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Analytical Note

Selenium speciation in *radix puerariae* using ultrasonic assisted extraction combined with reversed phase high performance liquid chromatography-inductively coupled plasma-mass spectrometry after magnetic solid-phase extraction with 5-sulfosalicylic acid functionalized magnetic nanoparticles

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ABSTRACT

A new method for determination of selenium species in *radix puerariae* was described. The method consists of sample enrichment with 5-sulfosalicylic acid (SSA)-functionalized silica-coated magnetic nanoparticles (SMNPs), high performance liquid chromatography (HPLC) separation, and online detection using inductively coupled plasma mass spectrometry (ICP-MS). The selenium species were extracted using ultrasonic extraction system with a mixture of protease K and lipase. The SSA-SMNPs were used to enrich trace amounts of selenite [Se(IV)], selenate [Se(VI)], selenomethionine (SeMet), and selenocystine ($SeCys_2$) from lower selenium containing samples. Under the optimal conditions, the limits of detection (3σ) for $SeCys_2$, Se(IV), SeMet and Se(VI) were observed as 0.0023, 0.0015, 0.0043, and 0.0016 ng mL^{-1}, respectively. The RSD values ($n = 6$) of method for intraday were observed between 0.5% and 0.9%. The RSD values of method for interday were less than 1.3%. The linear concentration ranges for SeCys2, Se(IV), SeMet and Se(VI) were 0.008–1000, 0.005–200, 0.015–500 and 0.006–200 ng mL^{-1}, respectively. The detection limits of this method were improved by 10 times due to the enrichment with the SSA-SMNP extraction. The contents of $SeCys_2$, Se(IV), SeMet, and Se(VI) in *radix puerariae* were determined as 0.0140, 0.171, 0.0178, and 0.0344 μg g^{-1}, respectively. The recoveries were in the range of 95.6%–99.4% and the RSDs (n = 6) of recoveries were less than 1.5%.

1. Introduction

Selenium is an essential micronutrient at low concentrations; however, it is toxic for animals and humans at higher concentrations [1]. The toxicity, biological effects, and bioavailability of selenium are highly dependent on its total amount and chemical forms [2]. The levels and bioavailability of selenium in foods have increased interest due to its antioxidant and anticancer properties. Selenium deficiency is associated with several diseases, such as Kashin-Beck, Kashan, and hypothyroidism [3].

Several quantification methods were used for the analysis of selenium, for instance, atomic absorption spectrometry (AAS) [1,4], atomic fluorescence spectroscopy (AFS) [5], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [6], and inductively coupled plasma mass spectrometry (ICP-MS) [7–10]. Among them, ICP-MS method has been proved to be the most powerful technique for the determination of trace elements due to its attractive features: high sensitivity, good precision, multi-element capability, and wide linearity. However, main disadvantage for the determination of selenium by quadrupole ICP-MS is associated with isobaric interferences from $^{40}Ar^{40}Ar^{+}$ on $^{80}Se^{+}$, and $^{38}Ar^{40}Ar^{+}$ on $^{78}Se^{+}$. Meanwhile, the concentrations of selenium species are usually very low in food, biological, and environmental samples. Therefore, a preconcentration step is usually required prior to quantitative analysis to improve sensitivity and precision of applied techniques [11,12]. Different preconcentration techniques, such as coprecipitation with hydroxides, extraction, microextraction [13], isotachophoresis, and anion exchange chromatography have been recently proposed for selenium analysis. Among these techniques, solid phase extraction (SPE) features simple device and can enrich the interesting species, make it a desirable procedure for species analysis [14,15].

Magnetic solid-phase extraction (MSPE) is a kind of magnetic or magnetizable material as absorbent matrix solid-phase extraction technique [16]. Compared with common solid-phase extraction technique, MSPE has many advantages, including, easy automation, simple

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operation, short extraction time, and low organic solvent consumption [17]. Recently, magnetic materials have been exploited in many fields, for instance as catalysis [18], in cell imaging [19], biomedicine [20], and separation science [21]. Chen et al. developed a MSPE method for the determination of selenoaminoacids in yeast cells using HPLC-ICP-MS analytical technique [2]. The MSPE method was established using sulfonated polystyrene-coated magnetic nanoparticles as adsorption material for MSPE of selenoamino acids and selenopeptide. Kocot et al. developed dispersive micro-solid-phase extraction technique in which graphene was used as solid adsorbent and ammonium pyrrolidinedithiocarbamate as chelating agent for species and determination of inorganic selenium using the energy-dispersive X-ray fluorescence spectrometry technique [11]. Among the magnetic materials, iron oxides (for example, magnetite Fe_3O_4) is widely used in MSPE technique [22,23].

Radix puerariae (Gegen, Kudzu root) is the dried root of *Pueraria lobata* (Willd.) ohwi and *Pueraria thomsonii* Benth from leguminous plants [24]. *Radix puerariae* has been frequently described in East Asia for its antipyretic, antidiarrhetic, diaphoretic, antiemetic, and antialcoholic characters alone or in combination with other traditional medications, such as *Panax notoginseng* and *Salvia miltiorrhiza*. The root, stem, leaf, flower, and seed of *radix puerariae* have also been used in traditional Chinese medicine [25]. *Radix puerariae* powder and its extract have attracted a growing attention as a supplement throughout many countries [26], for instance, in the United States of America, the United Kingdom, and Australia. *Radix puerariae* has many essential amino acids, such as, methionine, lysine, isoleucine, leucine, and phenylalanine, and contains several trace elements, for instance, selenium, zinc, manganese, and germanium. The selenium species for *radix puerariae* has not been reported. In this study, sulfonated Fe_3O_4 magnetite nanoparticles were used to enrich selenium species from *radix puerariae* and the effects of analysis conditions of selenium species were investigated. The developed method of MSPE combined with reversed phase (RP) HPLC-ICP-MS technique was applied to determine selenium species present in *radix puerariae*.

2. Experimental

2.1. Instrumentation

The separation of selenium species was performed on-line through coupling of an HPLC system with ICP-MS as detector. The chromatographic system (Perkin Elmer, USA) included a Flexar Binary LC Pump, Flexar Solvent Manager, and Flexar LC Autosampler. Separation of $SeCys_2$, Se(IV), SeMet, and Se(VI) with HPLC were performed on a Hamilton PRP-×100 (250 mm length × 4.1 mm i.d., 10 μm particle size) analytical column (Hamilton, Reno, USA) connected to a guard column Hamilton PRP-×100 (20 mm length × 2.1 mm i.d., 10 μm particle size). NexIon 300× ICP-MS instrument (Perkin Elmer, USA), equipped with a Universal Cell Technology TM (UCT), which integrated standard, collision and dynamic reaction cell system to eliminate interference. The samples were introduced to the system using a glass Meinhard nebulizer and a glass cyclonic spray chamber. The chromatographic system was connected to the ICP-MS instrument with a PEEK tube. Titan MPS microwave digestion system was purchased from PerkinElmer (USA). TG16-W high speed micro-centrifuge was purchased from Hunan Xiangyi Laboratory Instrument Development (Hunan, China). The SK3310HP Ultrasonic cleaner was purchased from Shanghai Kudos Ultrasonic Instrument of China. DZF-300 vacuum drying oven was produced by Zhengzhou Greatwall Scientific Industrial and Trade of China. Instrumental parameters and experiments conditions are listed in Table 1.

2.2. Reagents

Selenium standard solution (1000 μg mL^{-1}, GSB G62029-90(3401)) was obtained from National Analysis Center of Iron and Steel of China.

Table 1
Operating conditions for the HPLC and ICP-MS examinations.

Chromatographic conditions	
Column	Hamilton PRP-×100 anion exchange column (10 μm, 4.1 mm × 250 mm)
Guard column	Hamilton PRP-×100 guard column (10 μm, 2.1 mm × 20 mm)
Mobile phase	8 mmol L^{-1} citric acid (pH 5 using 20% $NH_3 \cdot H_2O$ control)
Flow rate	1.5 mL min^{-1}
Injection volumex	50 μL
ICP-MS conditions	
RF power	1250 W
Plasma flow	16 L min^{-1}
Auxiliary flow	1.1 L min^{-1}
Nebulizer flow	0.95 L min^{-1}
Pulse state voltage	1250 V
RPq	0.80
Cell gas flow rate DRC $-CH_4$	1.20 mL min^{-1}
Monitored species	^{80}Se

Selenium stock solutions of 1000 μg mL^{-1} for each species were prepared from selenium oxide (SeO_2), sodium selenate (Sigma, St. Louis, MO, USA), SeMet (DL-selenomethionine, Alfa, USA), and $SeCys_2$ (L-selenocystine, Beijing Chemical Reagent Company, Beijing, China). Nitric acid (65%, UPS), hydrogen peroxide solution (30%, UPS) were obtained from Suzhou Crystal Clear Chemical, Jiangsu, China. Lipase and proteinase K came from Sigma (St. Louis, MO, USA) and Tiangen Biotech (Beijing, China), respectively. Citric acid, ferrous chloride, ferric chloride, tetraethyl orthosilicate, 5-sulfosalicylic acid and ammonium hydroxide were supplied by Xilong Chemical of Guangdong, China. Thionyl chloride was obtained from Tianjin Zhiyuan Chemical (Tianjin, China). Helium (99.999%), argon (99.999%), oxygen (99.995%) and methane (99.999%) were produced by Dalian Special Gases of Liaoning, China. ICP-MS daily performance was checked using a multielemental standard solution containing Be, Mg, In, U and Ce at concentrations of 1 μg L^{-1} (Perkin Elmer, USA). Ultrapure water (18.2 MΩcm, ELGA Purelab Option) was used in the experiments. Standard solutions of the desired concentrations were prepared daily *via* stepwise dilution of their stock solutions. All solutions were filtered through a 0.45 μm membrane filter (Shanghai Xinya Purification Material Factory, Shanghai, China) before use.

2.3. Samples pretreatment

2.3.1. Digestion of samples

Radix puerariae was purchased from a drugstore in Guilin of China. It was placed in a vacuum drying oven at 70 °C until constant weight was reached, then made it into a 100-mesh-size powder. The *radix puerariae* powder samples were accurately weighed 0.2000 g into a Teflon microwave digestion vessel, and then digested with Titan MPS microwave sample preparation system. Digestion was performed in mixture solution of 6.0 mL of 65% (w/v) HNO_3 and 1.0 mL of 30% (w/v) H_2O_2. The microwave vessels were subjected to two different digestion conditions: Temperature (in °C), ramp time (in minutes), hold time (in minutes), and power (in %) were adjusted at 140/15/8/80 and 220/10/25/90, respectively. Digested samples were cooled and diluted to 25 mL with ultrapure water and analyzed for the total content of selenium using ICP-MS analytical system. Six independent replicates were made, and the reagent blank solutions were prepared using the same preparation method of sample.

2.3.2. Selenium species extraction

0.5000 g *radix puerariae* were added into a 15 mL polyethylene centrifuge tube, after which 5 mL of ultrapure water, 1 mL of 20 mg mL^{-1} of protease K, and 20 mg of lipase were added. The mixtures were