by mixing 100 g (dry weight) of five honeys of different origins of Argentina. Honey samples free from any traces of OPPs obtained from organic beekeepers were used for blend preparation and utilized for method development and validation. Honey blend was spiked at 50 ng g^{-1} with each OPP. A 50 g L^{-1} honey solution was prepared form 50 ng g^{-1} honey blend and used without further treatment.

The samples analyzed included honey from agricultural Argentinean areas: Santa Fe (Sample 1), La Pampa (Sample 2) and Santa Rosa, Mendoza (Samples 3 and 4); and a commercially honey available in regular market (Sample 5). Honey samples were stored in dark flasks at 4 °C. All samples were analyzed in triplicate with the optimized CME-UABE-GC–MS methodology.

2.4. Coacervative microextraction ultrasound-assisted back-extraction procedure

Ten milliliters of 50 g L^{-1} honey blend solution was placed into a 15-mL glass-centrifuge tube. One hundred microliters of 0.1 mol L^{-1} hydrochloric acid and 100 μL of Triton X-114 100 g L^{-1} were subsequently added and mixed-up by handshaking. The centrifuge tube was thermostatized at 85 °C for 5 min. Under these conditions, the micelles agglomeration was favored and the coacervate phase started to get separated from the sample bulk. The tube was centrifuged at 3500 rpm (1852.2 × g) for 5 min to accelerate the coacervate phase decantation. In order to increase the coacervate-phase viscosity and extract easily the aqueous supernatant, the centrifuge tube was placed into an ice bath for 2 min. The ultrasound-assisted back-extraction was carried out by adding 60 μL of hexane into the resulting coacervate phase and sonicating the system for 5 min. Again, two phases were formed: the coacervate phase and the hexane. The analytes remained in the hexane phase, which result on the top of the coacervate phase. A 1 μL of the hexane phase was injected and analyzed into the GC–MS.

3. Results and discussion

The affinity of an analyte for a particular surfactant aggregate depends on the analyte and micelles nature. The functional groups of the monomers lead different micro-mediums in the micelle with slight physicochemical differences which can be selectively tuned for enhance the affinity for certain analytes. Therefore, the efficiency of the CME technique can be altered by modifying different physicochemical variables that affect the analytes and/or the micelles characteristic such as pH, surfactant concentration, equilibration time and temperature, and matrix modifiers. Thus, to enhance the extraction efficiency of the OPPs from honey, these variables needed to be studied and optimized in order to establish the optimum working conditions. Furthermore, honey matrix has different components such as sugars, organic acids and insoluble matter that can modify the structure of the micelles and need to be considered. These studies were carried out following the procedure described in Section 2.4 modifying one of the variables at the time keeping the remaining constant. A 10 mL honey blend solution containing 50 ng g^{-1} of OPPs was used to perform the assays which were done in triplicate. The relative chromatographic peak area was used to evaluate the influence of those variables on the extraction efficiency of CME-UABE technique.

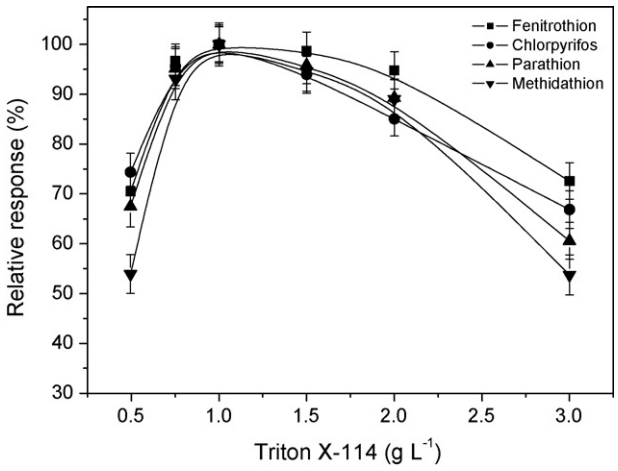

Fig. 1. Surfactant concentration effect on the relative response of target OPPs. Extraction conditions: 10 mL honey blend solution, 50 ng g^{-1} each OPPs; equilibration temperature and time, 70 °C, 10 min; centrifugation time, 5 min; ultrasound-assisted back-extraction, 100 μL hexane, 5 min.

3.1. Surfactant type and concentration effects

Two non-ionic surfactants were studied to carry out the CME of the target OPPs: Triton X-100 and Triton X-114. The cloud points of Triton X-100 and Triton X-114 are about 65 and 24 °C, respectively [25]. The extraction procedure was the one described above. Both surfactants reached the cloud point and the coacervate phases were separated from the aqueous bulk; however, Triton X-114 showed higher relative responses for the analytes than Triton X-100. Triton X-100 leaded to higher coacervate phase volumes, which difficult the back-extraction process. It was observed that for the back-extraction, it was necessary to work with a minimum volume equal to the coacervate phase to avoid forming a stable emulsion and achieve a quantitative extraction of the analytes. Therefore, due to the experimental and analytical convenience, Triton X-114 was chosen for further studies.

As it is well known, surfactant concentration above the critical micellar concentration is required to achieve the cloud point of the system and thus, get a coacervate phase to extract the analytes [22]. High surfactant concentrations would lead into an increment of the extraction efficiency of the technique. However, excessively high surfactant concentration would deteriorate the EF of the technique due to an increment in the resulting coacervate phase. Therefore, it was found necessary to study the surfactant concentration in order to achieve the maximum extraction efficiency without deteriorating the EF of the technique. The surfactant concentration study was carried out within the range 0.50–3.00 g L^{-1} Triton X-114, which is above its critical micellar concentration (CMC: 0.13 g L^{-1}). As can be observed from Fig. 1, the greater relative response for the target OPPs was achieved for the concentration range: 0.75–1.50 g L^{-1}. Excessive surfactant concentration decreased the EF and made the back-extraction process unpractical. Smaller concentrations than 0.75 g L^{-1} lead to low relative responses since the surfactant was not enough to quantitatively extract the analytes. Therefore, 1.0 g L^{-1} Triton X-114 was selected as optimum for OPPs-related CME technique.

3.2. pH and buffers effects

To the knowledge of the authors, CME was not previously applied for OPPs extraction in any matrix; therefore there was

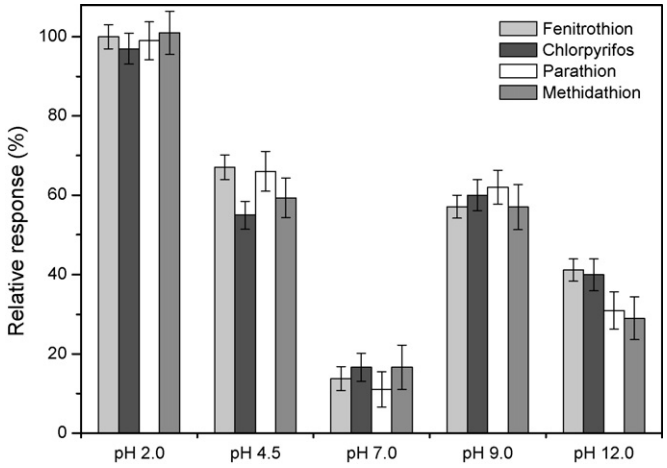

Fig. 2. pH effect on the relative response of target OPPs. Extraction conditions: 10 mL honey blend solution, 50 ng g^{-1} each OPPs; 100 μL 100 g L^{-1} Triton X-114; equilibration temperature and time, 70 °C, 10 min; centrifugation time, 5 min; ultrasound-assisted back-extraction, 100 μL hexane, 5 min.

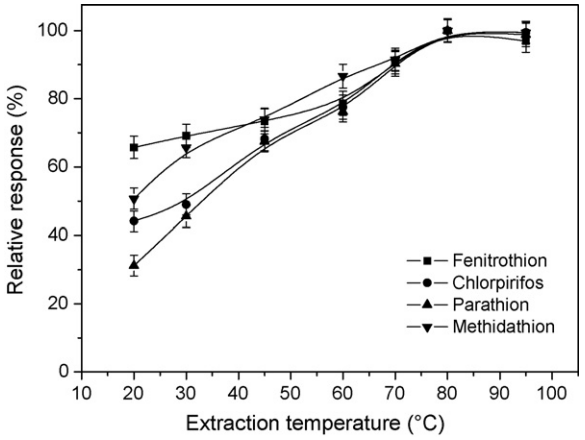

Fig. 3. Equilibration temperature effect on the relative response of target OPPs. Extraction conditions: 10 mL honey blend solution, 50 ng g^{-1} each OPPs; 100 μL 0.1 mol L^{-1} hydrochloric acid; 100 μL 100 g L^{-1} Triton X-114; equilibration time,10 min; centrifugation time, 5 min; ultrasound-assisted back-extraction, 100 μL hexane, 5 min.

no evidence on the effect of pH on CME extraction efficiency for the studied analytes. It is well known that honey matrix contains a number of natural buffers including amino acids (0.05–0.1%), organic acids (ca. 0.57%, such as acetic, butyric, citric, formic, gluconic, lactic, malic, pyroglutamic and succinic) and lactone, which are sensible to pH changes. It was previously reported that the presence of organic acids can affect the extraction efficiency of the CME technique, even though for those analytes that cannot exchange protons [25]. Regarding the analytes, they have an intermediate to high polarity ($\log K_{o/w}$ ca. 2.4); but they cannot exchange protons with the medium [31]. Therefore, it was expected that the OPPs were extracted by adsorption on the palisade layer of the micellar surface by interactions with the hydrophilic head groups of monomers [19,22]. Taking into account these considerations, it was found interesting to study the pH effect on the relative response of the microextraction technique within the range of 2–12 adjusting it with hydrochloric acid and sodium hydroxide, respectively. The pH of the sample blend was 4.5. The extraction procedure was the one described above. Within the studied pH it was observed that the appearance of the supernatant bulk after CME was changing as the pH was modified. The results are presented in Fig. 2. The best relative responses for all OPPs were observed at pH 2. At this pH, it was also observed a notorious reduction of the chromatograms' base line compared with the extractions carried out at higher pH. This fact led to an increment of the sensitivity of the technique favoring the LODs of each analyte. The results suggest that at pH 2, smaller amounts of concomitant were extracted by reducing their affinity by the micelles, while the OPPs remained invariant. Thus, a selectivity effect favored the extraction efficiency of the CME technique for the studied OPPs. Additionally to these results, it was studied the effect of the use of an organic acid for buffering the CME system. Thus, potassium phthalate buffer (pH 2.2) was compared with hydrochloric acid (pH 2.0). The results revealed that the greater relative responses were observed when 100 μL 0.1 mol L^{-1} hydrochloric acid was used to adjust the pH. Therefore, the pH of all sample solutions was adjusted to 2 by adding 100 μL 0.1 mol L^{-1} hydrochloric acid.

3.3. Equilibration temperature and time effects

Equilibration temperature and time play important roles in the CME performance. For non-ionic surfactants the temperature effect depends mainly on the analytes nature. Non-polar compounds with greater affinity for the micellar core show an increment in their solubility by increasing temperature. Polar analytes solubilized in the palisade layer generally exhibit a behavior that depend on their structure and the type of sample matrix [22]. Equilibration temperature and time govern the micelles dehydration, which is desirable to achieve smaller coacervate-phase volumes, and also to accelerate the phase-separation process. By increasing the equilibration temperature or time the micelles dehydration phenomenon is favored, and thus smaller coacervate-phase volumes are finally achieved [21]. Additionally, by increasing the extraction temperature above the cloud-point temperature, the aqueous solubility of the micelles diminishes [22]. Therefore, the EF and percent recovery of the CME technique increase as the equilibration temperature is progressively increased. Furthermore, small coacervate-phase volumes are desired since they favored the ultrasound-assisted back-extraction process and enhanced the EF of the technique. Considering all these aspects, the temperature study was carried out within the temperature range of 20–95 °C keeping the equilibration time constant at 10 min (Fig. 3). The extraction procedure was the one described above. An increment in the relative response of the analytical signal was observed for the temperature range: 20–80 °C. After 80 °C the relative response of the OPPs remained invariant. Therefore, 85 °C was chosen for further studies as the working equilibration temperature. To determine the influence of the extraction time, it was varied within the range of 1–15 min keeping the equilibration temperature constant at 85 °C. It was observed that by increasing the equilibration time, the relative response increases, reaching the maximum value at 5 min; after which, remained invariant. Therefore, 5 min equilibration time was chosen as working conditions for further studies.

3.4. Matrix modifiers effect

Fructose and other sugars are considered as micellar structure-makers because exert their influence through modification of the bulk water structure and decreasing the CMC [22]. In presence of carbohydrates, water–water interaction is replaced by water–sugar interaction. Thus, the chances of micelles formation to protect the monomers tend to diminish. As a consequence micelle formation is favored and CMC is lowered [22,32]. Honey has a ca. 75% of sugars (fructose, glucose and sucrose) and those components can affect the structure of the micelles; and therefore the OPPs affinity for the micelles. In order to determine the honey matrix effect on the relative responses of OPPs, two different experiments were carried out. A 10 mL honey blend solution and a 10 mL water sample

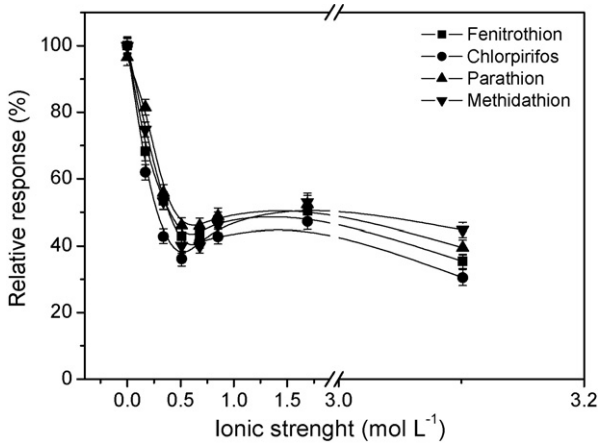

Fig. 4. Ionic strength effect on the relative response of target OPPs. Extraction conditions: 10 mL honey blend solution, 50 ng g^{-1} each OPPs; 100 μL 0.1 mol L^{-1} hydrochloric acid; equilibration temperature and time, 85 °C, 5 min; centrifugation time, 5 min; ultrasound-assisted back-extraction, 100 μL hexane, 5 min.

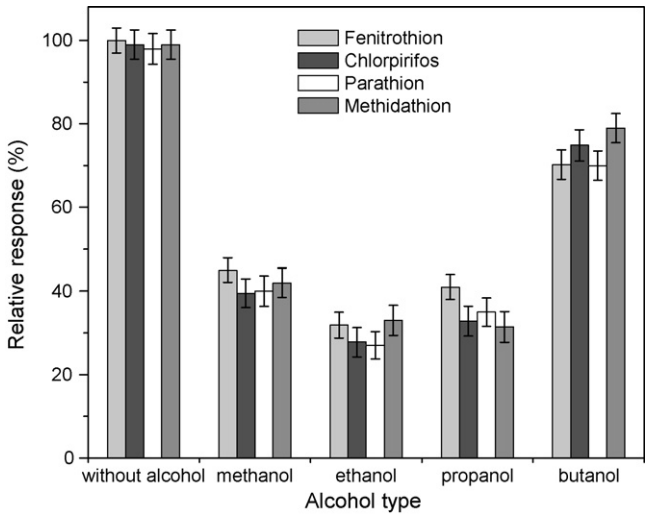

Fig. 5. Alcohol type effect on the relative responses of target OPPs. Extraction conditions as described in Fig. 4.

spiked with 50 ng g^{-1} of OPPs were extracted as described above. It was observed that the relative responses obtained for OPPs in honey were ca. 36% higher than in water. Therefore, it is possible to conclude that the sugar content is a natural matrix modifier of the microextraction technique that enhances the extraction efficiency of it.

It is well known that the ionic strength of the aqueous medium can affect the phase-separation process of micellar systems based on non-ionic surfactant [22]. The addition of small amounts of inert salts can facilitate the phase-separation process since it alters the density of the bulk aqueous phase by increasing the micellar size and the aggregation number [21]. Furthermore, the cloud-point temperature of the system is diminished by increasing the ionic strength. However, the addition of small electrolytes concentration results in a decrement of the solubility of analytes located in the palisade region of the micelles [22,33,34]. The ionic strength of honey blend solution was determined by electrical conductivity and was 0.004 mol L^{-1}. The salting out study was carried out by adding different volumes of NaCl 6.15 mol L^{-1} (Fig. 4). The best relative response for all OPPs was observed when no NaCl was added to the extraction solution. As the ionic strength of the medium was increased, their relative response decreased up to ca. 50%, after which it remained constant. It could be due to the presence of electrolytes change the physical properties of the palisade layer in which the OPPs are preferentially extracted [33,34]. Therefore, no additionally NaCl 6.15 mol L^{-1} was added for further studies.

Alcohols can also have significant influence on the micelles characteristics and polar concomitant of the matrix. Regarding the effect of the alcohols on the micelles, they can be adsorbed on the micelle–water interfacial region favoring the dehydration of the micelle and decreasing the cloud-point temperature [35,36]. Additionally, these interactions can change the polar character of the micelles and thus, modify the analytes affinity for it [22]. On the other hand, alcohols can also interact with the hydroxilated compounds of the matrix, including sugars, by hydrogen bonding [37]. Therefore, alcohols could affect the concomitants affinity for the micelles and thus, the selectivity of extraction technique. In order to study the influence of the alcoholic matrix modifier on the extraction technique, four different alcohols, including methanol, ethanol, 1-propanol and 1-butanol were evaluated. The alcoholic matrix modifier study was carried out by adding 100 μL of each alcohol to the extraction system. The extraction procedure was the one described above. As can be seen from Fig. 5, the addition of alcohol deteriorates the analytical signal for the OPPs. Among the studied alcohols, methanol, ethanol and 1-propanol showed lower relatives responses than 1-butanol. Short-chain alcohols have a tendency to interact with the polar-palisade branch of the monomers turning them into a less polar area [36]. Short-chain alcohols could fit better in between the micelle palisades; while longer chain alcohols would be conditioned by steric effects. Thus, larger number of short-chain alcohol molecules would fit in between the palisade diminishing the polar character of it [36]. Therefore, it is to be expected that the extraction efficiency of polar analytes, such OPPs, for the micelles diminish, too. Additionally, short-chain alcohols have more polar character and higher water solubility than the longer one, which might favor the solubility of OPPs into the aqueous phase worsen their affinity for the micelles. This phenomenon would deteriorate the extraction efficiency of the microextraction technique for polar compounds. Taking into consideration the effect of 1-butanol compared with the other alcohols, it was found interesting to study the addition of different volumes to the extraction system. It was observed that by increasing the 1-butanol volume from 50 to 500 μL, the resulting coacervate phase volume diminish and also the relative responses of OPPs. It is in agreement with the results about the reduction of CMC using increasing concentrations of long chain alcohols achieved by Alauddin et al. [35]. The increment of 1-butanol volume disfavored the micellation phenomenon and phase separation of the system; therefore there was a shortage of micelles available to extract the analytes leading to a decrement of the extraction efficiency of the technique. Based on the results, it is possible to conclude that none of the studied alcohols favored the extraction efficiency of the studied OPPs. As it was mentioned above, alcohols can also interact with sugars through their hydroxyl groups. Therefore, these alcohol–sugar interactions might be interfering with sugar–micelles interaction, contributing to the deterioration of the extraction efficiency of the CME technique for OPPs. In view of the above results, non-alcohol or salts was added in further studies.

3.5. Ultrasound-assisted back-extraction

Due to the high viscosity and low volatility of the surfactant-rich phase, it cannot be injected directly into the GC. Therefore, after CME procedure and before the injection, a supplemental stage was required in order to avoid clogging the injector and deteriorate the column. Ultrasound-assisted back-extraction was selected as a suitable approach for coupling CME to GC–MS.

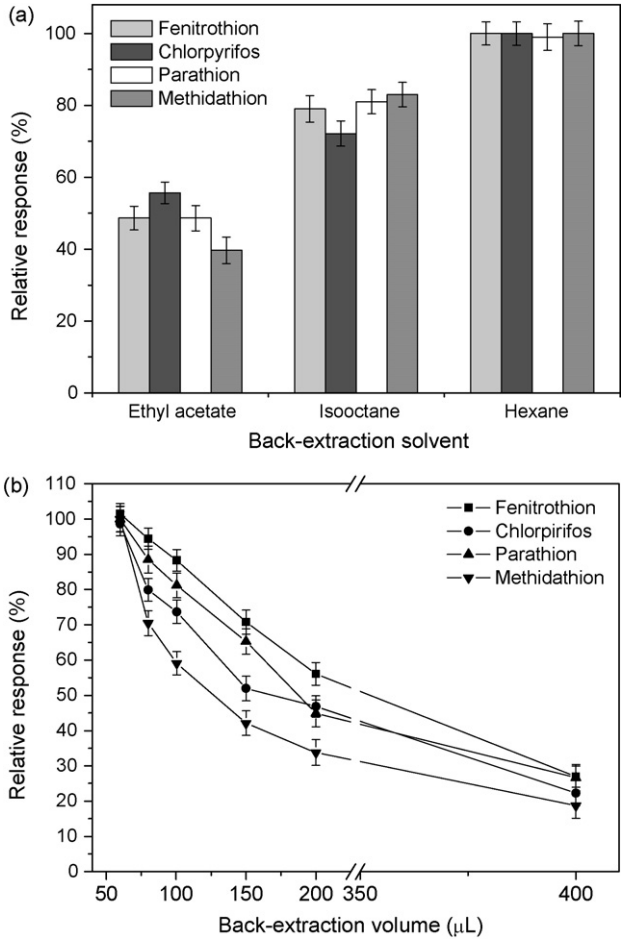

Fig. 6. (a) Back-extraction solvent effect on the relative response of target OPPs. (b) Hexane volume effect on the relative response of target OPPs. Extraction conditions as described in Fig. 4.

Different water-immiscible solvents (hexane, isooctane and ethyl acetate) were studied in order to evaluate their back-extraction efficiencies for extracting the target analytes from coacervate phase. The study was carried out by adding 100 μL of the studied solvents to the coacervate phase and sonicating the resulting mix for 10 min. The results revealed that the relative responses of OPPs in hexane are higher than isooctane and ethyl acetate (Fig. 6a). Thereby, hexane was selected as the back-extracting solvent for further studies.

The volume of back-extraction solvent was studied within a volume range of 60–400 μL with a view to recover the target OPPs from the coacervate phase yielding the highest EF with the minimum solvent consumption. Fig. 6b shows that the greater relative response for the target OPPs were obtained when 60 μL hexane were used. When hexane volumes smaller than 60 μL were used, a stable emulsion with the coacervate phase was formed. Larger volumes than 60 μL result in a gradual decrement of the relative response of the analytes due to subsequent dilution. Therefore, 60 μL of hexane was selected to develop further studies.

The sonication time was studied within the range of 1–15 min. It was observed that the relative responses of the OPPs increased as the time increase, reaching a maximum at 4 min. No significant differences were observed when longer time periods were assayed. Similar results were reported in previous works [25,26]. Thus, 5 min were chosen as the ultrasound-assisted back-extraction working time for further studies.

Table 1
CME-UABE-GC–MS analytical performance for OPPs determination.

Analyte	RSD%[a,b]	r^2	LODs[a] (ng g^{-1})	Linear range[a] (ng g^{-1})
Fenitrothion	2.9	0.9996	0.06	1.0–1000
Chlorpirifos	5.8	0.9992	0.03	0.3–1000
Parathion	7.2	0.9997	0.09	1.0–1000
Methidathion	9.5	0.9994	0.47	1.0–1000

Extraction conditions as described in Section 2.4.
[a] 95% confidence interval; $n = 5$.
[b] OPPs concentration 1 ng g^{-1}, dry weight.

3.6. Analytical performance

The calibration curves were made under optimized conditions with a honey blend solution spiked at different concentration of target OPPs. The analytical figures of merits were summarized in Table 1. The EF of the proposed methodology was calculated as previous works [25,38] and was 167. The LOD of the analytes for the preconcentration of 0.5 g honey sample spiked at 1 ng g^{-1}, calculated as $S/N = 3$, were 0.06, 0.03, 0.09 and 0.47 ng g^{-1} for fenitrothion, chlorpirifos, parathion and methidathion, respectively. The precision was evaluated over five replicates resulting values of RSDs ≤ 9.5%. The calibration curves showed a satisfactory linearity within the concentration range: 0.3–1000 ng g^{-1} for chlorpirifos and 1.0–1000 ng g^{-1} for fenitrothion, parathion and methidathion, respectively, and the coefficient of correlation (r^2) exceeded 0.9992 for all analytes. Validation of the analytical methodology was performed by standard addition method at two concentration levels (2 and 20 ng g^{-1}) over the real honey samples. Recoveries and repeatabilities values of the fortified samples at the different concentrations were evaluated. This study led to a satisfactory robustness achieving recoveries ≥90% with RSDs < 2.9, 6.7, 7.3 and 10.1 for fenitrothion, chlorpirifos, parathion and methidathion, respectively (Table 2).

3.7. Application of the method to real samples

CME-UABE-GC–MS was applied for the determination of OPPs in honey of five regions of Argentina. The sample results and the recovery study were performed in triplicate (Table 2). Although different types of honey samples were analyzed, only the presence of methidathion in Sample 4 and Sample 5 was detected. The concentrations were 1.2 and 2.3 ng g^{-1}, respectively, and were lower than MRLs of EU regulations. The OPPs concentration in the other analyzed samples was below the detection limit of the proposed methodology. In order to evaluate the matrix influence on the analyte signals, the slope of the calibration graph based on the matrix-matched standards was compared with the slope of the pure solvent based calibration graph. The sensitivity decreased from pure solvent calibration to matrix-matched calibration curves. This effect shows the need to perform quantification by external calibration using matrix-matched standards. In this sense, a matrix of honey, as representative as possible, was obtained as described in Section 2.3.

3.8. Comparison of CME-UABE-GC–MS with other analytical methodologies

The analytical performance of CME-UABE-GC–MS for OPPs determination in honey samples was compared with other previously reported analytical methodologies such as LLE-GC–MS, SPE-GC–MS and SPME-LC–MS [9–11]. It was observed that the LODs of CME-UABE-GC–MS were lower than the other methodologies previously used for OPPs determination in honey (3.4–6.5 ng g^{-1}, 0.1–1.4 ng g^{-1} and 300–500 μg kg^{-1} for LLE-GC–MS, SPE-GC–MS and SPME-LC–MS, respectively). The mean RSDs values were com-

Table 2
Recovery study of the four OPPs in different honey samples.

Sample	Pesticide	Level found	2 ng g^{-1} spiked		20 ng g^{-1} spiked	
			Found[a]	Recovery[b]	Found[a]	Recovery[b]
Honey 1	Fenitrothion	nd	1.9 ± 0.1	95	19.7 ± 1.4	98
	Chlorpirifos	nd	2.0 ± 0.3	100	20.1 ± 2.9	101
	Parathion	nd	1.8 ± 0.3	90	19.1 ± 3.4	95
	Methidathion	nd	1.8 ± 0.4	90	20.4 ± 4.8	102
Honey 2	Fenitrothion	nd	2.0 ± 0.1	100	19.3 ± 1.4	96
	Chlorpirifos	nd	2.1 ± 0.3	105	20.4 ± 2.9	102
	Parathion	nd	1.9 ± 0.3	95	21.5 ± 3.8	107
	Methidathion	nd	2.0 ± 0.5	100	21.2 ± 5.0	106
Honey 3	Fenitrothion	nd	1.8 ± 0.1	90	19.3 ± 1.4	96
	Chlorpirifos	nd	1.9 ± 0.2	95	20.4 ± 2.9	102
	Parathion	nd	1.9 ± 0.3	95	21.1 ± 3.8	105
	Methidathion	nd	2.0 ± 0.5	100	21.3 ± 5.0	106
Honey 4	Fenitrothion	nd	1.8 ± 0.1	90	18.4 ± 1.3	92
	Chlorpirifos	nd	1.8 ± 0.3	90	19.6 ± 2.8	98
	Parathion	nd	1.8 ± 0.3	90	18.2 ± 3.2	91
	Methidathion	1.2 ± 0.6	1.9 ± 0.4	95	20.4 ± 4.8	102
Honey 5	Fenitrothion	nd	2.0 ± 0.1	100	21.2 ± 1.5	106
	Chlorpirifos	nd	2.0 ± 0.3	100	20.8 ± 3.0	104
	Parathion	nd	1.8 ± 0.3	90	19.4 ± 3.4	97
	Methidathion	2.3 ± 0.5	1.8 ± 0.4	90	18.6 ± 4.4	93

Extraction conditions as described in Section 2.4.
nd: not detectable.
[a] Results expressed as $\bar{x} \pm t \times \mathrm{SD}/\sqrt{n}$; $n = 3$; 95% confidence interval; ng g^{-1} dry weight.
[b] [(Found − Base)/Added] × 100.

parables (3.2–8.6, 6.3–7.0 and 3.1–9.9, respectively). CME employs simple and inexpensive equipment so it is applicable for most of the analytical laboratories. Moreover, the extraction equilibrium is established within a few minutes in comparison to other methodologies such as SPME (ca. 150 min versus ca. 18 min, respectively). Furthermore, LLE and SPE require large volumes of organic solvents. CME-UABE use alternative solvents such as surfactants and only require 60 μL of hexane on the overall extraction procedure to achieve a satisfactory performance. CME-UABE-GC–MS is a sensitive, rapid, versatile and reproducible technique. Additionally, it is a low organic solvent consuming extraction technique, which turns it into a low cost and environmentally friendly technique.

4. Conclusions

CME is a sensitive and fast microextraction technique which was satisfactorily applied for the determination of OPPs at concentration lower than MRLs of EU regulations. Under optimized working conditions, detection limit in the order of low nanogram per gram with an acceptable precision were obtained. The back-extracted analytes were introduced to GC–MS successfully without declining the separation efficiency of the capillary column. The proposed methodology represents a large time-saving and requires lower volumes of organic solvents in comparison to methodologies previously reported. Furthermore, the developed CME-UABE provides good linearity, precision and quantitative recoveries. The proposed CME-UABE-GC–MS methodology has been applied for the extraction, preconcentration and determination of OPPs in real honey samples with satisfactory robustness. The proposed CME-UABE-GC–MS analysis is well suited as a potential methodology in routine analysis to determine trace levels of OPPs in honey due to their simplicity, ruggedness and cost effectiveness.

Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) (PICT-BID).

References

[1] A. Choudhary, D.C. Sharma, Bull. Environ. Contam. Toxicol. 80 (2008) 417.
[2] B. Morzycka, J. Chromatogr. A 982 (2002) 267.
[3] S.R. Rissato, M.S. Galhiane, M.V. de Almeida, M. Gerenutti, B.M. Apon, Food Chem. 101 (2007) 1719.
[4] C. Blasco, M. Fernández, A. Pena, C. Lino, M.I. Silveira, G. Font, Y. Picó, J. Agric. Food Chem. 51 (2003) 8132.
[5] http://www.argentinatradenet.gov.ar/sitio/estrategias/Informe_Sectorial_Miel.pdf.
[6] http://europa.eu/legislation_summaries/food_safety/.
[7] A. Herrera, C. Pérez-Arquillué, P. Conchello, S. Bayarri, R. Lázaro, C. Yagüe, A. Ariño, Anal. Bioanal. Chem. 381 (2005) 695.
[8] O. Erdogrul, Food Control 18 (2007) 866.
[9] Z. Jin, Z. Lin, M. Chen, Y. Ma, J. Tan, Y. Fan, J. Wen, Z. Chen, F. Tu, Chin. J. Chromatogr. 24 (2006) 440.
[10] B. Albero, C. Sánchez-Brunete, J.L. Tadeo, J. Agric. Food Chem. 52 (2004) 5828.
[11] C. Blasco, M. Fernández, Y. Picó, G. Font, J. Chromatogr. A 1030 (2004) 77.
[12] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[13] H. Prosen, L. Zupancic-Kralj, Trends Anal. Chem. 18 (1999) 272.
[14] N. Fontanals, T. Barri, S. Bergström, J.A. Jönsson, J. Chromatogr. A 1133 (2006) 41.
[15] F. Ahmadi, Y. Assadi, M.R. Milani Hosseini, M. Rezaee, J. Chromatogr. A 1101 (2006) 307.
[16] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1.
[17] A.R. Fontana, R.G. Wuilloud, L.D. Martínez, J.C. Altamirano, J. Chromatogr. A 1216 (2009) 147.
[18] A.R. Fontana, J.C. Altamirano, Talanta 81 (2010) 1536.
[19] F.J. López-Jiménez, S. Rubio, D. Pérez-Bendito, J. Chromatogr. A 1195 (2008) 25.
[20] R. Carabias-Martínez, E. Rodríguez-Gonzalo, B. Moreno-Cordero, J.L. Pérez-Pavón, C. García-Pinto, E. Fernández Laespada, J. Chromatogr. A 902 (2000) 251.
[21] Z. Sosa Ferrera, C. Padrón Sanz, C. Mahugo Santana, J.J. Santana Rodríguez, Trends Anal. Chem. 23 (2004) 469.
[22] E. Pramauro, E. Pelizzetti, in: S.G. Weber (Ed.), Surfactants in Analytical Chemistry, vol. XXXI, Elsevier Science B.V., Amsterdam, The Netherlands, 1996.
[23] J.C.A. De Wuilloud, R.G. Wuilloud, M.F. Silva, R.A. Olsina, L.D. Martinez, Spectrochim. Acta B 57 (2002) 365.
[24] Y. Takagai, W.L. Hinze, Anal. Chem. 81 (2009) 7113.
[25] A.R. Fontana, M.F. Silva, L.D. Martínez, R.G. Wuilloud, J.C. Altamirano, J. Chromatogr. A 1216 (2009) 4339.
[26] T.I. Sikalos, E.K. Paleologos, Anal. Chem. 77 (2005) 2544.
[27] A. Ohashi, M. Ogiwara, R. Ikeda, H. Okada, K. Ohashi, Anal. Sci. 20 (2004) 1353.
[28] B. Fröschl, G. Stangl, R. Niessner, Fresenius J. Anal. Chem. 357 (1997) 743.
[29] J. Shen, X. Shao, Anal. Chim. Acta 561 (2006) 83.
[30] P.D. Zygoura, E.K. Paleologos, K.A. Riganakos, M.G. Kontominas, J. Chromatogr. A 1093 (2005) 29.

[31] A. Moral, M.D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chim. Acta 608 (2008) 61.
[32] K.R. Acharya, S.C. Bhattacharyya, S.P. Moulik, J. Photochem. Photobiol. A 122 (1999) 47.
[33] A.M. Bolkhus, H. HÃjland, E. Gilje, S. Backlund, J. Colloid Interface Sci. 124 (1988) 125.
[34] J.A. Molina-Bolívar, J. Aguiar, C. Carnero Ruiz, J. Phys. Chem. B 106 (2002) 870.
[35] M. Alauddin, T. Parvin, T. Begum, J. Appl. Sci. 9 (2009) 2301.
[36] T. Gu, P.A. Galera-Gómez, Colloid Surf. A 147 (1999) 365.
[37] F. Montañés, A. Olano, E. Ibáñez, T. Fornari, AIChE J. 53 (2007) 2411.
[38] A.R. Fontana, S.H. Patil, K. Banerjee, J.C. Altamirano, J. Agric. Food Chem. 58 (2010) 4576.