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Dual extraction technique combined with HPLC-ICP-MS for speciation of seleno-amino acids in rice and yeast samples

Xueqin Guo, Man He, Kai Nan, He Yan, Beibei Chen, Bin Hu*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, P R China

Abstract: Magnetic solid phase extraction (MSPE) was combined with hollow fiber liquid liquid liquid microextraction (HF-LLLME) for the extraction of seleno-amino acids. Graphene oxide (GO) modified magnetic nanoparticles (MNPs) were prepared and loaded with Cu^{2+} for the adsorption of target seleno-amino acids based on the interaction between Cu^{2+} and seleno-amino acids. Aqueous ethylenediamine was employed as desorption solvent, and the desorption solution was directly employed as the donor solution in the subsequent HF-LLLME procedure for further preconcentration of target seleno-amino acids. In HF-LLLME, 1-octanol was employed as extraction solvent, ionic liquid trioctylmethylammonium chloride ($[MTOA]^+[Cl]^-$) was employed as carrier for the extraction of target seleno-amino acids, and aqueous $NaNO_3$ solution was employed as acceptor solution. The target seleno-amino acids were extracted from the donor solution into the organic phase and then back into the acceptor solution under the driving force of ion-pair formation between target seleno-amino acids and $[MTOA]^+[Cl]^-$ and the gradient counter ions between donor phase and the acceptor phase. Various factors influencing the extraction of target seleno-amino acids by MSPE and HF-LLLME were investigated thoroughly. Based on it, a method of MSPE-HF-LLLME coupling with high performance liquid chromatography (HPLC)-inductively coupled plasma mass spectrometry (ICP-MS) was developed and the analytical performance was evaluated under the optimized conditions. After preconcentration by MSPE-HF-LLLME, the enrichment factors (EFs) for target seleno-amino acids ranged from 152 to 278-fold, and the LODs for target seleno-amino acids was in the range of 0.0075-0.013 μg L^{-1}. A certified reference material of SELM-1 was used to validate the accuracy of the proposed method, and the determined value of SeMet was in good agreement with the certified values. The proposed method was successfully applied to the speciation of seleno-amino acids in rice and

* Corresponding author, tel: 86-27-68752162; fax: 86-27-68754067, email: binhu@whu.edu.cn

Se-enriched yeast cell samples.

Key words: magnetic solid phase extraction, hollow fiber liquid liquid liquid microextraction, HPLC-ICP-MS, seleno-amino acids, rice, yeast cell

1. Introduction

The essential trace element selenium (Se) has various functions including antioxidant effect, cancer-protective effect, protection of myocardium and retina and alleviating the toxicity of heavy metals[1]. The bioavailability and toxicity of Se is highly dependent on its total amount and chemical form[2]. Either deficient or excess doses are harmful to human health, and the adequate nutritional range of Se was 0.1-1.0 μg g^{-1}[3, 4]. Compared with inorganic selenium compounds (selenite and selenate), organic selenium compounds (seleno-amino acids, selenopeptides and selenoproteins, etc.) exhibit more bioavailability and less toxicity[5]. Food is the main source for human intake of Se[6, 7], and dietary intake of Se recommended by Chinese Nutrition Society was 60-200 μg per day. Since the distribution of Se in soil varied in different regions of China greatly, Se-supplements (Se-enriched yeast cell, Selenium salt, etc.) has been employed to improve the dietary intake of Sein Se-deficient area. Therefore, developing reliable and high sensitive analytical methods for speciation of Se in food samples is of great significance to study the relationship between disease and uptake of Se and its cancer prevention mechanisms.

Hyphenated techniques combining highly efficient separation techniques (e.g. high performance liquid chromatography, HPLC) with highly sensitive element-specific detectors (e.g. inductively coupled plasma mass spectrometry, ICP-MS)[8, 9] are the most widely used methods for Se speciation. However, speciation of Se in biological samples is still a challenging task because of the low concentration level of Se in biological samples (except selenium-enriched samples) and the complicated sample matrix. Hence, appropriate sample pretreatment techniques are often required prior to HPLC-ICP-MS analysis. Up to now, solid phase extraction (SPE)[10-12], solid phase microextraction (SPME)[13], stir bar sorptive extraction (SBSE)[5] and magnetic solid phase extraction (MSPE)[14] have been employed for the speciation of Se. Among them, the adsorption material plays a key role in obtaining efficient separation and preconcentration of target Se species. Based on the electrostatic interaction between the adsorption material and seleno-amino acids, the

prepared partially sulfonated polystyrene-titania (PSP-TiO_2) SBSE coating[5] and sulfonated polystyrene (Fe_3O_4@PSS) coated magnetic nanoparticles (MNPs)[14] exhibited efficient enrichment for target seleno-amino acids, while both of the developed method suffered from inorganic salts interference seriously. Vonderheide et al.[13] developed a method of SPME-gas chromatography (GC)-ICP-MS for speciation of seleno-amino acids, and the target seleno-amino acids should be derivatized by isobutylchloroformate prior to SPME extraction. Since Cu^{2+} can form stable complexes with seleno-amino acids, Duan et al.[10] developed a method of online SPE-ICP-MS for the speciation of $SeCys_2$ and SeMet with Cu^{2+} loaded nanometer-sized alumina as adsorption material. The developed method presented good anti-interference ability, while the enrichment factors were relatively low. MSPE was developed from SPE with the assistant of magnetic adsorbents, featuring of simple operation, fast separation and batch operations available. And the preparation of MNPs with good selectivity is important for high enrichment and separation of seleno-amino acids. Graphene oxide (GO), a precursor for graphene, consists of a hexagonal carbon network bearing hydroxyl and epoxide functional groups which has high adsorption capacity toward transition metal ions[15-18], and the application potential for seleno-amino acids speciation is expected.

Liquid phase microextraction (LPME)[19, 20] developed in 1996 possesses merits of solvent-less, low cost, high enrichment factors, easy-to-operate, and is suitable for analysis of non-polar compounds. In order to improve the extraction efficiency of polar compounds, derivatization and carrier mediated hollow fiber liquid liquid liquid microextraction (HF-LLLME) was developed[21]. Duan et al.[22] developed derivatization mediated HF-LLLME for the preconcentration of seleno-amino acids followed by GC-ICP-MS detection using ethyl chloroformate to improve the water immiscibility and volatility of seleno-amino acids. Guo et al.[23] developed a method of ionic liquid (IL) based carrier mediated HF-LLLME-HPLC-ICP-MS for speciation of phenylarsenic compounds in chicken and feed samples. With trioctylmethyl ammonium chloride ($[MTOA]^+[Cl]^-$) as carrier, the developed HF-LLLME method presented high enrichment factors (86-372-fold) for target arsenic species, while the anti-interference ability was not so good due to the ion exchange extraction mechanism. Since the polarity and water solubility of seleno-amino acids was relatively high, it is predicted that high extraction efficiency may be obtained by carrier mediated HF-LLLME.

Since the desorption solution of MSPE matches with the HF-LLLME well, the combination of MSPE with HF-LLLME will endow the dual extraction technique high enrichment factors and good anti-interference ability simultaneously. The aim of this work is to develop a method of dual extraction technique involving MSPE-HF-LLLME combined with HPLC-ICP-MS for speciation of polar seleno-amino acids in food and yeast cell samples. Various factors influencing the extraction of seleno-amino acids by MSPE and HF-LLLME were studied in detail. The developed method was applied to the speciation of target seleno-amino acids in certified reference material SELM-1, rice and yeast cell samples with satisfactory results.

2. Experimental

2.1 Standard solutions and reagents

A stock solution of $SeCys_2$ (1 mg mL^{-1} as Se) was prepared by dissolving appropriate amount of L-selenocystine (98%, Acros organics, USA) in 0.1 mol L^{-1} HCl. Stock solutions of MeSeCys, SeMet and SeEt were prepared by dissolving Se-methylseleno-L-cysteine (98%, Acros Organics, USA), DL-selenomethionine (99+%, Acros Organics, USA) and seleno-D, L-ethionine (Toronto Research Chemicals Inc., Canada) in high purity water, respectively. Working solutions were prepared daily by appropriate dilution of the stock solutions. Certified Reference Material of SELM-1 selenium-enriched yeast was purchased from the National Research Council of Canada (NRCC) (Ottawa, Canada). Methyltrioctyl ammonium chloride ($[MTOA]^+[Cl]^-$, 97%) were purchased from Shanghai Jingchun Industrial Co. Ltd (Shanghai, China). Sodium nitrate ($NaNO_3$, AR) was obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). High purity water was obtained by a Milli-Q water purification system (18.25 MΩ·cm, Millipore, Molsheim, France). Plastic and glass containers and all other employed laboratory materials that could come into contact with samples or standards were stored in 20% (v/v) nitric acid over 24 h, and rinsed with high purity water prior to use.

The Accurel Q3/2 polypropylene hollow fiber membrane (600 μm i.d., 200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). Hollow fibers were cut into 2.8 cm pieces, and sonicated for 5 min in acetone to remove the contaminants in the fiber. After ultrasonication, the fibers were removed from acetone and dried in the air prior to use.

2.2 Instrumentals

A quadrupole ICP-MS (Agilent 7500a, Japan) was interfaced to HPLC through a Babington nebulizer as an online detector. ^{77}Se and ^{82}Se were simultaneously monitored, while ^{82}Se was used for quantification. An HPLC system equipped with two LC-10AD high pressure pumps and a CTO-10A column oven (Shimadzu, Japan) was utilized for chromatographic separation. A CAPCELL PAK C18 column (5 μm, 4.6 × 250 mm, Shiseido, Japan) was employed for separation of different selenium species. The operating conditions for HPLC-ICP-MS are given in Table 1. The peak area obtained by HPLC-ICP-MS was used for quantification.

2.3 Sample preparation

Various rice samples were purchased from a local market. Selenium-enriched yeast cells were supplied by Prof. D.W. Pang (Wuhan University, Wuhan, China). Certified Reference Material (SELM-1) was extracted by methane sulfonic acid[24]. Briefly, 0.1 g SELM-1 powder was placed in a round bottom flask, and 10 mL 4 mol L^{-1} sulfonic acid was added, the solution was then refluxed for 16 h. Then, the extract was transferred to 100 mL volumetric flask and diluted to the calibrate with high purity water. The Se species in rice and yeast cell samples were extracted by hot water as described in Ref[5]. 0.1 g rice or 0.01 g selenium-enriched yeast cell were placed into the centrifuge tube, and 5 mL high purity water was added. The tube was put in a hot water bath at 50ºC for 24 h, and then centrifuged for 10 min at 7000 rpm. After centrifugation, the rice extract was transferred to a 50 mL calibrated tube, adjusted to the desire pH with diluted ammonia, and then calibrated to 40 mL for analysis. The yeast cell extract was diluted by 100-fold, and adjusted to pH 7.0 prior to the extraction and determination by MSPE-HF-LLLME-HPLC-ICP-MS.

2.4 Preparation of Cu^{2+} modified Fe_3O_4@SiO_2@polyaniline (PANI)-graphene oxide (GO) nanoparticles

The preparation of graphene oxide and Fe_3O_4@SiO_2@PANI@GO was according to Ref[25]. Charging of Fe_3O_4@SiO_2@PANI@GO magnetic nanoparticles (MNPs) with Cu^{2+} were prepared as follows: 0.5 g Fe_3O_4@SiO_2@PANI@GO MNPs were mixed with 50 mL 1 mg mL^{-1} $CuSO_4$ (as Cu^{2+}), and ultrasonicated for 30 min. After ultrasonication, the excess unbound Cu^{2+} was removed by high purity water.

2.5 Magnetic solid phase extraction procedure

An aliquot of 10 mL sample solution was transferred into the beaker, and 20 mg Cu^{2+} charged Fe_3O_4@SiO_2@PANI@GO MNPs was added to the sample solution followed by ultrasonication

for 10 min at room temperature. The MNPs was isolated from the suspension with an Nd-Fe-B strong magnet. Finally, the adsorbed analytes were desorbed from the isolated adsorbent with 1 mL 0.1 mol L^{-1} ethylenediamine by stirring for 5 min. And the eluent was transferred to a 1 mL vial for the subsequent HF-LLLME procedure directly.

2.6 Hollow fiber liquid liquid liquid microextraction procedure

The desorption solution of MSPE (~1 mL) was added into the extraction vial, and a home-made magnetic stir bar was placed in the sample solution. After 9 μL $NaNO_3$ was withdrawn into the HPLC syringe, the syringe was inserted into the hollow fiber and the $NaNO_3$ solution was introduced into the lumen of the hollow fiber followed by impregnation of the hollow fiber in the organic phase for about 20 s. Then the hollow fiber together with the HPLC syringe was put into the sample solution as soon as possible. After extraction for a certain time, the acceptor solution was injected into HPLC-ICP-MS for subsequent analysis.

3. Results and discussion

In order to obtain high enrichment factors and strong anti-interference ability simultaneously, dual extraction technique of MSPE and HF-LLLME was employed for extraction of target seleno-amino acids. Various factors influencing the extraction of seleno-amino acids were investigated and discussed thoroughly.

3.1 Optimization of MSPE procedure

Effect of sample pH

Since the existing form of seleno-amino acids was dependent on the sample pH, the effect of sample pH plays a vital role on the extraction of target seleno-amino acids by MSPE. Fig. 1 illustrated the effect of sample pH (3.0-11.0) on the adsorption efficiency of target seleno-amino acids. It could be seen that the adsorption efficiency of target seleno-amino acids was increased with the increase of sample pH from 3.0 to 6.0, and kept almost constant with further increase of sample pH to 10.0. The adsorption efficiency of all target seleno-amino acids was decreased rapidly when sample pH was higher than 10.0. The possible reason was that the target seleno-amino acids can form stable complexes with Cu^{2+} in the sample pH range of 6.0-10.0, while the interaction between Cu^{2+} and target seleno-amino acids become weaker when sample pH was lower than 5.0 or higher than 10.0. Therefore, sample pH of 7.0 was employed in the

following experiments.

Effect of adsorption time

The effect of adsorption time (1-20 min) on the adsorption efficiency of target seleno-amino acids was studied. The analytical results (Fig. S1) showed that the adsorption efficiency of all target seleno-amino acids was increased rapidly with increasing the time from 1 to 5 min, and kept almost constant with further increase of the adsorption time to 20 min. Hence, 10 min was selected as the extraction time for adsorption of target seleno-amino acids.

Selection of the elution solvent

Based on the effect of sample pH on the adsorption efficiency of target seleno-amino acids, it can be seen that low extraction efficiency was obtained for target seleno-amino acids under acidic or basic environment. Besides, Ma et al.[26] reported that imidazole as a competitive substrate can displace adsorbed protein to form a coordination compound with Cu^{2+} (charged on the surface of magnetic IDA-silica nanoparticles). Therefore, four kinds of desorption solvent including 0.5 mol L^{-1} HNO_3, 0.01 mol L^{-1} NaOH, 0.1 mol L^{-1} imidazole (pH=10.0, 11.0. 12.0) and 0.1 mol L^{-1} ethylenediamine (pH=11.5, 12.0) was studied in detail. The analytical results in Fig. 2 showed that 0.01 mol L^{-1} NaOH presented the lowest desorption signal intensity for all target seleno-amino acids, and the desorption signal intensity was increased with increase of the imidazole pH from 10.0 to 12.0. Ethylenediamine (pH=11.5, 12.0) showed higher desorption signal intensity than 0.5 mol L^{-1} HNO_3 and 0.1 mol L^{-1} imidazole (pH=12.0). Therefore, 0.1 mol L^{1} ethylenediamine (pH=11.5) was selected as the desorption solvent for subsequent experiment.

Effect of ethylenediamine concentration

The effect of ethylenediamine concentration on the desorption efficiency of target seleno-amino acids was studied and the results are shown in Fig. S2. It could be seen that the desorption signal intensity of all target seleno-amino acids was increased rapidly with increase of the ethylenediamine concentration from 0.01 to 0.05 mol L^{-1}, and kept almost constant with further increase of ethylenediamine concentration to 0.2 mol L^{-1}. Hence, 0.1 mol L^{-1} ethylenediamine was employed for further experiment.

Desorption mode and time

With 1 mL 0.1 mol L^{-1} ethylenediamine as desorption solution, and desorption time as 10 min, the effect of desorption mode (ultrasonication and stirring) on the desorption efficiency of target

seleno-amino acids was investigated. It was found that the desorption of target seleno-amino acids by ultrasonication exhibited similar desorption efficiency with desorption by stirring, while the sediment time for ultrasonication (30 min) was longer than stirring (5 min), and the isolation of MNPs from the suspension was incomplete, probably because the strong desorption energy destroyed the MNPs structure. Therefore, the desorption of target seleno-amino acids was processed by stirring. The effect of desorption time on the desorption efficiency of target seleno-amino acids was studied in the time range of 0.5-20 min. It could be seen from Fig. S3 that the Cu^{2+} charged MNPs illustrated fast desorption dynamics toward all target seleno-amino acids, and 2 min was enough to obtain the desorption equilibrium. Hence, desorption time of 2 min was selected in this work.

Under the optimal extraction and desorption conditions, with 1 mL 0.1 mol L^{-1} ethylenediamine as the desorption solution, the desorption of target seleno-amino acids was processed for two times continuously. It could be seen (Fig. S4) that only weak signal was found for $SeCys_2$ in the 2nd desorption solution, and the signal was less than 8% of that in 1st desorption. It indicated that 1 mL 0.1 mol L^{-1} ethylenediamine was enough for a complete desorption of target analytes and employed in subsequent experiment.

Effect of sample volume

The effect of sample volume ranging from 5 to 40 mL on the extraction efficiency of target seleno-amino acids (200 ng) was investigated. The results demonstrated that the sample volume has no obvious effect on the extraction efficiency of target seleno-amino acids in the whole investigated sample volume range. With respect to the limited biological sample volume, sample volume of 10 mL was employed.

3.2 Optimization of HF-LLLME conditions

Selection of the carrier reagent

Due to the high polarity and water solubility of seleno-amino acids, carrier mediated HF-LLLME was employed to obtain high extraction efficiency. And selection of an appropriate carrier plays a key role in the successful extraction of target seleno-amino acids. Since seleno-amino acids can be protonated or ionized by adjusting the sample pH to acidic or alkaline, four cationic carriers (OAS, sodium 1-octanesulfonate; STS, sodium p-toluenesulfonate; DBS, sodium lauryl benzenesulfate; SDS, sodium lauryl sulfate) and anionic carrier (trioctylmethyl ammonium chloride,

([MTOA]$^+$[Cl]$^-$) on the extraction efficiency of target seleno-amino acids was investigated and the results are illustrated in Fig. 3. It could be seen that the anionic carrier [MTOA]$^+$[Cl]$^-$ exhibited higher extraction efficiency than other four cationic carriers. Hence, [MTOA]$^+$[Cl]$^-$ was employed as carrier for the extraction of target seleno-amino acids in this work.

Effect of extraction solvent and [MTOA]$^+$[Cl]$^-$ percentage in 1-octanol

The type of extraction solvent in carrier mediated HF-LLLME should be immiscible with water, has excellent dissolving capability toward [MTOA]$^+$[Cl]$^-$ and high extraction efficiency for the carrier-seleno-amino acid complexes. Three kind of extraction solvent, including phenetole (ethoxybenzene), toluene and 1-octanol were tested for carrier mediated HF-LLLME, and the experimental results are shown in Fig. S5. It could be seen that 1-octanol exhibited the best extraction performance for all target seleno-amino acids, probably because the high polarity of 1-octanol and the formation of hydrogen bond between 1-octanol and seleno-amino acids. Therefore, 1-octanol was employed as extraction solvent for the subsequent experiments.

With 1-octanol as extraction solvent, the effect of [MTOA]$^+$[Cl]$^-$ percentage in 1-octanol on the extraction of target seleno-amino acids was studied. The experimental results in Fig.4 demonstrated that the signal intensity of all target seleno-amino acids was increased rapidly from 0 to 10%, and kept almost constant with further increase of [MTOA]$^+$[Cl]$^-$ percentage to 30%. High percentage of [MTOA]$^+$[Cl]$^-$ in 1-octanol may increase the viscosity of the membrane phase and reduce the mass transfer of the analytes from the donor to the acceptor phase, hence, 15% (v/v) [MTOA]$^+$[Cl]$^-$ in 1-octanol was selected as the extraction phase.

Effect of sample pH and ethylenediamine concentration

Sample pH is especially important for extraction of polar acidic, alkaline and amphiphilic compounds by LPME. The target seleno-amino acids could be ionized when sample pH was higher than their pKa_3 value (8.07-9.5), and thus be extracted by carrier mediated HF-LLLME. The effect of sample pH on the extraction efficiency of target seleno-amino acids was investigated in the pH range of 10.0-12.0 (Fig. S6). It was found that the signal intensity of all target seleno-amino acids was increased rapidly from pH 10.0 to 10.5, kept almost constant with further increase of pH to 11.5, and was slightly decreased when sample pH was higher than 11.5. Since the pH of MSPE desorption solvent (0.1 mol L^{-1} ethylenediamine) was about 11.5, the sample pH was fixed at 11.5 in the following experiments.

Besides, the effect of ethylenediamine concentration on the extraction of efficiency of target seleno-amino acids by HF-LLLME was studied. It was found that the ethylenediamine concentration had no obvious effect on the extraction of target seleno-amino acids in the concentration range of 0.01-0.2 mol L^{-1}, indicating that the desorption solution of MSPE can be directly employed for the subsequent HF-LLLME procedure.

Effect of $NaNO_3$ concentration

With $[MTOA]^+[Cl]^-$ as carrier, the main driving force for extraction of polar compounds by HF-LLLME was a gradient concentration of counter ions between the acceptor solution and the donor solution. In order to prevent the polyatomic interferences of $^{40}Ar^{37}Cl$ to ^{77}Se and $^{12}C^{35}Cl_2$, $^{81}Br^1H$ to ^{82}Se in ICP-MS analysis, $NaNO_3$ aqueous solution was employed as the acceptor solution in this work. The effect of $NaNO_3$ concentration in the range of 0-1.0 mol L^{-1} on the extraction efficiency of target seleno-amino acids was investigated, and the analytical results are illustrated in Fig. 5. It could be seen that the signal intensity of all target seleno-amino acids was increased rapidly with increase of $NaNO_3$ concentration from 0 to 0.3 mol L^{-1}, and kept almost constant with further increase of $NaNO_3$ concentration to 1.0 mol L^{-1}. Therefore, 0.6 mol L^{-1} $NaNO_3$ was employed in this work.

Effect of stirring rate

Stirring of the aqueous sample solution will enhance the mass transfer by decreasing the thickness of the Nernst diffusion film and thus reduce the extraction time. Hence, the effect of stirring speed on the extraction of target seleno-amino acids by HF-LLLME was studied, and the results (Fig. S7) indicated that the signal intensity of $SeCys_2$ was increased with the increase of stirring speed in the whole investigated range of 500-1200 rpm, while the signal intensity of other three seleno-amino acids was increased firstly and then kept almost constant when stirring speed was higher than 700 rpm. This may probably be related to the extraction mechanism and the structure/property of target seleno-amino acids. The extraction of target seleno-amino acids by HF-LLLME with $[MTOA]^+[Cl]^-$ as carrier was based on the ion exchange and the gradient counter ion concentration from the donor to the acceptor phase. Since $SeCys_2$ has two –COOH, the amount of $SeCys_2$ extracted into the acceptor phase was higher than other three seleno-amino acids under the same extraction time. Hence, faster stirring speed was necessary to ensure the fast mass transfer of $SeCys_2$ in the aqueous solution. Considering that too high stirring speed may create air bubbles,

organic solvent loss and thus poor precisions, the stirring rate was fixed at 1100 rpm for the subsequent experiment.

Effect of extraction time

By fixing the stirring speed at 1100 rpm, the effect of extraction time on the extraction efficiency of target seleno-amino acids was studied. As can be seen in Fig. S8, the signal intensity of all target seleno-amino acids was increased with increase of extraction time in the whole investigated range of 10-40 min. Trading off the extraction efficiency and sample preparation time, 30 min was selected as the extraction time for subsequent experiment.

The enrichment factors and relative standard deviations of HF-LLLME

Under the optimal extraction conditions of HF-LLLME, the enrichment factors and relative standard deviations of target seleno-amino acids was evaluated, and the analytical results were listed in Table 2. By using 1 mL sample solution, the EFs of target seleno-amino acids were 29, 22, 29 and 38-fold, respectively, with RSDs in the range of 4.7-7.2%.

3.3 Effect of coexisting ions

Under the optimum conditions, the interference of common coexisting ions on the extraction and determination of target seleno-amino acids (each at 1 μg L^{-1}) was studied. The tolerance limit was defined as the largest amount of coexisting ions with which the recovery of the target seleno-amino acids could be maintained in the range of 85-115%. It was found that 5000 μg mL^{-1} K^+、Na^+, 1000 μg mL^{-1} Ca^{2+}, Mg^{2+}, 7700 μg mL^{-1} Cl^-, 8000 μg mL^{-1} NO_3^-, or 4000 μg mL^{-1} SO_4^{2-} had no significant effect on the extraction and determination of target seleno-amino acids. With the introduction of MSPE to HF-LLLME for the extraction and separation of target seleno-amino acids, the anti-interference ability of the proposed method was greatly improved.

3.4 Analytical performance

The analytical performance of the proposed method was evaluated under the optimum conditions, and the results are listed in Table 2. The limits of detection (LODs, evaluated as the concentration corresponding to three times the standard deviation of 11 runs of the blank solution) were calculated to be 0.0075-0.013 ng mL^{-1} with the relative standard deviations (RSDs) ranging from 6.8 to 10.2% (c=0.2 ng mL^{-1}, n=7). The linear range of the proposed method covered 3 or 4 orders of magnitude with the correlation coefficient in the range of 0.9942-0.9979. The EFs (defined as the slope ratio of the calibrations with and without MSPE-HF-LLLME) for the target

seleno-amino acids varied from 152 to 278-fold.

A comparison of analytical performance achieved by MSPE-HF-LLLME-HPLC-ICP-MS with that achieved by several other approaches is summarized in Table 3. As can be seen, the developed method of MSPE-HF-LLLME-HPLC-ICP-MS for extraction of seleno-amino acids was the most sensitive, possessing the highest EFs and the lowest LODs. Compared with the reported HF-LPME[22] and SPME[13]-based methods, no derivatization was needed by the proposed method. Compared with the SBSE-based[5] and the MSPE-based method[14], the developed method exhibited high anti-interference ability. Compared with the dual-column online SPE-ICP-MS method for organic and inorganic selenium speciation[12], the developed method possessed high EFs and low LODs, and can be employed for multi-seleno-amino acids analysis.

3.5 Sample analysis

Certified reference material of SELM-1 yeast was applied to validate the accuracy of the proposed MSPE-HF-LLLME-HPLC-ICP-MS method. The analytical result for SeMet was 3388±72 μg SeMet g^{-1} by MSPE-HF-LLLME-HPLC-ICP-MS, which is in good agreement with the certified value (3389±173 μg SeMet g^{-1}). The total Se in SELM-1 determined by PN-ICP-MS was 1978±105 μg Se g^{-1}, which is also in good agreement with the certified value (2059±64 μg Se g^{-1}).

The proposed MSPE-HF-LLLME-HPLC-ICP-MS method was employed for speciation of seleno-amino acids in rice and Se-enriched yeast cell samples, and an external standard calibration curve was employed for the quantification. Hot water extraction was employed prior to MSPE-HF-LLLME-HPLC-ICP-MS, and the analytical results are summarized in Table 4. The chromatograms of seleno-amino acids in SELM-1, Se-enriched yeast cell and rice extract are shown in Fig. 6 and 7, respectively. It can be seen that $SeCys_2$ and MeSeCys was found in Se-enriched yeast cell, while none of seleno-amino acids were found in rice samples, probably because the concentration of target seleno-amino acid is lower than the LOD of the proposed method and/or the extraction of target species by hot water was not complete. It should be pointed out that the total Se concentrations in three kinds of rice are 0.13 ± 0.01, 0.078 ± 0.003 and 0.065 ± 0.008 μg g^{-1} fresh weight (Table 4), respectively, and for each seleno-amino acid, the concentration should be much lower than this level. To evaluate the accuracy of the developed method, spiking experiments at two levels were performed, and the recovery for the spiked samples is also listed in Table 4. As can be seen, the recovery ranged from 84.5 to 114% for the

spiked Se-enriched yeast cell and from 81.0 to 116% for the spiked rice samples.

4. Conclusions

A novel method of dual extraction technique MSPE-HF-LLLME combined with HPLC-ICP-MS for speciation of seleno-amino acids in rice and yeast cell samples has been developed. The desorption solution obtained from MSPE matches well with the subsequent HF-LLLME. And in combination of MSPE and HF-LLLME, the developed method exhibited high enrichment factors for target species and good anti-interference ability to real complicated matrix, demonstrating a good application potential for speciation of seleno-amino acids in real food samples.

Acknowledgements

The authors would like to thank the National Science Foundation of China (No. 21175102, 21375097) and Science Fund for Creative Research Groups of NSFC (No. 20621502, 20921062) for their financial supports. This work is also supported by "the Fundamental Research Funds for the Central Universities (114009)".

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Table 1 Operation conditions for HPLC-ICP-MS

HPLC	
Stationary phase	CAPCELL PAK C_{18}(250 mm × 4.6 mm, 5 μm particle size)
Mobile phase	25 mmol L^{-1} ammonium formate-formic acid, pH 3.0, methanol/water (3/97, v/v)
Flow rate	1.0 mL min^{-1}
Column temperature	55 ^{o}C
Injection volume	9.0 μL
ICP-MS	
Rf power	1150 W
Rf matching	1.5 V
Sampling depth	6.8 mm
Carrier gas	1.1 L min^{-1}
Time-Resolved data acquisition	
Scanning mode	Peak-hopping
Dwell time	100 ms
Integration mode	Peak area
Detected isotope	^{77}Se, ^{82}Se

Table 2 Analytical performance data obtained by MSPE- HF-LLLME-HPLC-ICP-MS

Analytes	MSPE-HF-LLLME-HPLC-ICP-MS						HF-LLLME-HPLC-ICP-MS	
	Linear range (ng mL^{-1})	Linear equation	Correlation coefficient (r^2)	LODs (ng mL^{-1})	EFs[a]	RSDs[b] (n=7)	EFs[c]	RSDs[d] (n=7)
$SeCys_2$	0.05-10	Y=39077.3x+1738.2	0.9963	0.013	278	10.2%	29	7.2
MeSeCys	0.05-10	Y=14264.9x+8256.2	0.9979	0.011	152	9.7%	22	6.2
SeMet	0.025-10	Y=23368.8x+2601.2	0.9946	0.0085	180	6.8%	29	6.6
SeEt	0.025-10	Y=31410.7x+8065.4	0.9942	0.0075	235	9.0%	38	4.7

[a]: EFs calculated by the slope ratio of the calibration curve obtained after and before ILs-carrier mediated HF-LLLME

[b]: c = 0.2 ng mL^{-1}, n = 7

[c]: EFs defined as the slope ratio of the calibrations with and without HF-LLLME;

[d]: C_{SeCys2} = 0.5 ng mL^{-1}, $C_{MeSeCys,\ SeMet,\ SeEt}$ = 2.0 ng mL^{-1}

Table 3 Comparison of LODs and EFs for seleno-amino acids found in literatures involving different analytical approaches

Methods	LODs (μg L^{-1})				EFs				Ref.
	$SeCys_2$	MeSeCys	SeMet	SeEt	$SeCys_2$	MeSeCys	SeMet	SeEt	
HF-LPME-GC-ICP-MS	-	0.023	0.015	0.011	-	-	-	-	22
SBSE-HPLC-ICP-MS	0.050	0.061	0.065	0.074	48.4	27.5	30.2	24.1	5
SPME-GC-ICP-MS	0.029	-	0.016	0.014	-	-	-	-	13
FI-SPE-ICP-MS	0.045	-	0.21	-	5	-	1	-	12
MSPE-HPLC-ICP-MS	0.025	0.035	0.090	0.056	34	26	10	17	14
MSPE-HF-LLLME-HPLC-ICP-MS	0.013	0.011	0.0085	0.0075	278	152	180	235	This work

Table 4 The analytical results of seleno-amino acids speciation in Se-enriched yeast cell and rice samples

Samples	Added (μg g^{-1})	$SeCys_2$		MeSeCys		SeMet		SeEt		Total Se (μg/g)
		Found	Recovery	Found	Recovery	Found	Recovery	Found	Recovery	
Se-enriched yeast cell	0	76.7±8	-	43.4±2	-	n.d.[a]	-	n.d.[a]	-	2167±100
	25	100±12	93.2%	69.6±7	105%	24.6±2	98.3%	28.4±1	114%	-
	100	168±10	91.4%	128±10	84.5%	101±13	101%	109±10	109%	-
Rice 1#	0	n.d.[a]	-	n.d.[a]	-	n.d.[a]	-	n.d.[a]	-	0.13±0.01
	0.08	0.074±0.002	92.8%	0.21±0.04	105%	0.071±0.01	89.2%	0.069±0.006	86.6%	-
	0.40	0.33±0.03	81.4%	0.46±0.04	116%	0.43±0.07	109%	0.44±0.03	111%	-
Rice 2#	0	n.d.[a]	-	n.d.[a]	-	n.d.[a]	-	n.d.[a]	-	0.078±0.003
	0.08	0.073±0.009	91.8%	0.084±0.007	104%	0.081±0.002	102%	0.080±0.001	101%	-
	0.40	0.35±0.04	86.6%	0.38±0.02	95.1%	0.39±0.06	96.8%	0.37±0.04	92.5%	-
Rice 3#	0	n.d.[a]	-	n.d.[a]	-	n.d.[a]	-	n.d.[a]	-	0.065±0.008
	0.08	0.067±0.004	83.9%	0.078±0.005	97.5%	0.075±0.006	93.8%	0.083±0.003	104%	-
	0.40	0.32±0.02	81.0%	0.36±0.03	89.4%	0.41±0.01	101%	0.38±0.02	95.4%	-

[a]: not found

Figure captions:

Fig. 1 Effect of sample pH on the adsorption efficiency of seleno-amino acids by MSPE (Conditions: $C_{SeCys2,\ MeSeCys,\ SeMet,\ SeEt}$=25 ng mL^{-1}; sample volume, 10 mL; extraction time, 10 min)

Fig. 2 Effect of different solvent type on the desorption of target seleno-amino acids (Conditions: $C_{SeCys2,\ MeSeCys,\ SeMet,\ SeEt}$=25 ng mL^{-1}; sample volume, 10 mL; sample pH, 7.0; extraction time, 10 min; desorption time, 10 min; desorption volume, 1 mL. A, 0.01 mol L^{-1} NaOH; B, 0.1 mol L^{-1} imidazole (pH=10.0); C, pH=11 0.1 mol L^{-1} imidazole; D, pH=12 0.1 mol L^{-1} imidazole; E, 0.5 mol L^{-1} HNO_3; F, 0.1 mol L^{-1}ethylenediamine (pH=11.5); G, pH=12 0.1 mol L^{-1} ethylenediamine)

Fig. 3 Effect of type of carrier on the extraction of target seleno-amino acids (Conditions: $C_{SeCys2,\ MeSeCys,\ SeMet,\ SeEt}$=10 ng mL^{-1}; carrier concentration, 1 mmol L^{-1} OAS, STS, DBS and SDS, 20% (v/v) $[MTOA]^+[Cl]^-$; with OAS, STS, DBS and SDS as carrier, sample pH was 2.5, extraction solvent was 1-octanol, acceptor solution was 0.4 mol L^{-1} HNO_3; with $[MTOA]^+[Cl]^-$ as carrier, sample pH was 11.0, extraction solvent was toluene, acceptor solution was 0.3 mol L^{-1} $NaNO_3$; stirring rate, 1000 rpm; extraction time, 20 min)

Fig. 4 Effect of $[MTOA]^+[Cl]^-$ percentage in 1-octanol on the extraction of target seleno-amino acids (Conditions: C_{SeCys2}=2.5 ng mL^{-1}; $C_{MeSeCys,\ SeMet,\ SeEt}$=10 ng mL^{-1}; extraction solvent, 1-octanol; acceptor phase, 0.6 mol L^{-1} $NaNO_3$; stirring speed, 1100 rpm; extraction time, 30 min)

Fig. 5 Effect of $NaNO_3$ concentration on the extraction of target seleno-amino acids (Conditions: C_{SeCys2}=2.5 ng mL^{-1}; $C_{MeSeCys,\ SeMet,\ SeEt}$=10 ng mL^{-1}; extraction phase: 20% (v/v) $[MTOA]^+[Cl]^-$ in toluene; stirring rate, 1000 rpm; extraction time, 30 min)

Fig. 6 Chromatograms of seleno-amino acids in Se-enriched yeast cell obtained by MSPE-HF-LLLME-HPLC-ICP-MS. (a) extraction blank; (b) SELM-1 extract; (c) yeast extract obtained by direct HPLC-ICP-MS; (d) yeast extract obtained by MSPE-HF-LLLME-HPLC-ICP-MS; (e) yeast extract spiked with 25 μg g^{-1} target seleno-amino acids obtained by MSPE-HF-LLLME-HPLC-ICP-MS; Peaks of 1 to 4 resprent $SeCys_2$, MeSeCys, SeMet and SeEt, respectively

Fig. 7 Chromatograms of seleno-amino acids in rice obtained by MSPE-HF-LLLME-HPLC-ICP-MS. (a) Extraction blank; (b) rice 1 extract; (c) rice 2 extract; (d) rice 3 extract; (e) rice 1 spiked with 0.08 μg g^{-1} target seleno-amino acids

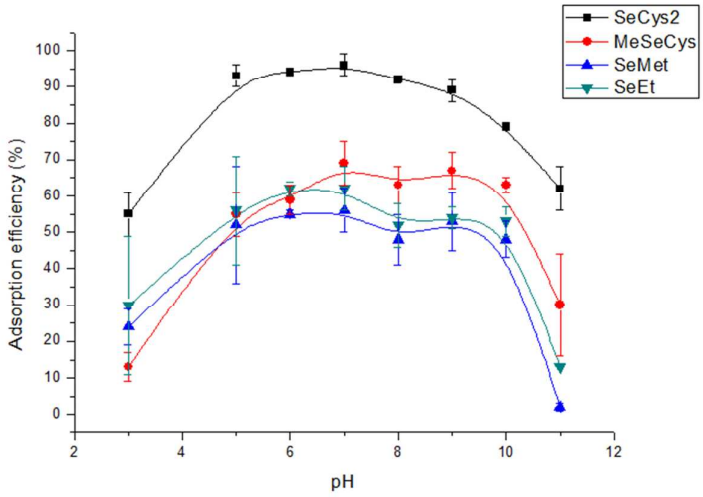


Fig. 1

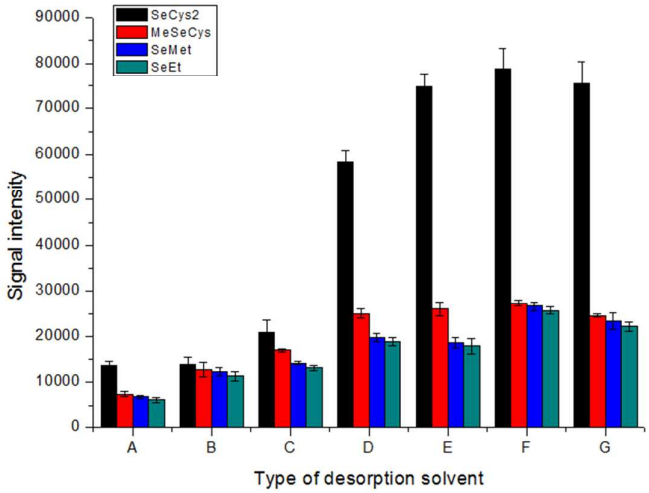


Fig. 2

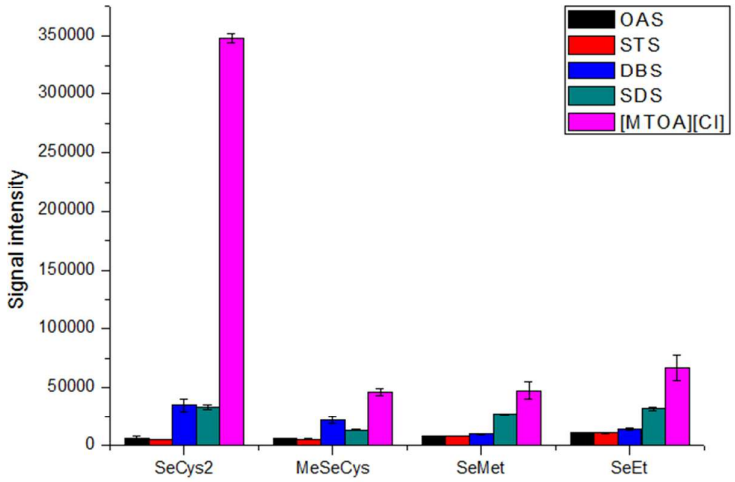


Fig. 3

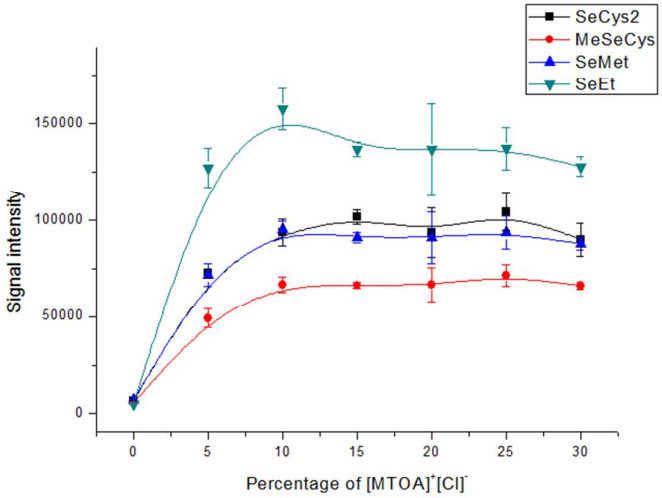


Fig. 4

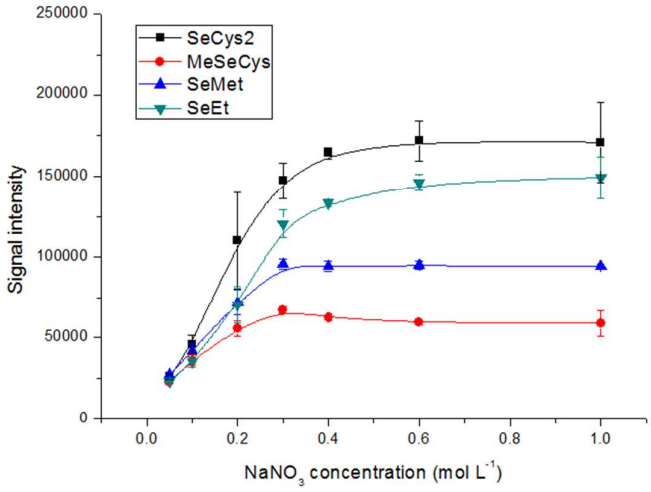


Fig. 5

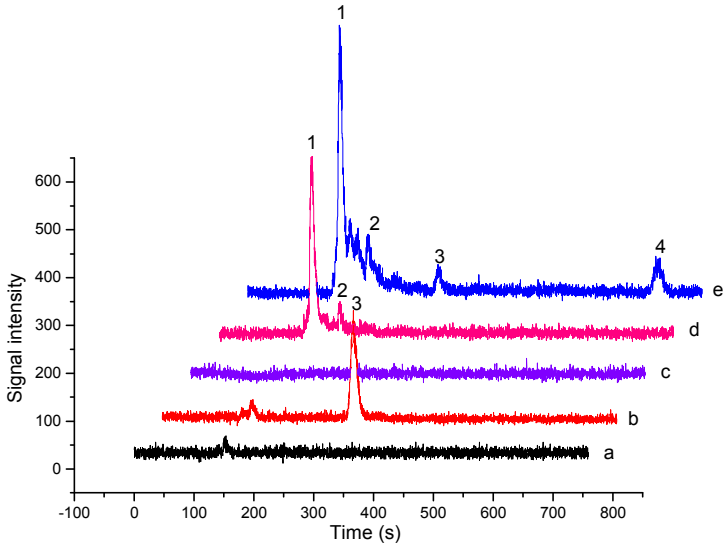


Fig. 6

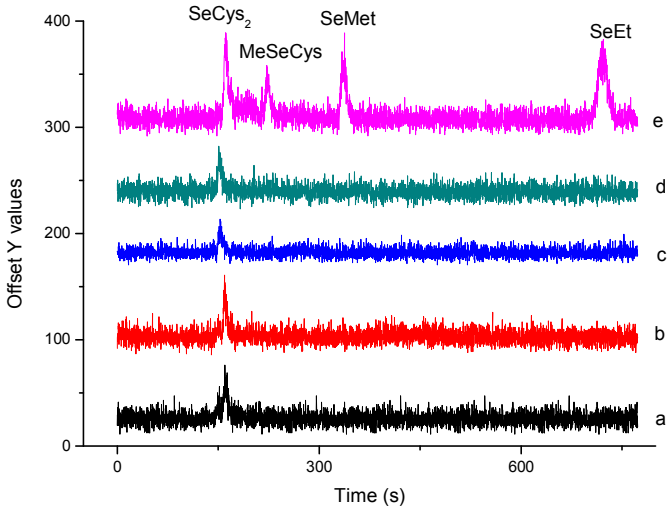
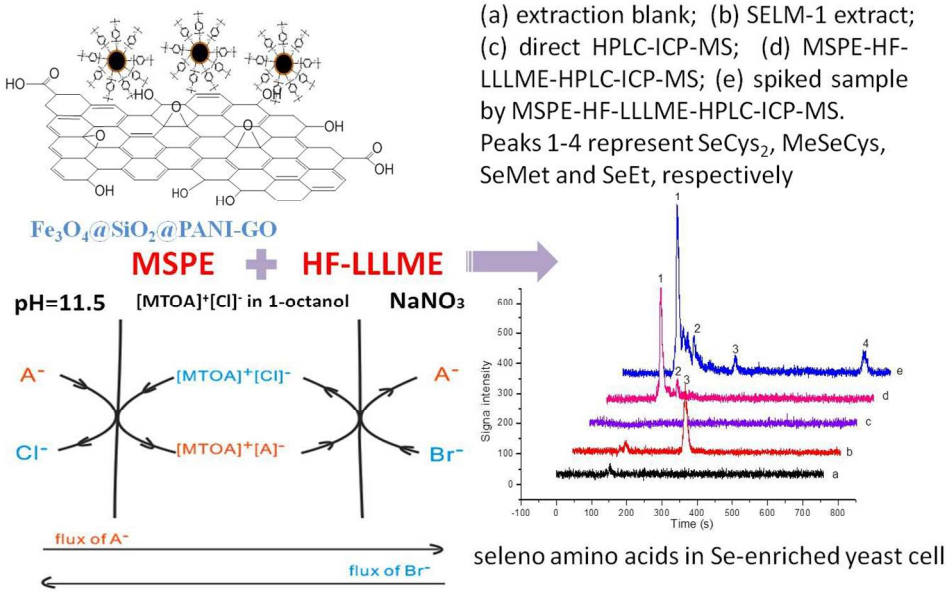


Fig. 7



MSPE combined with HF-LLLME for speciation of seleno amino acids with high EFs and good anti-interference ability
239x151mm (150 x 150 DPI)