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Analytical Methods

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

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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

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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