Food Chemistry 219 (2017) 102–108

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Inorganic selenium speciation analysis in *Allium* and *Brassica* vegetables by ionic liquid assisted liquid-liquid microextraction with multivariate optimization

Alexander Castro Grijalba, Estefanía M. Martinis, Rodolfo G. Wuilloud *

[a] *Laboratory of Analytical Chemistry for Research and Development (QUIANID), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre J. Contreras 1300, 5500 Mendoza, Argentina*
[b] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form 6 September 2016
Accepted 18 September 2016
Available online 19 September 2016

Keywords:
Selenium
Phosphonium ionic liquid
Liquid-liquid microextraction
Allium
Brassica

ABSTRACT

A highly sensitive vortex assisted liquid-liquid microextraction (VA-LLME) method was developed for inorganic Se [Se(IV) and Se(VI)] speciation analysis in *Allium* and *Brassica* vegetables. Trihexyl(tetradecyl)phosphonium decanoate phosphonium ionic liquid (IL) was applied for the extraction of Se(IV)-ammonium pyrrolidine dithiocarbamate (APDC) complex followed by Se determination with electrothermal atomic absorption spectrometry. A complete optimization of the graphite furnace temperature program was developed for accurate determination of Se in the IL-enriched extracts and multivariate statistical optimization was performed to define the conditions for the highest extraction efficiency. Significant factors of IL-VA-LLME method were sample volume, extraction pH, extraction time and APDC concentration. High extraction efficiency (90%), a 100-fold preconcentration factor and a detection limit of 5.0 ng/L were achieved. The high sensitivity obtained with preconcentration and the non-chromatographic separation of inorganic Se species in complex matrix samples such as garlic, onion, leek, broccoli and cauliflower, are the main advantages of IL-VA-LLME.

1. Introduction

Selenium is an essential trace element for many organisms but it can be toxic at high doses. However, toxicity of Se is also dependant on its chemical speciation, being inorganic forms more toxic than organic ones (Nordberg, Fowler, Nordberg, & Friberg, 2007). Thus, speciation analysis is very important to determine the possible impact of Se-containing food in human health. Some edible vegetable like *Allium* and *Brassica* plants are well-recognized for their nutritional properties and Se content. Moreover, it is widely known that they transform inorganic Se into bioactive organic species (*e.g.* selenoaminoacids) that show more benefits to health (Cornelis, Caruso, Crews, & Heumann, 2005). However, low concentrations (ng/g) of inorganic Se species can persist in these plants after metabolization (Pyrzynska, 2009), which imposes the need of developing preconcentration and highly sensitive techniques for its detection and quantification (Nordberg et al., 2007). One of the most used techniques for speciation analysis is high performance liquid chromatography (HPLC) coupled to sensitive inductively coupled plasma mass spectrometry (ICP-MS) detection. However, not all routine laboratories focused on food analysis might count with HPLC-ICP-MS due to the high cost of this instrumentation. On the other hand, non-chromatographic separation techniques coupled to widespread detectors such as flame atomic absorption spectrometry (FAAS), electrothermal AAS (ETAAS) or UV–Visible spectrophotometry have been successfully applied for Se speciation analysis with the additional advantage in the sensitivity enhancement obtained by analyte preconcentration (López-García, Vicente-Martínez, & Hernández-Córdoba, 2013).

In the last decade, important advances on analytical preconcentration have been registered by novel techniques based on liquid-liquid microextraction (LLME) (Dadfarnia, Haji Shabani, & Nozohor, 2014; Viñas, Campillo, López-García, & Hernández-Córdoba, 2013). In fact, recent developments in LLME have involved the replacement of volatile organic solvents by ionic liquids (ILs), which are organic salts with melting points close or below room temperature. This property provides a different set of applications to ILs compared with conventional molecular liquids (Koel, 2009). In fact, ILs have

* Corresponding author at: Laboratory of Analytical Chemistry for Research and Development (QUIANID), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre J. Contreras 1300, 5500 Mendoza, Argentina.
E-mail addresses: rodolfowuilloud@gmail.com, rwuilloud@mendoza-conicet.gob.ar (R.G. Wuilloud).

http://dx.doi.org/10.1016/j.foodchem.2016.09.117
0308-8146/

been applied as ion pair reagents and extractant phases in LLME to obtain high extraction efficiencies and low detection limits for different types of analytes (Escudero, Castro Grijalba, Martinis, & Wuilloud, 2013). In the case of Se, both inorganic and organic species have been separated and determined by IL-LLME techniques coupled to different detectors (López-García et al., 2013; Martinis et al., 2011; Rahnama & Abed, 2014; Tuzen & Pekiner, 2015). However, these methods have been applied for Se speciation in very simple samples such as water (Tuzen & Pekiner, 2015). Therefore, it is still required the development of novel preconcentration methods based on IL-LLME for samples containing highly complex matrices such as those occurring in *Allium* and *Brassica* plants-derived food. Furthermore, another important aspect to be considered in IL-LLME is the optimization of experimental conditions in order to obtain high extraction efficiency and preconcentration factor. The univariate approach involves the optimization of each factor while the remaining are kept constant, which leads to the need of performing multiple experiments. In addition, this approach does not consider the possible interactions occurred among the studied factors (Vera Candioti, De Zan, Cámara, & Goicoechea, 2014). On the other hand, multivariate approach reduces the time and the number of experiments, while modeling quantitatively the relationship between the studied factors and the analytical response. Therefore, a multivariate approach can contribute with important benefits to IL-LLME, such as lower detection limits and faster, in comparison to other reported methods (Stalikas, Fiamegos, Sakkas, & Albanis, 2009). However, multivariate optimization is still not widely applied during development of LLME methods (Viñas et al., 2013).

In this work, a novel LLME method based on a phosphonium IL was developed for separation and preconcentration of inorganic Se species in highly complex matrices. The IL trihexyl(tetradecyl)phos phonium decanoate was used as extractant phase for vortex assisted-LLME (VA-LLME) before ETAAS detection. Separation of Se species was achieved by formation of the complex Se(IV)-ammonium pyrrolidine dithiocarbamate (APDC) followed by VA-LLME. A multivariate optimization of the several factors influencing the IL-VA-LLME method was performed with the aim of obtaining optimal extraction conditions with a reduced number of experiments, which can be considered as a critical need of many routine analytical laboratories to achieve the highest productivity. The proposed methodology was demonstrated to be a valid alternative for the determination of Se(IV) and Se(VI) species at trace levels in garlic, broccoli, leek, onion and cauliflower.

2. Materials and methods

2.1. Instrumentation

Measurements were performed with a Perkin Elmer (Überlingen, Germany) Model 5100 ZL atomic absorption spectrometer equipped with a transversely heated graphite atomizer and a Zeeman-effect background correction system. A Se electrodeless discharge lamp (EDL) (PerkinElmer) was used. All measurements were performed based on absorbance signals (peak areas) with an integration time of 3 s. Instrumental conditions are listed in Table 1. A centrifuge (Luguimac, Buenos Aires, Argentina) model LC-15 was used for separation of phases. A vortex model Bio Vortex B1 (Boeco, Hamburg, Germany) was used for mixing the reagents. The temperature-controlled ultrasound bath (40 kHz and 600 W) was from Test Lab (Buenos Aires, Argentina). A Horiba F-51 pH meter (Kyoto, Japan) was used for pH determination. A Gilson (Villiers Le bell, France) Minipuls 3 peristaltic pump equipped with tygon-type pump tubes (Gilson) was employed to propel the solutions through a column used for cleaning of sample extracts.

2.2. Reagents

All the reagents were of analytical grade and the presence of Se was not detected within the working range. Stock standard solutions of inorganic Se(IV) and Se(VI) species (1000 mg/L) as sodium selenite (Na_2SeO_3) (99%) (Sigma-Aldrich, Milwaukee, WI, USA) and sodium selenate (Na_2SeO_4) (98%) (Sigma-Aldrich), respectively, were prepared in 0.1 mol L^{-1} HCl. Selenomethionine ($CH_3Se(CH_2)_2$-$CH(NH_2)CO_2H$) (99%) (Fluka, Buchs, Switzerland) and Se-(methyl) selenocysteine hydrochloride ($C_4H_9NO_2Se{\cdot}HCl$) ($\geqslant$95%) (Sigma-Aldrich) stock standard solutions (1000 mg/L) were prepared with ultrapure water and stored at 4 °C in amber-coloured HDPE bottles. A 500 mg/L palladium nitrate dehydrate solution [$Pd(NO_3)_2{\cdot}2H_2O$] ($\geqslant$99.99%) (Sigma-Aldrich) and 500 mg/L copper(II) nitrate hemi (pentahydrate) [$Cu(NO_3)_2{\cdot}2.5H_2O$] ($\geqslant$99.99%) (Sigma-Aldrich) were prepared and used as chemical modifiers (see Table 1). These solutions were prepared in 0.1% (v/v) HNO_3 (Ultrex® II Mallinckrodt Baker, Phillipsburg, NJ, USA). Hydrochloric acid (37%) was purchased from Merck. Trihexyl(tetradecyl)phosphonium decanoate (95%) was purchased from Sigma-Aldrich. A diluted solution at 50% (w/v) was prepared by weighting an accurate amount of the IL followed by dissolution in chloroform. Chloroform (99%) was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Citric acid (99.5%), ethanol (96%) and sodium hydroxide (98%) were purchased from Sigma-Aldrich. A 50% (w/v) sodium nitrate solution was prepared by dissolving 5 g of $NaNO_3$ (99.5%) (Merck) in 10 mL of ultrapure water. A 2% (w/v) ammonium pyrrolidinedithiocarbamate (APDC) (~99%) (Sigma-Aldrich) solution was prepared with ethanol. Multiwalled carbon nanotubes (MWCNTs), activated carbon and amberlite XAD-1180 polymeric resin were purchased from Sigma-Aldrich. A cartridge SEP-PAK C18 6 cc with 500 mg of sorbent [WATERS (Milford, Massachusetts)] was used to clean-up the acid extract of the samples. Ultrapure water (18 MΩ·cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). White clover BCR 402 was used as certified reference material (CRM). All the glassware was washed in 0.5 mol L^{-1} HNO_3 solution for 24 h and later rinsed with ultrapure water.

2.3. Sample collection and extraction of inorganic Se species

Garlic samples (red type clone "Fuego" and white type clone "Nieve") were obtained from the germplasm collection of INTA La Consulta (Mendoza, Argentina. Onion, leek, broccoli and cauliflower samples were collected from local stores of Mendoza (Argentina). The samples were washed with distilled water and hand-peeled. The edible parts were freezed, cut into small pieces, lyophilized and finally pulverized with a mill. The resulting fine powder was stored in polyethylene bags and kept inside a freezer at −20 °C. Ultrasound-assisted extraction of inorganic Se species was performed following a modification of a procedure described in a previous report (Zhong, Zhong, Hao, Luan, & Li, 2015). Briefly, 0.1 g of freeze-dried sample was accurately weighted inside a 15 mL-polyethylene tube and 10 mL of 0.1 mol/L HCl were added. The dispersion was sonicated for 10 min and the acid extract separated by centrifugation at 3500 rpm (2054.3 × g) for 10 min. The extract was collected with a Pasteur transference pipette and filtered through a 0.45 μm pore size nylon membrane filter (Millipore corporation, Bedford, MA, USA). Then, the extract was loaded into a column (2 mm i.d and 15 mm length) filled with 10 mg of MWCNTs at a flow rate of 1.0 mL/min. The column was preconditioned with 500 μL of 0.01 mol/L HCl solution. Every 5 clean-up cycles, the filling column material was washed with 500 μL of acetone followed by 500 μL of water. After the clean-up step, 1 mL of 37% (w/w) HCl was added to 5 mL of extract and heated on a hotplate at 100 °C for 30 min in order to reduce Se

Table 1
Instrumental and experimental conditions for Se species determination.

Wavelength				196 nm
Spectral bandwidth				2.0 nm
Lamp (EDL) current				210 mA
Matrix modifier				10 μg Cu [$Cu(NO_3)_2$]
				10 μg Pd [$Pd(NO_3)_2$]
Graphite furnace temperature program				
Step	T (°C)	Ramp time (s)	Hold time (s)	Argon flow (mL min^{-1})
Drying 1	110	1	30	250
Drying 2	130	15	30	250
Pyrolysis 1	180	90	30	250
Pyrolysis 2	600	90	30	250
Pyrolysis 3	1200	10	15	250
Cooling	300	1	15	250
Atomization	2100	0	3	0
Cleaning	2400	1	2	250
IL-VA-LLME conditions				
Pre-treated sample volume	5 mL			
APDC concentration	3.76×10^{-4} mol/L			
pH	2.3			
IL amount	25 mg			
IL phase solvent	Chloroform			
IL phase solvent volume	50 μL			
Extraction time	15 min			
Stirring mode	Vortex			

(VI) to Se(IV). The pH was adjusted to 2.3 with 5 mol/L NaOH solution and the final volume was made-up to 5 mL in a volumetric flask before total inorganic Se determination. For Se speciation analysis (Se(IV) determination), another 5 mL-aliquot of the cleaned sample extract was adjusted to pH 2.3 with 5 mol/L NaOH solution and submitted to the preconcentration and microextraction procedure.

2.4. IL-VA-LLME procedure for Se(IV) preconcentration and determination

A volume of 5 mL of the cleaned sample extract (pH fixed at 2.3) was placed in a 10 mL graduated glass centrifuge tube with 75 μL of 0.025 mol/L APDC solution (in ethanol) and mixed with vortex for 3 s. After formation of Se(IV)-APDC, 50 μL of 50% (w/v) tri hexyl(tetradecyl)phosphonium decanoate solution (prepared in chloroform) was added to the sample. After 15 min of extraction time with vortex stirring, the IL phase was dispersed and the Se (IV)-APDC complex was extracted. The dispersion was centrifuged at 3000 rpm for 5 min to form two well-defined phases. Then, the upper aqueous phase was manually removed with a Pasteur transference pipette while the IL phase was diluted with 50 μL of chloroform and injected into the graphite furnace of ETAAS for Se determination under the conditions given in Table 1. Calibration was performed against aqueous standards and blank solutions. Design Expert® 7.0 (Stat-Ease Inc., Minneapolis, USA) was the software employed to process the results obtained from factors screening, surface response and optimization with the desirability function.

3. Results and discussion

3.1. Optimization of ETAAS conditions for Se determination in IL-enriched matrix

The optimized temperature program used in ETAAS determinations is shown in Table 1. The high viscosity of the IL required a minor dilution of the extractant phase with an adequate solvent in order to obtain reproducible injection of the analyte into the graphite furnace of ETAAS instrument. Two solvents (toluene and chloroform) were assayed for this purpose. Individual additions of 50 μL of each solvent to the IL phase collected after IL-VA-LLME were studied. No significant differences were observed in Se absorbance upon the injection of these solvents. Therefore, chloroform was chosen for subsequent experiments. It has to be pointed out that the rich organic matrix composed of the IL, chloroform and APDC co-extracted along with the analyte (due to its low polarity), caused a significant reduction in Se sensitivity. In order to diminish potential matrix interference, a matrix modifier or a mix of modifiers was needed to obtain accurate ETAAS measurements. Experiments were performed by injecting 60 μL of 100 μg/L Se solution, 50 μL of IL/chloroform 50:50 (w/v) solution, 20 μL of 0.12 mol/L APDC and 20 μL of 500 mg/L Pd (10 μg of Pd). Two injection stages with intermediate drying were required for complete injection. Despite matrix modifier was increased up to 25 μg of Pd, no absorbance signal was observed for Se. This effect was attributed to the high volatilization of Se-APDC complex during pyrolysis (Kamada & Yamamoto, 1980). In order to solve this problem, Cu was injected along with Pd in the graphite furnace. Transition metals such as Cu are able to avoid sensitivity loss due to possible formation of stable metal selenides at high pyrolysis temperatures, thus avoiding the volatilization of the Se-APDC complex. Therefore, the injection of Cu and Pd in the mass range of 5–25 μg was evaluated in this work. A mix of matrix modifiers containing 10 μg Pd and 10 μg Cu was chosen to achieve the highest analytical sensitivity.

The effect of IL amount on Se determination was studied for optimal temperature program in ETAAS (Table 1). Different IL masses, i.e. 10, 25, 35 and 50 mg, were dissolved in chloroform and injected in ETAAS. The Se absorbance signal decreased by 48% after the injection of 35 mg of IL, while background signal increased. Thus, 25 mg of IL was established as the highest amount to be injected into the graphite furnace. However, since 25 mg IL was a considerable amount of matrix load to be pyrolized, different pyrolysis stages were needed to slowly decompose the organic matter while avoiding analyte volatilization. Two stages at 180 °C and 600 °C were included in the temperature program to achieve these goals. A third pyrolysis stage was also studied at 1100, 1200, 1300 and 1400 °C for full matrix decomposition. The highest

Se sensitivity was found at 1200 °C. Furthermore, a cooling step after pyrolysis and before atomization was applied to obtain well defined and Gaussian absorbance peaks (Escudero, Berton, Martinis, Olsina, & Wuilloud, 2012). Once pyrolysis temperature was selected, the effect of atomization on Se absorbance signal was evaluated within the range of 1900–2200 °C. The highest signal was observed at 2100 °C and a temperature of 2400 °C was chosen for the cleaning step.

3.2. Multivariate optimization of IL-VA-LLME

Seven parameters that could influence microextraction were chosen for the evaluation of significant factors within specific ranges: sample volume (2–10 mL), IL/chloroform extraction mix (25–100 μL), extraction pH (0.1–5.0), ionic strength (0–5% (w/v) as sodium nitrate), extraction type (vortex or ultrasound) and APDC concentration (6.3×10^{-7}–6.3×10^{-4} mol/L). A 2^{7-3} fractional factorial design (FFD) with 6 central points was used to evaluate most significant factors in the IL-LLME efficiency giving a total experiments number of 22. The response to be optimized was the area of peaks obtained by ETAAS.

The results obtained from FFD were studied by ANOVA (analysis of variance) for a 5% of significance of level. The pareto chart (Fig. 1) shows how the factor affects the response (negative with a gray filling bar or positive with a black filling bar), indicating that sample volume (negative effect), IL volume (positive effect), pH extraction (negative effect) and APDC concentration (positive effect) had a significant statistical influence. Likewise, the following interactions were significant: sample volume-extraction time (negative effect) and sample volume-extraction type (positive effect). The extraction time and extraction type were not significant factors individually, however, since they were part of a significant interaction, they were considered significant too. Ionic strength and other interactions (not listed in Fig. 1) were not significant factors in the studied range. From the six significant factors found, two were discarded for the next optimization step. The IL volume was fixed at 25 mg because it led to accurate measurements in ETAAS. Also, vortex stirring was chosen instead of ultrasound to obtain dispersions that were easily separated by centrifugation. Therefore, sample volume, extraction pH, extraction time and APDC concentration were the significant factors evaluated by CCD and multivariate response analysis.

A full CCD face centered design consisting of 24 experiments and 3 central points was applied to obtain the values of the factor

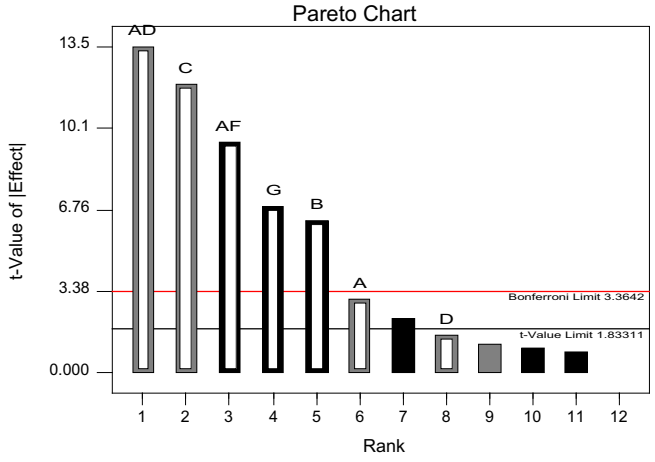

Fig. 1. Pareto graph used to determine significant factors. A. Sample Volume. B. IL volume. C. Extraction pH. D. Extraction time. F. Extraction type. G. APDC concentration. Black filling: positive effect. Gray filling: negative effect.

that lead to the highest Se absorbance signals. The selected ranges were the following: sample volume (2–10 mL), extraction pH (0.1–5.0), extraction time (1–15 min) and APDC concentration (6.3×10^{-8}–6.3×10^{-4} mol/L). Based on the results obtained after performing these experiments, the model was defined by a second order polynomial equation as follows:

$$\text{Se peak area} = 0.275 + 0.065A + 0.029B - 0.027C + 0.053D + 0.099B^2 - 0.153C^2 - 0.140D^2 \quad (1)$$

where A is the sample volume, B is the extraction time, C is the extraction pH and D is the APDC concentration. In Eq. (1), the positive and negative coefficients of the significant factors show how the response is affected by changes in the factors. In Fig. 2 are shown 3D response surfaces and contour plots of the obtained model for some of the possible configurations fixing two variables constant at the central point and showing the variation of the response against the other two variables. Fig. 2a shows that a maximum region for Se peak area is obtained with the maximum volume and extraction time evaluated. Fig. 2b shows the maximum point obtained when APDC concentration and pH extraction are at the central point of the evaluated range. This indicates a better Se-APDC microextraction at low pH and a Se:APDC molar ratio of 1:5000 approximately, showing an optimum value in a central point for APDC concentration. Higher Se:APDC molar ratios significantly affected the performance of IL-VA-LLME, probably due to saturation of the IL phase with high concentrations of unreacted APDC. Fig. 2c presents a maximum region when extraction time increases and the APDC concentration is in the central point of the evaluated range, corroborating the observation made on Fig. 2b.

ANOVA test was used to confirm that the fitted model was statistically significant (p value < 0.0001). The model was significant with a F value of 11.4 and the lack of fit did not cover the statistical requirements for a quadratic response surface model (Vera Candioti et al., 2014). The determination coefficients were acceptable ($R^2 = 0.81$ and $R^2_{adj} = 0.74$). These values were in agreement with those obtained in other works on microextraction (Di Carro, Ardini, & Magi, 2015).

Also, a volume of 10 mL yielded 50% extraction efficiency only. Therefore, a volume of 5 mL was fixed to improve Se extraction. The other significant factors were optimized considering the above-mentioned range. The highest desirability value obtained was 0.90 (Fig. 3) and the optimum values were: 5 mL sample volume, 15 min extraction time, pH 2.34 and 3.76×10^{-4} mol/L APDC concentration. Under these conditions an extraction efficiency of 90% was achieved. The experimental values provided by the optimization software were confirmed experimentally. The experimental peak area was compared with that predicted by the model and no significant difference was observed at a 95% confidence level.

3.3. Study of potential interfering species and sample clean-up evaluation

An interference study was performed on 5 mL of 0.5 μg/L Se standard solution. A concomitant ion was considered to interfere if variations of the analytical signal were higher than ±5%. The following elements: As(III), Bi(III), Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Mn(II), Ni(II), Pb(II), Sb(III), Sn(IV), V(V) and Zn(II), were tolerated up to at least 1500 μg/L. Analytical signal of the blank was not altered by the presence of the concomitant ions evaluated in this study.

The application of a clean-up step was considered due to sensitivity loss observed when IL-VA-LLME method was applied on untreated samples extracts. This effect was attributed to organic molecules extracted from vegetables matrices into the HCl

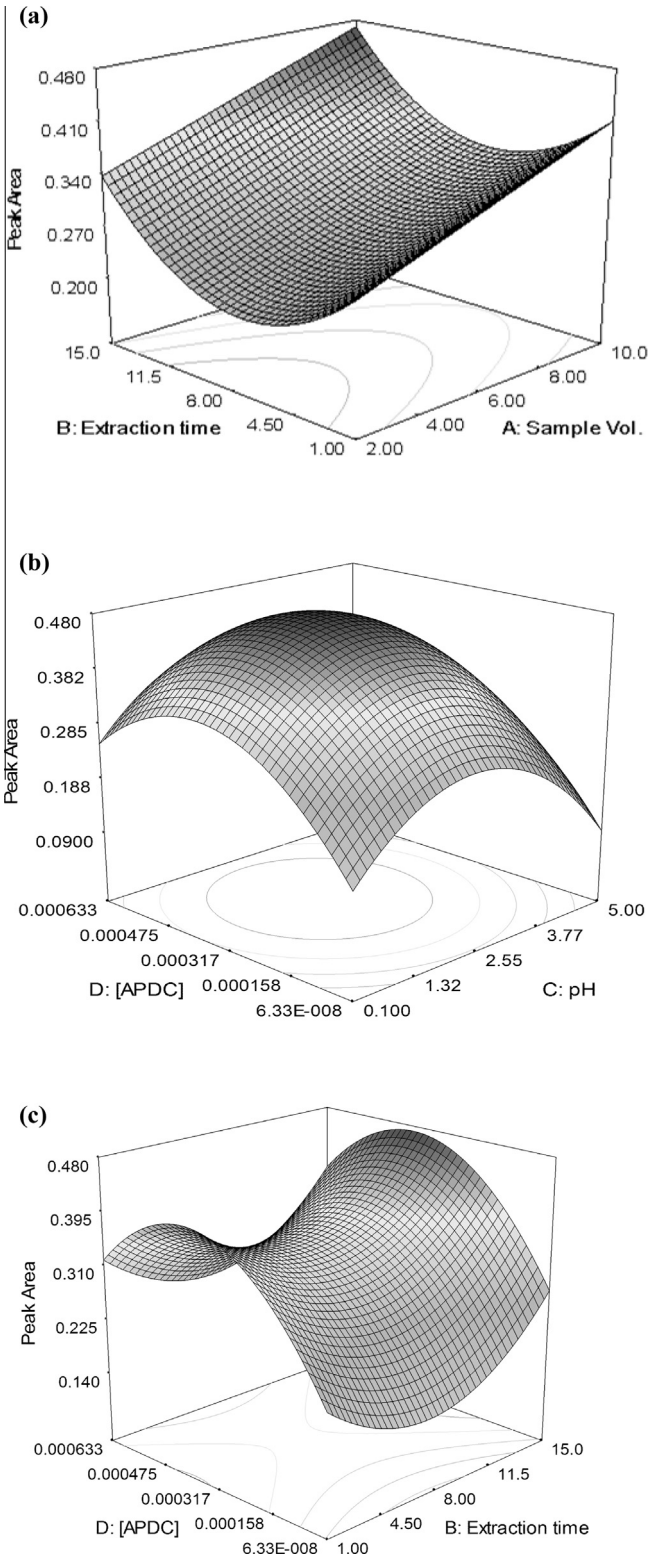

Fig. 2. Response surfaces of the models showing the variation of response (peak areas) as a function of a) Extraction time vs. Sample volume, b) APDC concentration vs. Extraction pH and c) APDC concentration vs. Extraction time.

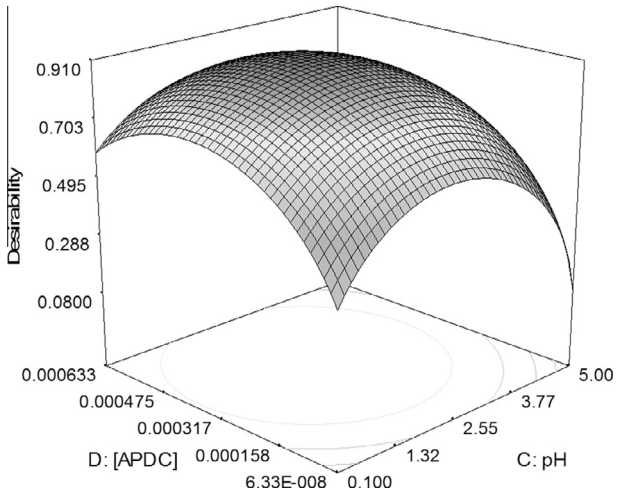

Fig. 3. Response surface plots corresponding to the desirability function.

medium used for Se extraction. In order to overcome this drawback, five different clean-up procedures including, 1) conventional liquid-liquid extraction (LLE) with chloroform (twice with 500 μL of chloroform added to 5 mL extract), solid phase extraction (SPE) with: 2) a C18 cartridge (loading the extract at 1 mL/min), 3) a column (2 mm i.d × 10 cm length) filled with XAD-1180 resin (loading the extract at 1 mL/min), 4) activated carbon (10 mg dispersed in 5 mL extract) or 5) MWCNTs (used as mentioned in Section 2.3) were evaluated. The Se recoveries obtained with LLE, C18 cartridge, XAD-1180 resin, activated carbon and MWCNTs were 75, 70, 43, 50 and 100%, respectively. Thus, the high surface area and the apolar character of MWCNTs could be responsible for the highest efficiency to clean-up the extract. Moreover, implementation of MWCNTs in a flow injection system permitted the reutilization of sorbent and avoided errors caused by handling of extracts.

3.4. Selectivity of Se species determination

The selectivity of the method for Se species determination was evaluated on 5 mL of 5.0 μg/L Se standard solutions prepared at different concentration ratios of Se(VI)/Se(IV), SeMet/Se(IV) and SeMetSeCys/Se(IV). In addition, the method was applied on solutions containing Se(VI), SeMet and SeMetSeCys species in the absence of Se(IV). The results showed that the developed method was highly selective towards Se(IV) species, mainly due to selective complexation with APDC, but also because the other species were not extracted. Acceptable recovery values were obtained for Se(IV) species under the evaluated conditions with values between 96% and 104% in the presence of the other species at molar ratios of 2, 5 and 10.

3.5. Analytical performance

The calibration curve for ETAAS determination of Se(IV) species was linear from levels near the limit of detection (LOD) up to at least 12.5 μg/L with a correlation coefficient of 0.9964. The LOD, calculated based on the signal at intercept and three times the standard deviation about regression of the calibration curve (Miller & Miller, 2001), was 5.0 ng/L. The relative standard deviation (RSD) for six replicate measurements at 0.5 μg/L of Se(IV) was 4.9%. The preconcentration factor (PF) was obtained from the slope ratio of calibration graphs after and before application of the IL-LLME procedure (Martinis, Berton, Olsina, Altamirano, & Wuilloud, 2009). A PF of 100 and extraction efficiency of 90% were obtained under optimal conditions (Table 1).

The frequency of analysis was 32 samples per hour taking into account that 16 tubes was the full capacity of the centrifuge used in our laboratory. However, this frequency can change according to the centrifugation equipment used. The frequency of analysis was calculated considering all steps: a) Se(IV) complexation with

Table 2
Analytical performance of the proposed method compared to others reporting IL-LLME for Se determination in different samples.

Method	IL	Speciation analysis	Sample	PF	LOD (ng/L)	RSD (%)	Sample volume (mL)	Analysis frequency (samples/h)	References
USA-IL-DLLME-ETAAS	$[C_6MIM][Tf_2N]$	Only in beverages	Beverages and several foods	150	12	4.2	10	N.R[a]	Tuzen and Pekiner (2015)
IL-DLLME-ETAAS	$[C_{12}MIM][Tf_2N]$	No[b]	Edible oils	129	0.04[c]	5.1	10[d]	N.R[a]	López-García et al. (2013)
IL-CI-AME-UV–vis	$[C_4MIM][PF_6]$	No	Rice and water	25	1500	1.2	25	N.R[a]	Rahnama and Abed (2014)
Online IL-DLLME-ETAAS	$[P_{6,6,6,14}][Cl]$	Yes	Water and garlic	20	15.0	5.1	4.0	4	Martinis et al. (2011)
IL-LLME-ETAAS	$[P_{6,6,6,14}][C_{10}H_{19}O_2]$	Yes	*Allium* and *Brassica* vegetables	100	5.0	4.9	5.0	32	This work

[a] Not reported.
[b] Extraction of organic selenoaminoacids.
[c] ng/g oil.
[d] Expressed in g of oil. $[C_6MIM][Tf_2N]$: 1-Hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_{12}MIM][Tf_2N]$: 1-Dodecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_4MIM][PF_6]$: 1-butyl-3-methylimidazolium hexafluorophosphate; $[P_{6,6,6,14}][Cl]$; Tetradecyl(trihexyl)phosphonium chloride; $[P_{6,6,6,14}][C_{10}H_{19}O_2]$: Tetradecyl(trihexyl)phosphonium decanoate; USA-IL-DLLME: ultrasound assisted ionic liquid dispersive liquid-liquid microextraction; IL-DLLME: ionic liquid dispersive liquid-liquid microextraction; IL-CI-AME: ionic liquid cold-induced aggregation microextraction.

APDC, b) extraction into the dispersed IL phase, c) centrifugation and d) separation of phases. Finally, a comparative evaluation on the analytical performance shows the strengths of the developed methodology with respect to others already reported in the literature using ILs for Se microextraction (Table 2). Our method shows analytical figures of merit that are comparable or better than those obtained in other methods. However, the feasibility of performing Se speciation analysis in samples with highly complex matrices is one of the greatest strengths of the proposed method. Moreover, IL-VA-LLME was compared with another microextraction method previously developed by our group using a flow injection system (Martinis et al., 2011). In the previous method, the analysis frequency was only of 4 samples per hour and many experimental steps had to be followed (i.e. column pre-conditioning, sample loading and washing with a surfactant solution prior to analyte elution). On the other hand, the IL-VA-LLME method is much faster, requires no further instrumentation and uses minimal amount of solvents than the previous method.

3.6. Validation study and analysis of samples

The developed method was applied to the determination of Se inorganic species in foods derived from *Allium* and *Brassica* plants. For the validation study, the proposed method was applied to a certified reference material (CRM), White clover BCR 402 with a declared total content for Se of 6.70 ± 0.25 mg/kg. An amount of 0.1 g of the CRM was used and total Se determination was performed. Since the certified concentration value in the CRM for Se was higher than the upper limit of the lineal range achieved by this method, a 6-fold dilution of the extract with 0.1 mol/L HCl solution had to be applied prior to analysis. By employing the IL-VA-LLME developed in this work, Se concentration found in the CRM was

Table 3
Determination of inorganic Se species in *Allium* and *Brassica* vegetables (95% confidence interval; n = 6).

Sample	Se added as (μg/L)		Se(IV) found (μg/L)	Recovery (%)[a]	Se(VI) found (μg/L)	Recovery (%)[a]
	Se(IV)	Se(VI)				
Garlic (Fuego)	–	–	0.08 ± 0.01	–	<LOD	–
	0.5	–	0.56 ± 0.03	96.5	<LOD	–
	–	0.5	0.08 ± 0.01	–	0.47 ± 0.03	94.0
	0.5	0.5	0.55 ± 0.03	94.8	0.49 ± 0.04	98.0
Garlic (Nieve)	–	–	0.12 ± 0.01	–	0.57 ± 0.03	–
	0.5	–	0.60 ± 0.03	96.8	0.57 ± 0.03	–
	–	0.5	0.12 ± 0.01	–	1.05 ± 0.06	98.1
	0.5	0.5	0.61 ± 0.03	98.4	1.06 ± 0.06	99.1
Onion	–	–	<LOD	–	0.08 ± 0.01	–
	0.5	–	0.49 ± 0.02	98.0	0.08 ± 0.01	–
	–	0.5	<LOD	–	0.56 ± 0.03	96.6
	0.5	0.5	0.48 ± 0.02	96.0	0.59 ± 0.03	102
Leek	–	–	<LOD	–	0.09 ± 0.01	–
	0.5	–	0.50 ± 0.03	100	0.09 ± 0.01	–
	–	0.5	<LOD	–	0.59 ± 0.03	100
	0.5	0.5	0.49 ± 0.03	98.0	0.58 ± 0.03	98.3
Broccoli	–	–	<LOD	–	<LOD	–
	0.5	–	0.48 ± 0.02	96.0	<LOD	–
	–	0.5	<LOD	–	0.49 ± 0.03	98.0
	0.5	0.5	0.47 ± 0.03	94.0	0.51 ± 0.03	102
Cauliflower	–	–	<LOD	–	<LOD	–
	0.5	–	0.50 ± 0.02	100	<LOD	–
	–	0.5	<LOD	–	0.50 ± 0.03	100
	0.5	0.5	0.48 ± 0.03	96.0	0.49 ± 0.03	98.0

[a] 100 × [(found − base)/added].

6.66 ± 0.43 mg/kg, indicating an acceptable accuracy of the method ($p < 0.01$). In addition, a recovery study can be performed as an alternative validation of the method in elemental speciation analysis. The recovery values obtained for Se(IV) and Se(IV) were in the range of 94–100% and 96.6–102%, respectively. These figures demonstrate the reliability of the proposed method for inorganic Se speciation analysis.

Finally, the concentrations of Se species in samples were in the range of <LOD – 0.12 μg/L for Se(IV) and <LOD – 0.57 μg/L for Se (VI) (Table 3). These values are in good agreement with those reported by other authors (Rayman, Infante, & Sargent, 2008). Concentrations of inorganic Se in the analyzed samples were acceptable considering the nutritional needs of this element. In fact, inorganic Se can be found in *Allium* and *Brassica* gender plants, indicating that Se taken by these plants is not fully metabolized into organic species (Pyrzynska, 2009).

4. Conclusions

The first application of the IL trihexyl(tetradecyl)phosphonium decanoate in a LLME procedure for inorganic Se species separation and determination is reported in this work. By using this IL, minimal amount of solvent was required for dispersion of the extractant phase, thus differing from extraction systems using imidazolium-ILs that usually must be accompanied by methanol or other volatile organic solvents. This can be considered as an important benefit in order to add chemical greenness to LLME methods in addition to the use of a low cost IL, with which a high extraction efficiency and preconcentration factor were achieved. Also, the possibility of applying the proposed methodology for the analysis of samples having complex matrices such as those raised by foods derived from *Allium* and *Brassica* plants was demonstrated. This represents a remarkable advantage that facilitates the direct implementation of the proposed method in routine laboratories working on Se speciation in foods. Moreover, the accurate determination of Se by ETAAS was feasible thanks to an appropriate optimization of the temperature program that allowed complete decomposition of the complex matrix presented by the IL-enriched phase, while the strong volatilization of Se caused by APDC was efficiently eliminated by injecting Cu into the graphite furnace. Furthermore, the use of a multivariate optimization approach reduced the number of experiments and steps needed during the development of the IL-VA-LLME method.

Acknowledgments

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET – Argentina), Agencia Nacional de Promoción Científica y Tecnológica (FonCyT – Argentina) (PICT-BID), Universidad Nacional de Cuyo (UNCuyo – Argentina) and the Organization for the Prohibition of Chemical Weapons (OPCW).

References

Cornelis, R., Caruso, J., Crews, H., & Heumann, K. (2005). *Handbook of elemental speciation II – Species in the environment, food, medicine and occupational health*.
Dadfarnia, S., Haji Shabani, A. M., & Nozohor, M. (2014). Dispersive liquid-liquid microextraction-solidified floating organic drop combined with spectrophotometry for the speciation and determination of ultratrace amounts of selenium. *Journal of the Brazilian Chemical Society, 25*(2), 229–237.
Di Carro, M., Ardini, F., & Magi, E. (2015). Multivariate optimization of headspace solid-phase microextraction followed by gas chromatography-mass spectrometry for the determination of methylpyrazines in cocoa liquors. *Microchemical Journal, 121*, 172–177.
Escudero, L. B., Berton, P., Martinis, E. M., Olsina, R. A., & Wuilloud, R. G. (2012). Dispersive liquid-liquid microextraction and preconcentration of thallium species in water samples by two ionic liquids applied as ion-pairing reagent and extractant phase. *Talanta, 88*, 277–283.
Escudero, L. B., Castro Grijalba, A., Martinis, E. M., & Wuilloud, R. G. (2013). Bioanalytical separation and preconcentration using ionic liquids. *Analytical and Bioanalytical Chemistry, 405*(24), 7597–7613.
Kamada, T., & Yamamoto, Y. (1980). Use of transition elements to enhance sensitivity for selenium determination by graphite-furnace atomic-absorption spectrophotometry combined with solvent extraction with the APDC-MIBK system. *Talanta, 27*(6), 473–476.
Koel, M. (2009). *Ionic liquids in chemical analysis*. Boca Raton, Florida: CRC Press.
López-García, I., Vicente-Martínez, Y., & Hernández-Córdoba, M. (2013). Nonchromatographic speciation of selenium in edible oils using dispersive liquid-liquid microextraction and electrothermal atomic absorption spectrometry. *Journal of Agricultural and Food Chemistry, 61*(39), 9356–9361.
Martinis, E. M., Berton, P., Olsina, R. A., Altamirano, J. C., & Wuilloud, R. G. (2009). Trace mercury determination in drinking and natural water samples by room temperature ionic liquid based-preconcentration and flow injection-cold vapor atomic absorption spectrometry. *Journal of Hazardous Materials, 167*(1–3), 475–481.
Martinis, E. M., Escudero, L. B., Berton, P., Monasterio, R. P., Filippini, M. F., & Wuilloud, R. G. (2011). Determination of inorganic selenium species in water and garlic samples with on-line ionic liquid dispersive microextraction and electrothermal atomic absorption spectrometry. *Talanta, 85*(4), 2182–2188.
Miller, J. N., & Miller, J. C. (2001). *Statistics and chemometrics for analytical chemistry*. New York: Prentice Hall.
Nordberg, G. F., Fowler, B. A., Nordberg, M., & Friberg, L. (2007). *Handbook of the toxicology of metals*. Waltham, Massachussetts: Academic Press.
Pyrzynska, K. (2009). Selenium speciation in enriched vegetables. *Food Chemistry, 114*(4), 1183–1191.
Rahnama, R., & Abed, Z. (2014). Application of cold-induced aggregation microextraction as a fast, simple, and organic solvent-free method for the separation and preconcentration of Se(IV) in rice and various water samples. *Environmental Monitoring and Assessment, 186*(7), 4209–4216.
Rayman, M. P., Infante, H. G., & Sargent, M. (2008). Food-chain selenium and human health: Spotlight on speciation. *British Journal of Nutrition, 100*(2), 238–253.
Stalikas, C., Fiamegos, Y., Sakkas, V., & Albanis, T. (2009). Developments on chemometric approaches to optimize and evaluate microextraction. *Journal of Chromatography A, 1216*(2), 175–189.
Tuzen, M., & Pekiner, O. Z. (2015). Ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction combined with graphite furnace atomic absorption spectrometric for selenium speciation in foods and beverages. *Food Chemistry, 188*, 619–624.
Vera Candioti, L., De Zan, M. M., Cámara, M. S., & Goicoechea, H. C. (2014). Experimental design and multiple response optimization. Using the desirability function in analytical methods development. *Talanta, 124*, 123–138.
Viñas, P., Campillo, N., López-García, I., & Hernández-Córdoba, M. (2013). Dispersive liquid-liquid microextraction in food analysis. A critical review. *Analytical and Bioanalytical Chemistry, 406*(8), 2067–2099.
Zhong, N., Zhong, L., Hao, L., Luan, C., & Li, X. (2015). Speciation of selenium in enriched garlic sprouts by high-performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry. *Analytical Letters, 48*(1), 180–187.