Microchemical Journal 129 (2016) 63–70

Contents lists available at ScienceDirect

Microchemical Journal

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

# Development of a dispersive liquid-liquid microextraction technique for the analysis of aryloxyphenoxy-propionate herbicides in soy-based foods

Ester Lubomirsky, Juan M. Padró, Mario R. Reta *

*Facultad de Ciencias Exactas, UNLP, CONICET, Laboratorio de Investigación y Desarrollo de Métodos Analíticos (LIDMA) y División Química Analítica, Calle 47 esq. 115, La Plata B1900AJL, Argentina*

ARTICLE INFO

*Article history:*
Received 3 March 2016
Received in revised form 9 June 2016
Accepted 10 June 2016
Available online 12 June 2016

*Keywords:*
Aryloxyphenoxy-propionate herbicides
Phosphonium based room temperature ionic liquids
Ionic liquid–dispersive liquid-liquid microextraction
Liquid chromatography
Soy sauce
Soy milk

ABSTRACT

In this work, an ionic liquid–dispersive liquid-liquid microextraction (IL-DLLME) method combined with liquid chromatography and diode-array detection (DAD) was used for the determination of four aryloxyphenoxy-propionate herbicides (fenoxaprop-*p*-ethyl, quizalofop-*p*-tefuryl, propaquizafop and haloxyfop-*p*-methyl) in two soy-based foods (soy milk and soy sauce) was used. For this purpose, the phosphonium-based room temperature ionic liquid (trihexyl(tetradecyl)phosphonium bistriflamide) was used as the extractant. The effect of the experimental parameters on extraction efficiency such as type of disperser solvent, disperser solvent/ ionic liquid volumes ratio, pH, nature and concentration of salt in the aqueous phase, sample volume, and centrifugation and extraction times were investigated and optimized. Since matrix effects were detected, the standard addition method was used for quantification. Under the optimized conditions, the proposed sample preparation method coupled to high performance liquid chromatography-diode array detection (HPLC-DAD) had a satisfactory performance to determine the four herbicides in soy sauce and soy milk. The enrichment factors ranged from 18 to 43 and recovery factors from 25 to 66%. Although the recoveries were not high because of the presence of organic solvent in the sample preparation step, the inter-day reproducibility was 8.4% or less, depending on the analyte, the limits of detection (S/N = 3) were obtained in the range of 0.12–0.34 mg $L^{-1}$, the limits of quantification (S/N = 10) between 0.36 and 1.04 mg $L^{-1}$, and linear ranges from LOQs to 9.26 mg $L^{-1}$. Finally, the IL-DLLME methodology is inexpensive, simple, fast, and environmentally friendly for the determination of the studied herbicides in soy sauce and soy milk. This study constitutes the first application of an IL-DLLME methodology to aryloxyphenoxy-propionate herbicides analysis in commercial soy-derived foods.



## 1. Introduction

The aryloxyphenoxy-propionate herbicides (AOPPs) constitute a kind of selective post-emergence herbicides [1], which were registered for controlling annual and perennial grassy weeds for many crops as soy, rice, corn or peanut. AOPPs are toxic to aquatic organisms [2], especially fish, and could be inducers of liver toxicity and injury [3]. The widespread use of those compounds contributes to their presence in ground water, soil and other environmental matrices [4,5]. Recently there have been a few reported studies on the determination of some AOPPs in water [6–11], soils [12,13], crops [12,14], fruits and vegetables [9,15]. However, any analytical methodology to determine AOPPs in processed foods, specifically in crops-derived ones such as soy-sauce or soy milk, have been developed. Therefore, more sensitive and robust methods to analyze AOPPs in those complex matrices are required.

Conventional methods for the isolation and/or enrichment of AOPPs-related chemicals from water involve liquid–liquid extraction (LLE) or solid-phase extraction (SPE) [5,16]. However, SPE is a convenient technique very recommended to pre-concentrate analytes at trace levels and requires much less amounts of organic solvents but it suffers from some drawbacks such as low recoveries and low batch-to-batch reproducibility. In recent years, a lot of less solvent-consuming microextraction techniques have been used in extraction of AOPPs in water, such as solvent microextraction (SME) [4], microextraction in packed syringe (MEPS) [11] and IL-DLLME [7]. Recently, a faster, sensitive and environmentally friendly method for determination of AOPPs in environmental water samples by coupling both the dispersive magnetic SPE (d-MSEP) with both HPLC-DAD and ultrahigh pressure liquid chromatography with triple quadrupole mass spectrometer (UHPLC-MS/MS) was developed [6].

The use of Room Temperature Ionic Liquids (RTILs) in different areas of Analytical Chemistry has increased considerably in recent years due to their advantages over conventional organic solvents such as low toxicity, flammability and volatility [17]. As a counterpart, due to the typical high viscosities of the RTILs, a dilution with solvents such as methanol or

* Corresponding author at: LIDMA (Laboratorio de Investigación y Desarrollo de Métodos Analíticos) y División Química Analítica, Universidad Nacional de La Plata, 47 y 115, 1900 La Plata, Argentina.
*E-mail address:* mreta@quimica.unlp.edu.ar (M.R. Reta).

http://dx.doi.org/10.1016/j.microc.2016.06.015
0026-265X/

acetonitrile can be necessary before injection into the HPLC column, which decreases the enrichment factor [18].

Although phosphonium-based RTILs (PB-RTILs) have been known and synthesized for years, they have been more or less neglected in the literature, mainly in LLE, as compared to their imidazolium- or pyrrolidinium-based counterparts [19,20]. PB-RTILs are made of tetraalkylphosphonium cations with different anions and have some additional advantages compared to the nitrogen-based RTILs (NB-RTILs), such as very high thermal and chemical stability, higher solvation properties and they are cheaper [18]. There are about 20 different types of PB-RTILs commercially available. Cytec Industries Inc. sells phosphonium salts under the Cyphos® trade name [21].

The (IL-DLLME) has been developed as an efficient sample preparation and preconcentration method. The advantages of IL-DLLME are the use of a small volume of ionic liquid, ease of operation, rapidity, low cost, high recovery for several compounds, high enrichment factors and environmentally friendly nature [22,23]. The extraction by IL-DLLME is based on a ternary solvent system: the aqueous sample, the dispersive solvent and the extraction solvent. An appropriate mixture of the extraction solvent (an organic solvent or an ionic liquid) and the dispersive solvent (a water-organic miscible solvent) is rapidly injected into the aqueous sample with a syringe and a cloudy solution is formed. After centrifugation, the analytes are collected into the small volume (a single drop) of the extraction solvent while the dispersive solvent remains in the initial aqueous solution [22]. There has been a large number of works that use nitrogen-based RTILs (NB-RTILs) as the solvent extraction in LLE, especially those with the cation dialkylimidazolium, while the use of phosphonium-based RTILs (PB-RTILs) is very scarce. Recently, a flow injection system for online IL-DLLME using the less dense than water IL tetradecyl(trihexyl)phosphonium chloride (Cyphos 101) for preconcentration of cobalt (Co) was presented [24]. Also, the use of tetradecyl(triehexyl)phosphonium *bis*-2,4,4-trimethylpentylphosphinate (Cyphos 104) as an effective extractant of lactic acid (LA) in aqueous systems by LLE have been measured [25].

In this study a simple and sensitive analytical method to determine aryloxyphenoxy-propionate herbicide residues (AOPPs) in soy sauce and soy milk was developed. The IL-DLLME methodology using a PB-RTIL as extractant and final analysis by HPLC-DAD were used. Special attention to the optimization of IL-DLLME parameters to maximize the extraction efficiency and to assure ruggedness has been given. The most important figures of merit of the analytical methodology were obtained.

This work represents the first proposal to determine aryloxyphenoxy-propionate herbicides residues in soy sauce and soy milk.

## 2. Material and methods

### 2.1. Chemicals and materials

Trihexyl(tetradecyl)phosphonium bistriflamide, (Cyphos 109, $[(C_6)_3C_{14}P][NTf_2]$) was purchased from Cytec (New Jersey, USA). Reagents were of analytical grade or better: haloxyfop-*p*-methyl, quizalofop-*p*-tefuryl, fenoxaprop-*p*-ethyl y propaquizafop from Agrofina (Bs. As., Argentina) (Fig. 1), acetic acid anhydrous (Merck, Hohenbrunn, Germany), sodium acetate anhydrous (J.T. Baker, México), sodium chloride and potassium chloride (Anedra, Argentina), magnesium sulfate 7-hydrate (Biopack, Argentina), potassium phosphate (Merck, Hohenbrunn, Germany), isopropanol HPLC grade (Sintorgan, Bs. As., Argentina), phosphoric acid 85% (Merck, Hohenbrunn, Germany), potassium dihydrogen phosphate (Merck, Hohenbrunn, Germany), monoacid potassium phosphate (Carlo Erba, Divisione Chimica Industriale- Milano, Italy), citric acid (Panreac, Castellar del Vallès, Spain), formic acid (Anedra, Bs. As., Argentina), sodium hydroxide (Analar, Poole, United Kingdom), sodium tartrate (J. T. Baker, Estado de México, México), potassium hydrogen phthalate (Anedra, Bs. As., Argentina), *tris* (tris(hydroxymethyl)aminomethane) (Carlo Erba, Divisione Chimica Industriale- Milano, Italy), chlorhidric acid 37% (Anedra, Bs. As., Argentina), acetone (Biopack, Bs. As., Argentina), methanol and acetonitrile grade HPLC (J. T. Baker, Estado de México, México) and ethanol (Carlo Erba, Divisione Chimica Industriale- Milano, Italy). Solutions were prepared with MilliQ® water.

Conical graduated polypropylene light-blue screw-capped test tubes (17 × 120 mm, 15 mL) for the IL-DLLME experiments and sample conditioning were used. The samples were filtered through a Micro-Mate TM interchangeable syringe (Popper & Sons Inc., New Hyde Park, NY, USA) containing a 0.22 mm cellulose–nitrate membrane.

### 2.2. Instrumentation and chromatographic conditions

All chromatographic studies were performed on an HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA) equipped with vacuum degasser, binary pump, autosampler, thermostatted column device, and photodiode array detector (DAD). The column was a Symmetry C-18 (3.9 × 150 mm; 5 μm) from Waters (Milford, USA). Optimum separation was achieved with a water-methanol mobile phase at a flow rate of 1 mL $min^{-1}$ and temperature of 25 °C. The gradient elution program was (solvent A: water; solvent B: methanol): 70% B, 0 min; 76% B, 15 min; 100% B, 20 min; 70% B, 28 min. The sample injection volume was 20 μL. All mobile phases were filtered through 0.22-μm nylon membranes (Osmonics-Magna) for organic solvents

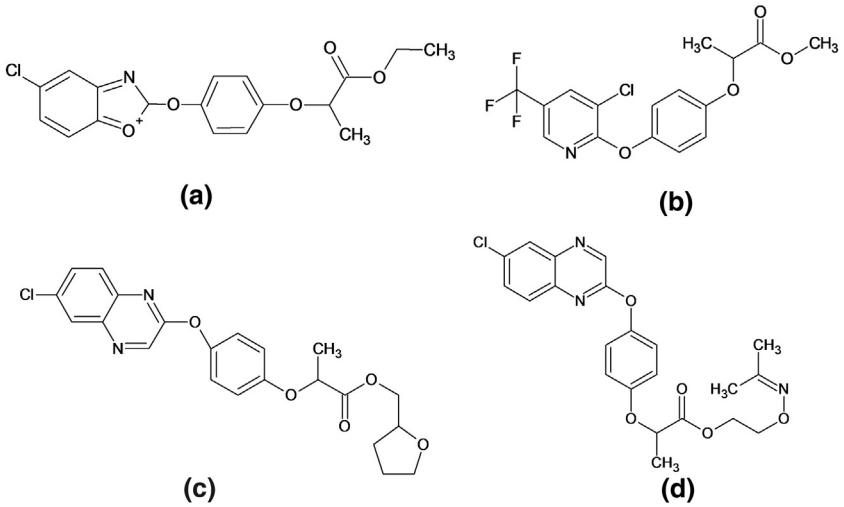


**Fig. 1.** Chemical structures of (a) fenoxaprop-*p*-ethyl; (b) haloxyfop-*p*-methyl; (c) quizalofop-*p*-tefuryl; (d) propaquizafop.

and 0.45-μm cellulose-nitrate filters (Micron Separations) for aqueous phases. The detector was set at 230 nm for all analytes. At this wavelength the RTIL absorb radiation but the elution of all analytes occurs at longer retention times.

A LUGUIMAC LC-20 centrifuge operating at 4000 rpm with 15 mL polypropylene tubes for the optimization experiments was used. A combined glass Metrohm electrode in a commercial Accument AR25pH/mV/Ion/Meter (Fisher Scientific) pH meter for the pH measurements was used. Water was purified with a Milli-Q system (Millipore Co.).

### 2.3. Extraction method for the optimization experiments

Stock solutions of fenoxaprop-*p*-ethyl, quizalofop-*p*-tefuryl, propaquizafop and haloxyfop-*p*-methyl were prepared by dissolving the compounds in methanol at concentrations of 480, 520, 500 and 500 mg $mL^{-1}$, respectively. The solutions were sonicated for a few minutes. These stock solutions were stored in the refrigerator for up to 1 month and their preservation status was checked daily by comparing the peak areas with the corresponding values obtained immediately after the solutions were prepared. From these solutions, a standard solution in acetic acid/acetate buffer (pH = 4.63, 0.1 mol $L^{-1}$), was prepared to reach a final concentration of 0.5 mg $L^{-1}$.

The IL-DLLME was performed as follows: 300 μL of a mixture containing $[(C_6)_3C_{14}P][NTf_2]$ and isopropanol in a ratio 1:5 were added to 9.00 mL of standard solution and a cloudy solution was formed. To maximize the extraction efficiency, the emulsion formed was sonicated for 3 min and then centrifugated for 15 min to separate the phases. After removing the upper phase, 20 μL of the resulting sedimented RTIL was injected into the HPLC column and analyzed under the aforementioned chromatographic conditions.

### 2.4. Extraction method for soy milk

A conditioning process adapted from the literature [26] previous to the IL-DLLME method was as follows: 3.00 mL of soy milk were spiked with the analytes standard mixture and 3.00 mL of acetone/acetonitrile (5:1) were added to remove the suspended material. To accelerate the proteins coagulation, the mixture was sonicated for 10 min and then centrifugated 30 min. The supernatant phase was transferred to a 15 mL centrifuge tube and 3.50 mL of an acetic/acetate buffer (pH = 4.63, 0.1 mol $L^{-1}$) were added to reach a total volume of 9.00 mL. Then, 300 μL of a mixture containing $[(C_6)_3C_{14}P][NTf_2]$ and isopropanol (1:5) were added and a cloudy solution was formed. To maximize the extraction efficiency, the emulsion formed was sonicated for 3 min and then centrifugated for 15 min to separate the phases. After removing the upper phase, 20 μL of the resulting sedimented RTIL phase was injected into the HPLC column.

### 2.5. Extraction method for soy sauce

A conditioning step adapted from the literature [26] was as follows: 3.00 mL of the sample were spiked with the analytes standard mixture

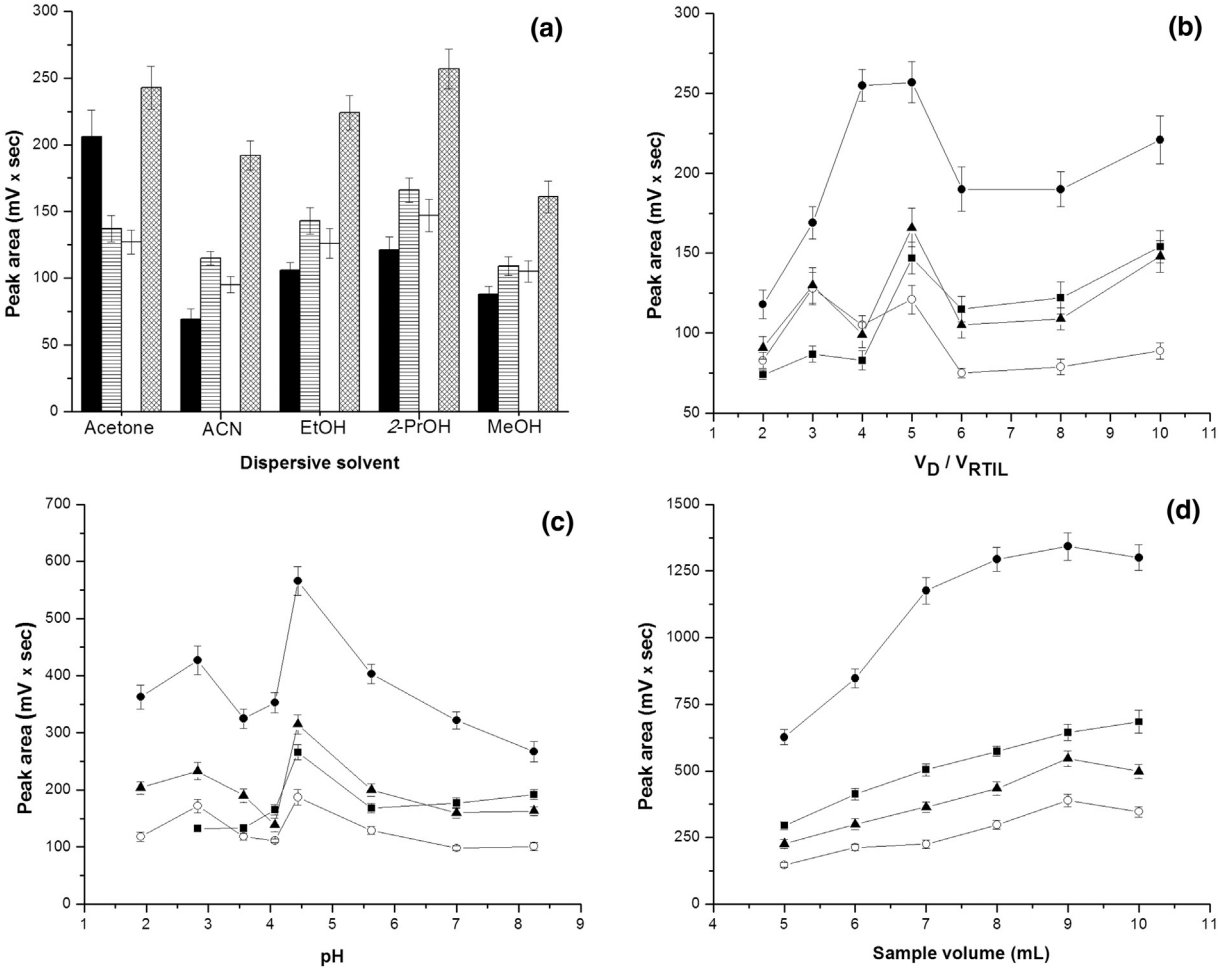


**Fig. 2.** Effects on extraction efficiency of: a) disperser solvent (filled bars, haloxyfop-*p*-methyl; empty bars, fenoxaprop-*p*-ethyl; striped bars, quizalofop-*p*-tefuryl; squared bars, propaquizafop) (working conditions in Section 3.1.2.); b) disperser solvent/ionic liquid volumes ratio (working conditions in Section 3.1.3.); c) pH (working conditions in Section 3.1.4.); d) sample volume (working conditions in Section 3.1.5) (● propaquizafop; ■ fenoxaprop-*p*-ethyl; ○ haloxyfop-*p*-methyl; □ quizalofop-*p*-tefuryl).

and 4.50 mL of acetone were added to eliminate the suspended material. The two phase system was sonicated for 10 min to promote the contact between them and then, it was centrifugated for 10 min. Then, 3.00 mL of the upper phase were taken and placed in a 15 mL centrifuge tube. Finally, 6.00 mL of an acetic/acetate buffer (pH = 4.63, 0.1 mol $L^{-1}$) were added and the IL-DLLME methodology was performed as described before.

## 3. Results and discussions

### 3.1. Optimization of IL-DLLME

In order to find the best experimental conditions for the IL-DLLME method, a *step-by-step* optimization was developed. Some variables that affect the performance of the experimental procedure were studied, such as, type of disperser solvent, disperser solvent to ionic liquid volumes ratio, sample volume, pH, type and salt concentration, extraction and centrifugation times. All experiments were performed by triplicate.

#### 3.1.1. Type of extraction solvent

The convenience of working with denser-than-water RTILs is that it is easy to take the extractant from the bottom of a conical tube, which can be simply pulled with a syringe and directly be injected into the HPLC column. However, it is necessary the addition of an organic solvent to decrease the viscosity and allow the HPLC injector to take correctly the injection volume. The low viscosity, very low water solubility and higher density than water of $[(C_6)_3C_{14}P][NTf_2]$ makes this RTIL a good candidate for the DLLME. Thus, this ionic liquid was selected in this work.

#### 3.1.2. Type of dispersive solvent

Dispersive solvents need to be soluble in both phases, in this case water and the selected PB-RTIL. Thus, to the water solution containing the herbicides, a mixture of the selected ionic liquid $[(C_6)_3C_{14}P][NTf_2]$ and different organic solvents in a ratio of 1:5 were rapidly injected. Acetone, methanol, ethanol, isopropanol and acetonitrile as the dispersive solvents were tested. Chromatographic peak areas corresponding to the IL phase were used as a parameter proportional to the extraction efficiency for each solvent, since in all cases the same starting AOPPs standard solution was used. It can be seen from Fig. 2.a that acetone and isopropanol as dispersive solvents allowed to obtain the greatest peak areas. However, for acetone some peaks showed shoulders. Thus, isopropanol has been chosen as the dispersive solvent in the following experiments.

#### 3.1.3. Disperser solvent/ionic liquid volumes ratio

The dispersive solvent/IL ratio is very important on the IL-DLLME process because a high proportion of IL increase (up to reach a plateau) the amount of analytes extracted. However, a high volume of dispersive

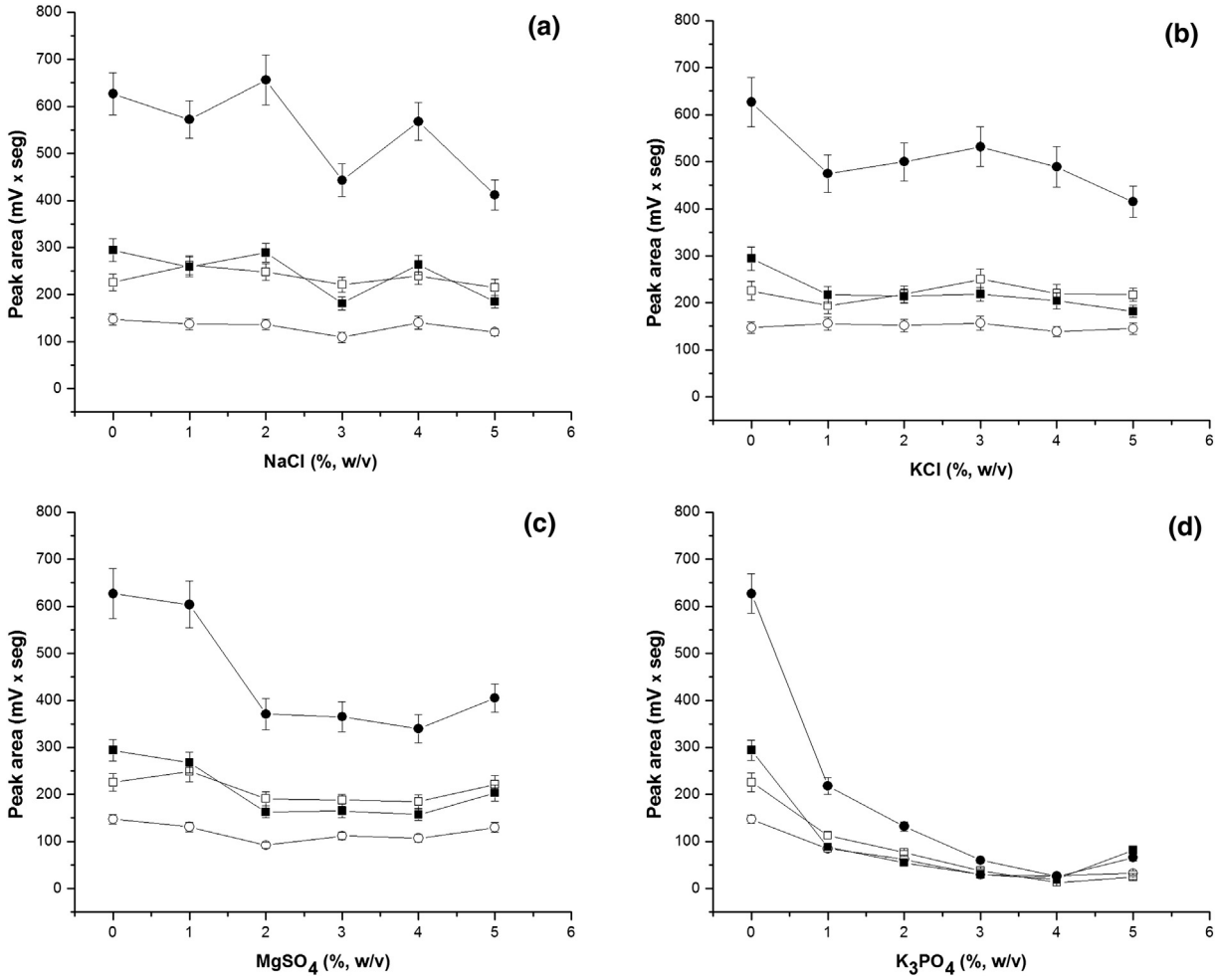


**Fig. 3.** Effect of the type and concentration of different salts on extraction efficiency (● propaquizafop; ■ fenoxaprop-*p*-ethyl; ○ haloxyfop-*p*-methyl; □ quizalofop-*p*-tefuryl) (working conditions in Section 3.1.6.).

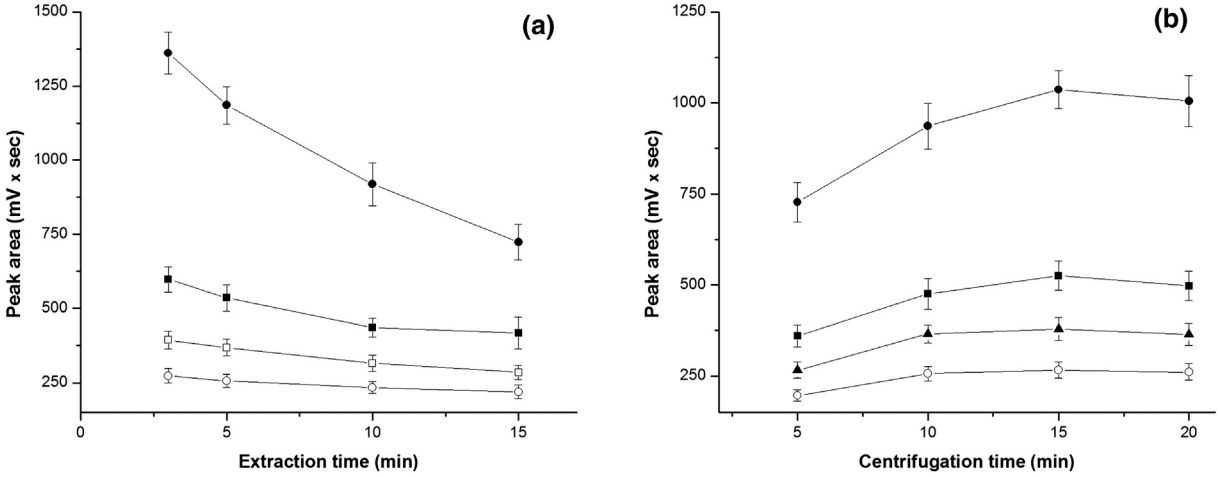


**Fig. 4.** Effect of centrifugation and extraction times on extraction efficiency (● propaquizafop; ■ fenoxaprop-*p*-ethyl; ○ haloxyfop-*p*-methyl; □ quizalofop-*p*-tefuryl) (working conditions in Section 3.1.7.).

solvent will affect or even avoid the formation of the emulsion into the aqueous sample, decreasing the extraction performance. Due to the high viscosity of the RTILs, it is preferred to calculate the volume by using the density instead of measuring it. A constant volume of 50 ± 1 μL of IL (density = 1.0652 g $mL^{-1}$ at 25 °C from the CYTEC product data sheet) was used and different amounts of isopropanol were added in order to have volume ratios from 2 to 10. In Fig. 2.b an optimum value is observed at 1:5 ratio. Thus, this composition was used in further experiments.

### 3.1.4. pH of the aqueous sample

It can be seen from Fig. 1 that the studied analytes have ionizable nitrogen atoms. The only $pK_a$ value available in the literature was found for : fenoxaprop-*p*-ethyl, which is 4.60 [27]. Buffers with a constant ionic strength of 0.1 mol $L^{-1}$ and different pHs close to their maximum buffer capacity were prepared (fosforic acid/diacidic phosphate, pH = 1.91; citric acid/citrate, pH = 2.83; formic acid/formate, pH = 3.57; tartaric acid/tartrate, pH = 4.07; acetic acid/acetate, pH = 4.44; hydrogen phthalate/phthalate, pH = 5.63; dihydrogen phosphate/ hydrogen phosphate, pH = 7.00 and *tris*(hydoxymethyl)aminomethane/tris hydrochloride, pH = 8.25) and then spiked with the analytes. The resulting solution was used as the sample for the IL-DLLME. The extraction efficiency for all the analytes showed a maximum at a pH of 4.44 (with an acetic acid/acetate buffer) (Fig. 2.c). Thus, this buffer and pH were used for the next experiments.

### 3.1.5. Volume of aqueous sample

Although it is convenient to use a high aqueous/IL volume ratio to accomplish a better enrichment factor, a high aqueous volume can cause a partial dissolution of the IL. For these experiments a constant amount of IL was used (50 μL) and increasing volumes of aqueous samples were tested. The better extraction efficiency was reached with 9.00 mL of aqueous sample (Fig. 2.d).

### 3.1.6. Salt effect

The effect of four types of salts and their concentrations on the extraction efficiency were tested: NaCl, KCl, $MgSO_4$ and $K_3PO_4$. Salt addition to the aqueous phase can concentrate the analytes in the IL phase because of the well-known salting-out effect. However, increasing the ionic strength may cause a partial solubilization of the IL, which is promoted by coulombic interactions [20]. Fig. 3 shows that extraction efficiency decreased with the presence of the studied salts, which can be attributed to the solubilization of the RTIL into the aqueous phase. Thus, any salt was used in the developed IL-DLLME method.

### 3.1.7. Extraction and centrifugation times

Extraction time is defined as the interval between the addition of the RTIL to the sample solution until the time in which the samples were put in the centrifuge. Extraction times between 3 and 15 min were tested, obtaining a maximum at 3 min (Fig. 4.a), showing that the equilibrium is fast.

**Table 1**
Calibration curves of the AOPPs in the different studied matrixes and the calculated *t*-values for the statistical comparison of the slopes (see Section 3.2).

| Matrix | Analyte | Linear regresion* | $R^{**}$ | $SD^{**}$ | $N^{**}$ | *t*-Values | *t′*-Values |
|---|---|---|---|---|---|---|---|
| Soy sauce | Haloxyfop-*p*-methyl | $y = 0.44(\pm0.01) \times -0.03(\pm0.07)$ | 0.9958 | 5.78 | 25 | 4.158 | 2.064 |
| | Quizalofop-*p*-tefuryl | $y = 0.33(\pm0.01) \times +0.04(\pm0.08)$ | 0.9894 | 4.37 | 25 | 2.345 | 2.064 |
| | Fenoxaprop-*p*-ethyl | $y = 0.276(\pm0.007) \times -0.05(\pm0.04)$ | 0.9965 | 3.63 | 25 | 30.381 | 2.064 |
| | Propaquizafop | $y = 0.71(\pm0.03) \times -0.1(\pm0.2)$ | 0.9963 | 9.05 | 24 | 7.289 | 2.069 |
| Soy milk | Haloxyfop-*p*-methyl | $y = 0.44(\pm0.02) \times -0.1(\pm0.1)$ | 0.983 | 5.82 | 25 | 3.040 | 2.064 |
| | Quizalofop-*p*-tefuryl | $y = 0.32(\pm0.01) \times +0.004(\pm0.06)$ | 0.9936 | 3.60 | 31 | 18.690 | 2.058 |
| | Fenoxaprop-*p*-ethyl | $y = 0.285(\pm0.006) \times -0.04(\pm0.04)$ | 0.9972 | 3.52 | 27 | 5.798 | 2.056 |
| | Propaquizafop | $y = 0.66(\pm0.02) \times +0.02(\pm0.1)$ | 0.9927 | 7.83 | 31 | 16.915 | 2.139 |
| Water | Haloxyfop-*p*-methyl | $y = 0.41(\pm0.01) \times -0.01(\pm0.06)$ | 0.9963 | 5.34 | 25 | – | – |
| | Quizalofop-*p*-tefuryl | $y = 0.36(\pm0.02) \times -0.04(\pm0.09)$ | 0.9881 | 4.67 | 25 | – | – |
| | Fenoxaprop-*p*-ethyl | $y = 0.306(\pm0.007) \times -0.04(\pm0.04)$ | 0.997 | 4.03 | 25 | – | – |
| | Propaquizafop | $y = 0.75(\pm0.05) \times -0.04(\pm0.3)$ | 0.9803 | 9.83 | 25 | – | – |

*SD* = standard deviation; *N* = number of points; *t′-values*: calculated from tabulated *t-values* considering the different variances of the slopes.
* In parenthesis ($s_a$. $t_{crit}$ and $s_b$. $t_{crit}$, with N-2 degrees of freedom).
** *R* = regression coefficient.

**Table 2**
Figures of merit of the IL-DLLME coupled to HPLC-DAD methodology for the studied compounds in the two studied samples.

| Matrix | Analyte | LOD[a] (mg mL$^{-1}$) | LOQ[a] (mg mL$^{-1}$) | LOD[b] (mg mL$^{-1}$) | LOQ[c] (mg mL$^{-1}$) | LR (mg mL$^{-1}$) |
|---|---|---|---|---|---|---|
| Soy sauce | Haloxyfop-*p*-methyl | 0.55 | 1.67 | 0.12 | 0.36 | LOQ – 9.26 |
| | Quizalofop-*p*-tefuryl | 0.79 | 2.40 | 0.29 | 0.87 | LOQ – 9.26 |
| | Fenoxaprop-*p*-ethyl | 0.42 | 1.28 | 0.34 | 1.04 | LOQ – 9.26 |
| | Propaquizafop | 0.45 | 1.37 | 0.13 | 0.41 | LOQ – 9.26 |
| Soy milk | Haloxyfop-*p*-methyl | 0.97 | 2.95 | 0.13 | 0.40 | LOQ – 9.26 |
| | Quizalofop-*p*-tefuryl | 0.55 | 1.67 | 0.29 | 0.87 | LOQ – 9.26 |
| | Fenoxaprop-*p*-ethyl | 0.38 | 1.16 | 0.32 | 0.98 | LOQ – 9.26 |
| | Propaquizafop | 0.59 | 1.78 | 0.14 | 0.42 | LOQ – 9.26 |

[a] From calibration curve (IUPAC definition).
[b] S/N = 3.
[c] S/N = 10.

Centrifugation speed up phase separation at the end of the IL-DLLME. The centrifugation time in the range of 5 to 20 min and a constant speed of 4000 rpm (the maximum speed of the centrifuge) was studied. As observed in Fig. 4.b, peak areas decrease after 15 min. Thus, this time was selected as optimal for centrifugation.

### 3.2. Evaluation of method performance

The IL-DLLME method was then applied to the determination of AOPPs herbicides in soy sauce and soy milk. The following figures of merit were evaluated under the optimized conditions: linear range (LR), reproducibility (RSD%), limits of detection (LODs), limits of quantification (LOQs), enrichment factor (EF) and recovery (R%).

Any herbicide was detected in the two studied matrices with the developed IL-DLLME method. Thus, all experiments have been made by spiking with standards. Calibration curves shown in Table 1 were made by using the internal standard (IS) method. Since the two more retained chromatographic peaks were not completely resolved, peak heights instead of peak areas were selected as signals in both matrices. Thus, calibration curves were made as peak height of the spiked analyte/peak height of the IS *vs.* the analyte concentration spiked in the sample (7 levels, each one by triplicate). We have used haloxyfop-*p*-methyl as IS to perform the calibration curve of the other three herbicides, and propaquizafop as IS to calibrate haloxyfop-*p*-methyl. In order to investigate if matrix effects were present in the quantitative determinations a *t-test* for comparison of the calibration curves slopes obtained in water with those obtained in the soy sauce and the soy milk samples was used [28,29]. In Table 1 the obtained *t-values* are compared with the theoretical ones, *t'-values*, according to a Cochran test for slopes with unequal variances.

It can be observed that the slopes are significantly different (*t-values* > *t'-values*) and thus, matrix effects are therefore present. As a consequence, for the quantitative determinations of the four aryloxyphenoxy-propionate herbicides in the two studied soy-derived food samples, the standard addition method was used.

The LODs in soy sauce and soy milk were calculated by two procedures in order to make comparisons with other studies in the literature: the signal to noise ratio (S/N) of 3.0 and by using the IUPAC definition of LOD = 3.29 $s_0$ [28] based on the standard deflection of the concentration predicted for a blank sample, $s_0$. Also, the lower LOQ (the beginning of the linear range) was evaluated by the S/N of 10, and by the IUPAC definition of LOQ = 10 $s_0$ [28].

The upper point of the LR, was determined by the lack-of-fit test (eliminating the highest value and applying the statistical test again with the remaining points) [28]. This process was repeated until the data could be adjusted to a straight line. The results for the LODs, LOQs and LRs are shown in Table 2.

The enrichment factors (EFs) and recoveries (R%) were calculated by means of Eqs. (1) and (2), respectively:

$$EF = \frac{C_{IL}}{C_w} \quad (1)$$

$C_{IL}$ and $C_w$ are the analyte concentrations in the IL phase and the initial aqueous solution, respectively.

$$R\% = 100\frac{C_{IL}V_{IL}}{C_wV_w} = 100EF\varphi \quad (2)$$

$V_{IL}$ and $V_w$ are the volumes of the IL phase and the sample solution, respectively, and $\varphi$ is the phase ratio. The EFs (Table 3) were obtained by spiking the soy-derived foods and then comparing the resulting chromatographic peak heights from the solutions obtained before and after the IL-DLLME method. The analyte was left in contact with the sample matrix for 1 h before extraction. The concentrations of the target analytes in the extracts were within the LR of the calibration curves. The obtained EFs were between 18 and 43, which reflects a very satisfactory preconcentration capacity of the analytical methodology. Fig. 5 shows typical chromatograms of the four aryloxyphenoxy-propionate herbicides in spiked samples of soy milk and soy sauce before and after the IL-DLLME method.

The R% values were determined by measuring the initial volume of spiked soy sauce or soy milk and the final volume of RTIL phase and using the corresponding EFs values according to Eq. (2). As can be observed in Table 3, R% values are not very high because of the presence of organic solvent in the aqueous matrix, necessary in the sample conditioning step to precipitate the suspended material (see Sections 2.4 and 2.5), which consequently increase the LOD and LOQ values. In spite of

**Table 3**
Enrichment (EF) and recovery (R%) factors for different spiked amounts of AOPPs after the IL-DLLME.

| Matrix | Analyte | Spiked amount (mg mL$^{-1}$) | EF | R% | RSD%[a] |
|---|---|---|---|---|---|
| Soy sauce | Haloxyfop-*p*-methyl | 5.24 | 31 | 45.7 | 5.5 |
| | | 7.94 | 31 | 39.9 | 6.4 |
| | Quizalofop-*p*-tefuril | 5.24 | 24 | 35.6 | 4.9 |
| | | 7.94 | 18 | 25.4 | 6.6 |
| | Fenoxaprop-*p*-ethyl | 5.24 | 33 | 48.9 | 3.1 |
| | | 7.94 | 25 | 34.2 | 3.8 |
| | Propaquizafop | 5.24 | 31 | 46.2 | 6.9 |
| | | 7.94 | 22 | 30.1 | 5.8 |
| Soy milk | Haloxyfop-*p*-methyl | 3.38 | 34 | 52.9 | 7.9 |
| | | 5.24 | 33 | 43 | 5.3 |
| | Quizalofop-*p*-tefuril | 3.38 | 28 | 42.9 | 5.0 |
| | | 5.24 | 27 | 41.3 | 2.2 |
| | Fenoxaprop-*p*-ethyl | 3.38 | 43 | 65 | 4.6 |
| | | 5.24 | 43 | 66.8 | 1.8 |
| | Propaquizafop | 3.38 | 40 | 60.2 | 8.4 |
| | | 5.24 | 36 | 56.8 | 2.5 |

[a] Relative standard deviation of the recovery.

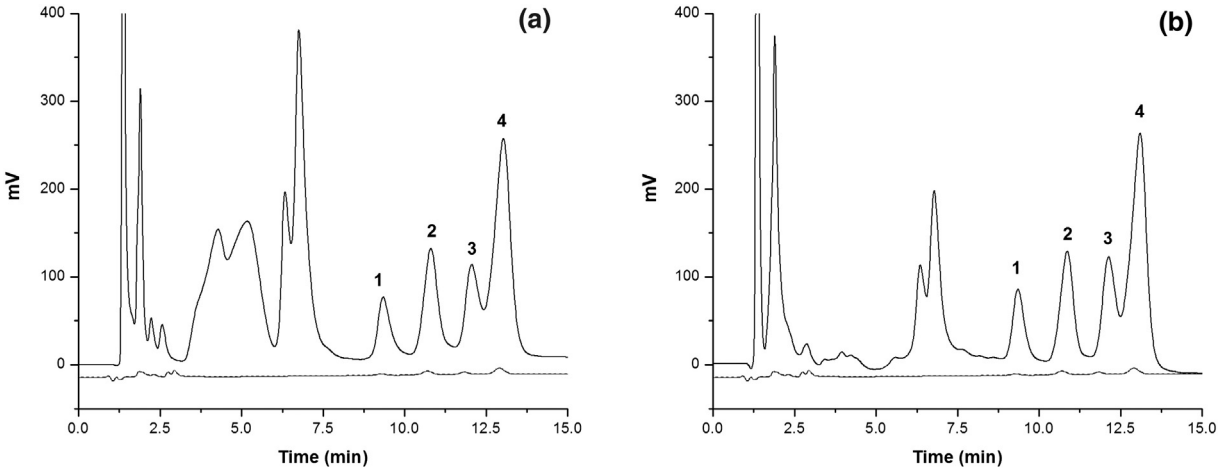


**Fig. 5.** Chromatograms for spiked (5.24 mg $mL^{-1}$) samples before (grey line) and after (black line) the IL-DLLME method. a) Soy sauce, b) soy milk. 1: Haloxyfop-*p*-methyl; 2: quizalofop-*p*-tefuryl; 3: fenoxaprop-*p*-ethyl; 4: propaquizafop.

this, the obtained results for these two figures of merit are low. The precision of the determinations was evaluated by the inter-day reproducibility and expressed as the percent relative standard deviation (RSD%) with respect to measurements made in quintuplicate and over three consecutive days. For that purpose, each sample was spiked with the target herbicide at two different concentrations levels within the LR. In Table 3 the RSD% values are shown.

### 3.3. Comparison of the proposed methodology with some existing in the literature

The comparison made in this Section between the proposed analytical method with others from the scientific literature is quite limited since the four AOPPs studied in this work have never been analyzed in soy-derived foods. Thus, the conclusions from this Section will have a general character. The comparisons were made mostly with other techniques in other samples such as water, crops and environmental matrixes analyzed by HPLC with MS/MS or DAD detection. The LOD, LOQ, LR, RSD%, amount and kind of sample necessary for the analysis, type and amount of solvent, and R% are gathered in Table 4. Looking at the results, we could conclude that with the IL-DLLME coupled to HPLC-DAD method developed in this work, very low limits of detection and quantification, and very good inter-day reproducibility can be achieved. Additionally, the procedure is fast, requires small amounts of extraction solvent (here a few microliters of a cheap ionic liquid) and exhibits a wide range of linearity.

As expected, lower limits of detection and quantification than the ones obtained here can be achieved, but with very specific, expensive and sensitive detectors such as HPLC-MS/MS, which are not available in common laboratories.

**Table 4**
Comparison of the proposed IL-DLLME method coupled with HPLC-DAD with other procedures described in the literature for the determination of AOPPs herbicides in different matrixes.

| Analyte | Matrix | Extraction procedure | Detection | LOD (mg $L^{-1}$) | LOQ (mg $L^{-1}$) | R% | RSD% | LR (mg $L^{-1}$) | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Fenoxaprop-*p*-ethyl | Soil | Extraction with organic solvent and purification with alumina or florisil | HPLC-DAD | 0.02a,b | – | 81.6–95.1 | 1.61–11.72 | – | [30] |
| | Soil and wheat | Extraction with acetonitrile and solvent evaporation | HPLC-MS/MS | 0.005 | 0.01b (soil) 0.02–0.05b (wheat) | 75.36–112.34 | – | – | [31] |
| | Soil and wheat | Soxhlet | HPLC-DAD | 0.1 | | 50–82.4 | – | LOQ-10 | [32] |
| | Rice plants, paddy water and soil | QuEChERS | HPLC-MS/MS | 0.003[1] | 0.01b | 79.2–102.8 | 1.8–6.7 | – | [12] |
| | Water | Sequential DLLME | HPLC-DAD | 0.00150 | 0.010 | 82.2 | 4.23 | 0.010–0.300 | [7] |
| | Soy sauce | Our method | HPLC-DAD | 0.34 | 1.04 | 34.2–48.9 | 3.1–3.8 | LOQ-9.26 | – |
| | Soy milk | Our method | HPLC-DAD | 0.32 | 0.98 | 65.0–66.8 | 1.8–4.6 | LOQ-9.26 | – |
| Quizalofop-*p*-ethyl | Peanut | Modified QuEChERS | HPLC-MS/MS | – | 0.005b | 81.3–88.0 | 2.9–16.6 | 0.001–0.050 | [33] |
| | Wheat, spinach, carrot, apple, citrus, peanut | QuEChERS and multi-plug filtration cleanup | HPLC-MS/MS | – | 0.002–0.005b | 15–88 | – | – | [34] |
| | Environmental water | Dispersive magnetic SPE | UPLC-MS/MS | $2.3 \times 10^{-6}$ | $5.9 \times 10^{-6}$ | 62.5–65.6 | 5.8 | – | [6] |
| Quizalofop-*p*-tefuryl | Soy sauce | Our method | HPLC-DAD | 0.29 | 0.87 | 30.1–46.2 | 5.8–6.9 | LOQ-9.26 | – |
| | Soy milk | Our method | HPLC-DAD | 0.29 | 0.87 | 41.3–42.9 | 2.2–5.0 | LOQ-9.26 | – |
| Haloxyfop-*p*-methyl | Water | Sequential DLLME | HPLC-DAD | 0.00435 | 0.015 | 78.4 | 3.12 | 0.015–0.300 | [7] |
| | Environmental water | Dispersive magnetic SPE | UPLC-MS/MS | $1.9 \times 10^{-6}$ | $4.3 \times 10^{-6}$ | 72.1–85.3 | 6.1–7.5 | | [6] |
| | Soy sauce | Our method | HPLC-DAD | 0.12 | 0.36 | 39.9–45.7 | 5.5–6.4 | LOQ-9.26 | – |
| | Soy milk | Our method | HPLC-DAD | 0.13 | 0.40 | 43–52.9 | 5.3–7.9 | LOQ-9.26 | – |
| Propaquizafop | Soy sauce | Our method | HPLC-DAD | 0.13 | 0.41 | 25.4–35.6 | 4.9–6.6 | LOQ-9.26 | – |
| | Soy milk | Our method | HPLC-DAD | 0.14 | 0.42 | 56.8–60.2 | 2.5–8.4 | LOQ-9.26 | – |

[a] Calculated with a S/N ratio of 2.
[b] Expressed as mg $kg^{-1}$.

### 3.4. Conclusions

A one-step IL-DLLME method followed by HPLC-DAD analysis have been developed and successfully applied to the determination of four aryloxyphenoxy-propionate herbicides in soy milk and soy sauce This study constitutes the first application of the developed methodology to the analysis of those compounds in commercial soy-derived foods. Optimization was carried out by using a *step-by-step* experimental design. The figures of merit for the proposed methodology were very satisfactory although, recovery factors were not high enough because the presence of an organic solvent in the sample preparation step, necessary to precipitate proteins and cleaning the sample. However, LODs and LOQs were between 0.1 and 0.3 mg $L^{-1}$ and 0.4–1 mg $L^{-1}$, respectively. The reproducibility of the methodology was less than 8.4%, and the linear ranges were very broad. Additionally, the developed methodology easily implementable in any laboratory, it is fast and the consumption of reagents and materials is low, which make the method more environmentally friendly. These characteristics make the proposed methodology feasible in the food industrial control.

## Acknowledgments

Financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 0244) and Agencia Nacional de Promoción Científica y Técnica (ANPCYT 2014-3597) is gratefully acknowledged. Dr. Prof. Héctor Di Loreto for providing the aryloxyphenoxy-propionate herbicides. M. Reta is a scientific member of CONICET and Professor of Analytical Chemistry at the Universidad Nacional de La Plata (Argentina). J.M. Padró and E. Lubomirsky are research fellows of CONICET. The authors want to thank to all of these scientific organizations.

## References

[1] L. Lucini, G. Pietro Molinari, Detection of the herbicide fenoxaprop-p-ethyl, its agronomic safener isoxadifen ethyl and their metabolites residue in rice, Qual. Assur. Saf. Crops Food 3 (2011) 63–68.
[2] X. Cai, W. Liu, G. Sheng, Enantioselective degradation and ecotoxicity of the chiral herbicide diclofop in three freshwater alga cultures, J. Agric. Food Chem. 56 (2008) 2139–2146.
[3] I.S. Elefsiniotis, G.D. Liatsos, D. Stamelakis, A. Moulakakis, Case report: mixed cholestatic/hepatocellular liver injury induced by the herbicide quizalofop-p-ethyl, Environ. Health Perspect. 115 (2007) 1479–1481.
[4] H. Bagheri, A. Es'haghi, A. Es-haghi, Immersed solvent microextraction of aryloxyphenoxypropionate herbicides from aquatic media, Int. J. Environ. Anal. Chem. 93 (2013) 450–460.
[5] R. Curini, A. Gentili, S. Marchese, A. Marino, D. Perret, Solid-phase extraction followed by high-performance liquid chromatography-ionspray interface-mass spectrometry for monitoring of herbicides in environmental water, J. Chromatogr. A 874 (2000) 187–198.
[6] M. Luo, D. Liu, L. Zhao, J. Han, Y. Liang, P. Wang, et al., A novel magnetic ionic liquid modified carbon nanotube for the simultaneous determination of aryloxyphenoxy-propionate herbicides and their metabolites in water, Anal. Chim. Acta 852 (2014) 88–96.
[7] S. Li, P. Gao, J. Zhang, Y. Li, B. Peng, H. Gao, et al., Sequential dispersive liquid–liquid microextraction for the determination of aryloxyphenoxy-propionate herbicides in water, J. Sep. Sci. 35 (2012) 3389–3395.
[8] H. Bagheri, M. Dehghan, A. Es'haghi, M. Naderi, A conically fixed position single drop microextraction method for isolation of aryloxyphenoxypropionate herbicides from aquatic media, Anal. Methods 5 (2013) 4846–4851.
[9] H. Bao, S. Fang, Z. Liu, H. Shi, Y. Ye, M. Wang, Development of an enzyme-linked immunosorbent assay for the rapid detection of haloxyfop-p-methyl, J. Agric. Food Chem. 58 (2010) 8167–8170.
[10] L.C. Marube, S.S. Caldas, K.L. Soares, E.G. Primel, Dispersive liquid-liquid microextraction with solidification of floating organic droplets for simultaneous extraction of pesticides, pharmaceuticals and personal care products, Microsc. Acta 182 (2015) 1765–1774.
[11] M.T. Jafari, M. Saraji, S. Yousefi, Negative electrospray ionization ion mobility spectrometry combined with microextraction in packed syringe for direct analysis of phenoxyacid herbicides in environmental waters, J. Chromatogr. A 1249 (2012) 41–47.
[12] J.X. Wu, Y. Zhang, K. Wang, H.Y. Zhang, Residue analysis and dissipation of fenoxaprop-P-ethyl and its metabolite fenoxaprop-P in rice ecosystem, J. Anal. Chem. 70 (2015) 897–902.
[13] W. Guan, H. Zhang, Determination and study on residue and dissipation of benazolin-ethyl and quizalofop-p-ethyl in rape and soil, Int. J. Environ. Anal. Chem. 93 (2013) 679–691.
[14] S.J.P. De Carvalho, M. Nicolai, R.R. Ferreira, A.V.D.O. Figueira, P.J. Christoffoleti, Herbicide selectivity by differential metabolism: considerations for reducing crop damages, Sci. Agric. 66 (2009) 136–142.
[15] E.A. Araújo, M.C.R. Lara, M.R. Dos Reis, R.L.S. Viriato, R.A.R. Rocha, R.G.L. Gonçalves, et al., Determination of haloxyfop-methyl, linuron, and procymidone pesticides in carrot using SLE-LTP extraction and GC–MS, Food Anal. Methods (2015).
[16] N. Rosales-Conrado, V. Guillén-Casla, L.V. Pérez-Arribas, M.E. de León-González, L.M. Polo-Díez, Simultaneous enantiomeric determination of acidic herbicides in apple juice samples by liquid chromatography on a teicoplanin chiral stationary phase, Food Anal. Methods 6 (2013) 535–547.
[17] P. Sun, D.W. Armstrong, Ionic liquids in analytical chemistry, Anal. Chim. Acta 661 (2010) 1–16.
[18] J.M. Padró, R.B. Pellegrino Vidal, M. Reta, Partition coefficients of organic compounds between water and imidazolium-, pyridinium-, and phosphonium-based ionic liquids, Anal. Bioanal. Chem. 406 (2014) 8021–8031.
[19] H.R. Cascon, S.K. Choudhari, G.M. Nisola, E.L. Vivas, D.-J. Lee, W.-J. Chung, Partitioning of butanol and other fermentation broth components in phosphonium and ammonium-based ionic liquids and their toxicity to solventogenic clostridia, Sep. Purif. Technol. 78 (2011) 164–174.
[20] J.M. Padró, A. Ponzinibbio, L.B.A. Mesa, M. Reta, Predicting the partitioning of biological compounds between room-temperature ionic liquids and water by means of the solvation-parameter model, Anal. Bioanal. Chem. 399 (2011) 2807–2820.
[21] D.R. Fraser, K.J. McFarlane, Phosphonium-based ionic liquids: an overview, Aust. J. Chem. 62 (2009) 309–332.
[22] Y. Liu, E. Zhao, W. Zhu, H. Gao, Z. Zhou, Determination of four heterocyclic insecticides by ionic liquid dispersive liquid–liquid microextraction in water samples, J. Chromatogr. A 1216 (2009) 885–891.
[23] J.M. Padró, R.B. Pellegrino Vidal, R.N. Echevarria, A.N. Califano, M.R. Reta, Development of an ionic-liquid-based dispersive liquid–liquid microextraction method for the determination of antichagasic drugs in human breast milk: optimization by central composite design, J. Sep. Sci. 38 (2015) 1591–1600.
[24] P. Berton, R.G. Wuilloud, An online ionic liquid-based microextraction system coupled to electrothermal atomic absorption spectrometry for cobalt determination in environmental samples and pharmaceutical formulations, Anal. Methods 3 (2011) 664–672.
[25] J. Marták, S. Schlosser, Extraction of lactic acid by phosphonium ionic liquids, Sep. Purif. Technol. 57 (2007) 483–494.
[26] Y. Zhu, Y. Yang, Z. Zhou, G. Li, M. Jiang, C. Zhang, et al., Direct determination of free tryptophan contents in soy sauces and its application as an index of soy sauce adulteration, Food Chem. 118 (2010) 159–162.
[27] S. Solonesky, M. Larramendy, Herbicides, Theory and Applications, InTech, Rijeka, Croatia, 2011.
[28] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. Dejong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A. Elsevier, Amsterdam, 1997.
[29] J.M. Padró, M.E. Marson, G.E. Mastrantonio, J. Altcheh, F. García-Bournissen, M. Reta, Development of an ionic liquid-based dispersive liquid-liquid microextraction method for the determination of nifurtimox and benznidazole in human plasma, Talanta 107 (2013) 95–102.
[30] L. Celi, M. Négre, M. Gennari, HPLC determination of fenoxaprop and fenoxaprop-ethyl in different soils, Pestic. Sci. 38 (1993) 43–47.
[31] X. Chen, S. Yu, L. Han, S. Sun, Y. Zhi, W. Li, Residues and dissipation of the herbicide fenoxaprop-P-ethyl and its metabolite in wheat and soil, Bull. Environ. Contam. Toxicol. 87 (2011) 50–53.
[32] S.B. Singh, T.K. Das, G. Kulshrestha, Persistence of herbicide fenoxaprop ethyl and its acid metabolite in soil and wheat crop under Indian tropical conditions, J. Environ. Sci. Health B 48 (2013) 324–330.
[33] A. Saha, T.P. Ahammed Shabeer, K. Banerjee, S. Hingmire, D. Bhaduri, N.K. Jain, et al., Simultaneous analysis of herbicides pendimethalin, oxyfluorfen, imazethapyr and quizalofop-p-ethyl by LC–MS/MS and safety evaluation of their harvest time residues in peanut (*Arachis hypogaea* L.), J. Food Sci. Technol. 52 (2015) 4001–4014.
[34] Y. Qin, P. Zhao, S. Fan, Y. Han, Y. Li, N. Zou, et al., The comparison of dispersive solid phase extraction and multi-plug filtration cleanup method based on multi-walled carbon nanotubes for pesticides multi-residue analysis by liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1385 (2015) 1–11.