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Selenium Species Determination in Se-Enriched Grain Crops with Foliar Spray of Sodium Selenite by IP-RP-HPLC-UV-HG-AFS

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Abstract

A simple, rapid, and accurate method for determination of selenocystine ($SeCys_2$), methyl selenocysteine (MeSeCys), selenourea (SeUr), selenite (Se(IV)), selenotmethionine (SeMet), and selenate (Se(VI)) in grain crops was developed, based on the coupling of ion-pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC) and ultraviolet irradiation hydride generation atomic fluorescence spectrometry (UV-HG-AFS). The conditions of IP-RP-HPLC-UV-HG -AFS were studied. The conditions of the pretreatment sample, such as types and dosage of proteinases, were investigated. The detection limits (LODs) for six Se species were between 0.77 and 1.77 $\mu g \cdot L^{-1}$. Then, the proposed method has been successfully used to analyze selenium (Se) species in Se-enriched rice paddy (*Oryza sativa L.*), soybean (*Glycine max (Linn.) Merr.*), and sweet potato (*Ipomoea batatas (L.) Lam.*) with foliar application of sodium selenite solution. SeMet was the major Se spices in Se-enriched grain crops, ranged from 77–90% of the total Se contents. The capabilities of Se enrichment were relatively larger differences in three-grain corps as follows: soybean > rice paddy > sweet potato.

Keywords Se species · Ion-pair HPLC · UV-HG-AFS · Foliar spray · Se-enriched grain crops

Introduction

Selenium (Se) plays an important role in enzyme glutathione peroxidase, such as endocrine modulation, cancer prevention, inhibition of the toxicity of heavy metals, and the protection of nerves, blood vessel, liver, skeletal, and cardiac muscles (Gladyshev and Hatfield 1999; Ju et al. 2017; Gopalakrishna et al. 2018). Se deficiency in the human body is associated with Kashan, Kashin-Beck, hypothyroidism, Crohn diseases, and so on (Dai et al. 2017; Lima Barros et al. 2020; Pirola et al. 2020), whereas high dietary intake of Se can also have toxic effects (Lenz and Lens 2009; Hadrup and Ravn-Haren 2020). The Se level range between deficiency and toxicity is narrow (Tinggi 2003). The recommended safe intake and the upper safe intake levels of Se are 50–200 and 400 ug day^{-1} for adults, respectively (WHO 1996).

The main source of Se intake for human is diet (Combs 2001; Natasha et al. 2018). Se species in food may be inorganic Se (selenite (Se(IV)) and selenate (Se(VI)) and/or organic Se (e.g., selenocystine ($SeCys_2$), methyl selenocysteine (MeSeCys), and selenotmethionine (SeMet)). The nutritional value of Se in food depends not only on the total concentration but even more importantly on the species (Thiry et al. 2012). It is generally accepted that long-term intake of inorganic Se is harmful to the human body, while organic Se is beneficial. Therefore, it is necessary and important to analyze Se species in Se-enriched food.

The analytical method of determination of inorganic Se and organic Se in various samples by different instruments have been reported, such as in water samples by spectrometric techniques (e.g., ETAAS) (Panhwar et al. 2017; Acikkapi et al. 2019), in yeast by GC-MS (Yang et al. 2004), in garlic by LC-MS (Dumont et al.

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2006), in soybean by HPLC-MS-MS (Tie et al. 2015), in radix puerariae by HPLC-ICP-MS (Cao et al. 2016), and in strawberry by HPLC-AFS (Sánchez-Rodas et al. 2016). The reversed-phase (RP) and anion-exchange (AE) chromatography were widely used for Se species separation in the HPLC system. For Se species separation with RP-HPLC system, ion-pairing agent, such as tetramethylammonium hydroxide (Oliveira et al. 2016), trifluoroacetic acid (Thosaikham et al. 2014), pentafluoropropionic (Jitaru et al. 2010), and heptafluorobutyric acid (Shah et al. 2004) were often adopted. There was almost no report on using tetrabutylammonium bromide (TBAB) as an ion-pairing agent for Se species separation. Simultaneous determination of $SeCys_2$, MeSeCys, SeUr, Se(IV), SeMet, and Se(VI) in plants was barely reported.

Rice is one of the world major grain crops, and more than 60% Chinese take it as their staple diet. Soybean and sweet potato are also the main grain crops in China and are widely planted. The sown areas of rice, soybean, and sweet potato in China in 2019 were 29.7, 11.1, and 7.1 million hectares respectively, and the yields were 209.6, 21.3, and 28.8 million tons respectively (NBSC 2020). However, approximately 51% of China's agricultural land area is Se deficiency, and 39%–61% of Chinese have very low daily Se intakes (Dinh et al. 2018). Fortunately, plants can be accumulated Se fertilizer by using the exogenous Se. In the last decades, many researchers studied Se-enriched plant foods, such as rice (Dai et al. 2019; Yin et al. 2019), maize (Wang et al. 2013), wheat (Eiche et al. 2015), peanut (Gao et al. 2019), potato (Zhang et al. 2019), and pumpkin (Polona et al. 2006). However, less reports were about bean crops and tuber crops. As far as we know, Tie et al. (2015) studied Se-enriched soybean with root application of sodium selenite. It was almost no studies on the capacities of Se enrichment between different grain crops, not to mention the distribution characteristics of Se species on the three-grain crops. SeUr was generally supposed to be a synthetic product and is less attention in the past. But, Zhang et al. (2020) found that SeUr existed in Se-enriched pig muscles. It was aroused our interests that whether Se-enriched grain crops contained SeUr. Thus, a method was developed to analyze as much Se species as possible (especial for SeUr) in plants in our study.

The aim of this study was firstly built a simple, rapid, and accurate method for the determination of six Se species contents by using TBAB as an ion-pair agent with the RP-IP-HPLC-UV-HG-AFS system, and then, the proposed method was used to analyze accumulation characteristics of Se species by foliar spray of sodium selenite solution on leaves in three Se-enriched grain crops.

Materials and Methods

Instrumentation

Se species contents in grain crop samples were determined by RP-RP-HPLC-UV-HG-AFS (SA-20, Jitian Corporation, China). Reverse phase C18 column (SunFire C18, Waters, USA) and anion-exchange column (PRP X-100, Hamilton, USA) with corresponding mobile phase were both studied (Table 1). The aliquots of 100 uL were injected using an autosampler. The contents of total Se in all samples were carried out by HG-AFS (AFS-9320, Jitian Corporation, China).

A vacuum freeze drier (pilot5-8E, Biocool Corporation, China) was used for the freeze-drying of samples. An ultrasonic cell crusher (HN-150Y, Hanuo Corporation, China) and microwave digestion system (Xpress 6, CEM Corporation, USA) were used for extraction of Se species. A high-speed refrigerated centrifuge (CR21N, Hitachi Corporation, Japan) was adopted for centrifuging sample solution. A pH meter (S2, Meter Corporation, Switzerland) was used for pH measurements. A hot plate (EG37C, Labtech Corporation, China) was adopted for the digestion of total Se.

Standard Substances and Chemicals

The liquid standard substances of Se(IV), Se(VI), MeSeCys, and SeMet were from the National Institute of Metrology of China. The solid standard substances, $SeCys_2$ and SeUr, were obtained from Toronto Research Chemicals of Canada and Alfa Aesar of the USA, respectively. $SeCys_2$ was dissolved in 0.1 $mol{\cdot}L^{-1}$ hydrochloric acid, and standard substances of other Se species were dissolved in water. Solid standard substances were stored at – 20 °C. Standard solutions were stored at 0 °C~4 °C. The standard working solutions were diluted with water immediately before use. A certified reference material, selenium-enriched yeast (SLEM-1, National Research Council of Canada) was used for the accuracy check of Se species determination. Another certified reference material, rice flour (GBW(E) 080684a, Academy of State Administration of Grain of China), was used for the accuracy check of total Se determination.

The chemicals diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, ACS grade), formic acid (HPLC grade), methanol (MeOH, HPLC grade), potassium iodide (KI, GR grade), potassium borohydride (KBH_4, AR grade), hydrochloric acid (HCl, GR grade), potassium hydroxide (KOH, GR grade), nitric acid (GR grade), perchloric acid (GR grade), potassium ferricyanide (AR grade), tetrabutylammonium bromide (TBAB, AR grade), pepsin (≥ 2500 units/mg, from the porcine stomach), and proteinase K (≥ 30 units/mg) were purchased from Aladdin of China. Lipase (30,000 U/g, from porcine pancreas, J&K corporation, China) and trypsin (2500

Table 1 HPLC-UV-HG-AFS operating conditions for Se species

IP-RP-HPLC conditions for separation of $SeCys_2$, MeSeCys, SeUr, Se(IV), SeMet, and Se(VI)	
Column	Waters SunFire C18 (250 × 4.6 mm id, 5 μm)
Column temperature	35 °C
Mobile phase mode	25 mmol·L^{-1} $(NH_4)_2HPO_4$, 0.5 mmol·L^{-1} TBAB, and 5% (v/v) MeOH at pH6.0
Flow rate	0.6 mL·min^{-1} (Isocratic)
AE-HPLC conditions for separation of $SeCys_2$, MeSeCys, Se(IV), SeMet, and Se(VI)	
Column	Hamilton PRP X-100 (250 × 4.1 mm id, 10 μm)
Column temperature	35 °C
Mobile phase mode	40 mmol·L^{-1} $(NH_4)_2HPO_4$ at pH6.0
Flow rate	1.0 mL·min^{-1} (isocratic)
HG-AFS conditions	
Reducing agent 1	0.5% (w/v) KI in 0.35% (w/v) KOH
Reducing agent 2	2% (w/v) KBH_4 in 0.35% (w/v) KOH
Carrying current	10% (v/v) HCl
Voltage and current of Se lamp	310 V and 110 mA
Flow efficiencies of carrier and shielding gas	300 mL·min^{-1} and 800 mL·min^{-1}

units/mg, from porcine pancreas, J&K corporation, China) and proteinase XIV (≥ 3.5 units/mg, from *Streptomyces griseus*, Sigma-Aldrich, Germany) were used. High-purity water (resistivity > 18 MΩ cm) from a Milli-Q water system (Millipore, American) was used to prepare aqueous solution and for ware washing.

Samples Production and Preparation

A field experiment was conducted for the enrichment of Se in rice, soybean, and sweet potato in Nanchang, the capital of Jiangxi Province, China. The rice, soybean, and sweet potato were selected as local conventional varieties. Each treatment of the Se-enriched grain crop area was 50 m^2 and sets control treatments. The sowing time for rice, soybean, and sweet potato was in late May, late May, and early May in 2019, respectively. The time of foliar spraying sodium selenite solution for rice, soybean, and sweet potato was at the full heading stage (late August), podding stages (early August), and vigorous growing stage of steam and leaf (middle August), respectively. The spraying concentration of sodium selenite solution was 40 mg·L^{-1}. The harvest times of rice, soybean, and sweet potato were in early October, early November, and late October in 2019, respectively. Se-enriched samples and control samples were collected at harvest times. Three replicate samples were collected from the same treated grain crops, and the weight of each sample was about 1 kg.

Rice and soybean samples were hulled; sweet potato samples with skin were rinsed with ultrapure water and cut into thin slices. All samples were freeze-dried. Each sample with about 500 g was crushed and stored at − 20 °C until it was weighed.

Determination of Se Species Contents in Samples

About 0.25 g of the sample was weighed into the tube, 5 mL of water was added, and put into an ultrasonic cell crusher by ultrasonic for 3 min. Proteinase K (20 mg) was added into the solution and covered caps and shaked up. The solution was transferred into the microwave system and extracted by microwave radiation at 55 °C for 30 min. When the extracting program ended, the solution was quantitatively transferred to a centrifuge tube and centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to microstep (3KDa, Millipore) and centrifuged at 5000 rpm for 15 min. The purified solution filtered through a 0.22-μm nylon filter and stored at 0 °C~4 °C until analysis. The blank test and recovery test were both done at the same time. The Se species contents of the extracting solution were determined by HPLC-UV-HG-AFS.

Determination of Total Se Contents in Samples

According to the Chinese national standard method (GB 5009.93-2017), about 1.0 g of the sample was weighed into a conical flask; 15 mL of nitric acid and 0.5 mL of perchloric acid were added and covered with a bent neck hopper and put on an electric hot plate for heating at 150 °C for 2 h and then heated at 250 °C until the white smoke went out, and added 5 mL 50% (v/v) hydrochloric acid heating at 100 °C for 5 min. After cooling, the digestion solution was rinsed with water to 25 mL of the volumetric flask, and 2.5 mL 10% (w/v) potassium ferricyanide was added into and diluted with water to scale. The blank test and recovery test were both carried out at the same time. The total Se content of the digestion solution was determined by HG-AFS.

Results and Discussion

Optimization of HPLC Conditions

Due to different ionic characteristics of Se species, their separation by HPLC may not be easy. In order to separate six Se species successfully, IP-RP-HPLC and AE-HPLC system were studied and compared in this study.

For the IP-RP-HPLC system, six Se species were separated well by using a RP C18 column and phosphate buffer solution with TBAB as mobile phase (Table 1). If the mobile phase only contained phosphate buffer solution, some Se species such as MeSeCys, SeUr, and Se(IV) cannot be separated well even through reducing the flow rate or changing the pH of the mobile phase. These Se species were successfully separated by adding TBAB as an ion-pairing agent. An ODS-3 column (250 × 4.6 mm id, 5 μm, GL Science, Japan) was also carried out to repeat the process. Similar results were obtained, which indicating the good reproducibility of the IP-RP-HPLC system.

For the AE-HPLC system, only five Se species ($SeCys_2$, MeSeCys, Se(IV), SeMet, and Se(VI)) were determined by using an anion-exchange column (Hamilton PRP X-100) and phosphate buffer solution as mobile phase. Compared with the IP-RP-HPLC system, the sensitivities of organic Se (especial for SeMet) were poorer, and the retention time of Se(VI) was significantly longer (Fig. 1). Obviously, the most Se species in the IP-RP-HPLC system were cuter and showed a better resolution than that in the AE-HPLC system (Fig. 1). Strangely, the peak of SeUr could not be found in the AE-HPLC system, while SeUr could be determined by using an anion-exchange column (Dionex AS 11) with potassium hydroxide as mobile phase (Ochsenkühn-Petropoulou et al. 2003) and a ZORBAX SB-Aq column with citric acid as mobile phase (Zhang et al. 2020). We believed that the mobile phase and the column jointly suppressed SeUr from converting to a hydride-forming species, and the mobile phase of phosphate buffer solution is the maximum likelihood. At last, the IP-RP-HPLC system was adopted for the determination of six Se species contents in Se-enriched grain crop samples.

In order to obtain the best conditions of separations and sensitivities for Se species, TBAB concentrations, phosphate buffer concentrations, pH, and the amount of methanol were detailed investigated. As shown in Fig. 2, TBAB concentrations showed a significant effect on the retention time of Se(VI), while had a minor effect on retention times of Se(IV) and organic Se species. Ultimately, the concentration of TBAB was set at 0.5 $mmol{\cdot}L^{-1}$. While phosphate buffer concentrations increase, the retention time of Se(VI) is shortened significantly and the signal of Se species increased markedly. However, retention times of organic Se species have a little change by adjusting phosphate buffer concentrations. Too high phosphate buffer concentrations would lead to the peak overlapping of Se species (e.g., MeSeCys, SeUr, and Se(IV)). Therefore, 25 $mmol{\cdot}L^{-1}$ phosphate buffer concentration was used (Fig. 3). The influence of pH of the mobile phase on the retention times of Se species was similar to that of phosphate buffer concentrations. Good resolutions for separation of Se species were obtained at a pH of 6 (Fig. 4). It was found that adding the amount of methanol could improve sensitivities of Se species and decrease significantly the retention time of Se(VI). The retention times of organic Se species were almost unaffected by the methanol content (Fig. 5). Although adding the amount of methanol will increase the sensitivity of Se species, it may have a negative impact on hydride generation. So, 5% (v/v) methanol was selected.

Besides, the column temperature and the flow rate of the mobile phase were also researched. Column temperatures were performed at 30 °C, 35 °C, 40 °C, and 45 °C. The resolutions of Se species improved little with rising the column temperature. At last, the column temperature was heated at 35 °C. Since an extra equilibration time is often required, a gradient program for the separation of the Se species was tried

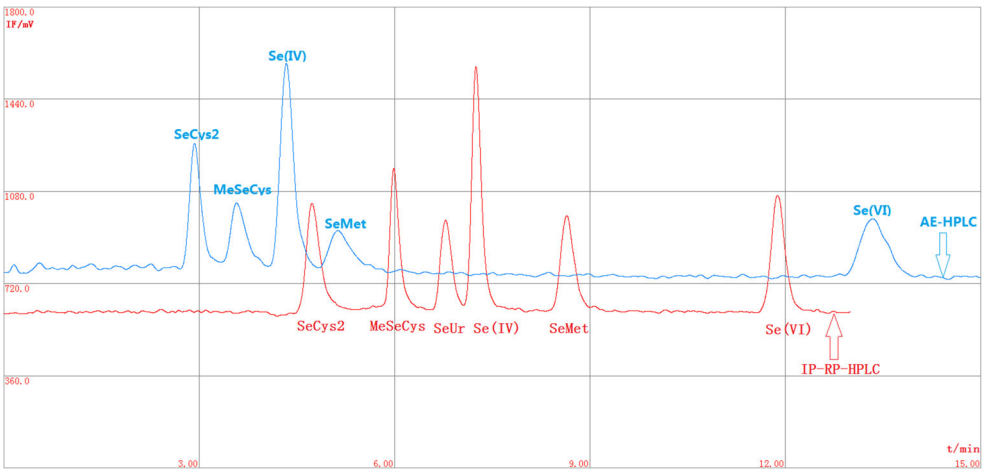

Fig. 1 Chromatogram of Se species on IP-RP-HPLC system and AE-HPLC system for the standard solutions

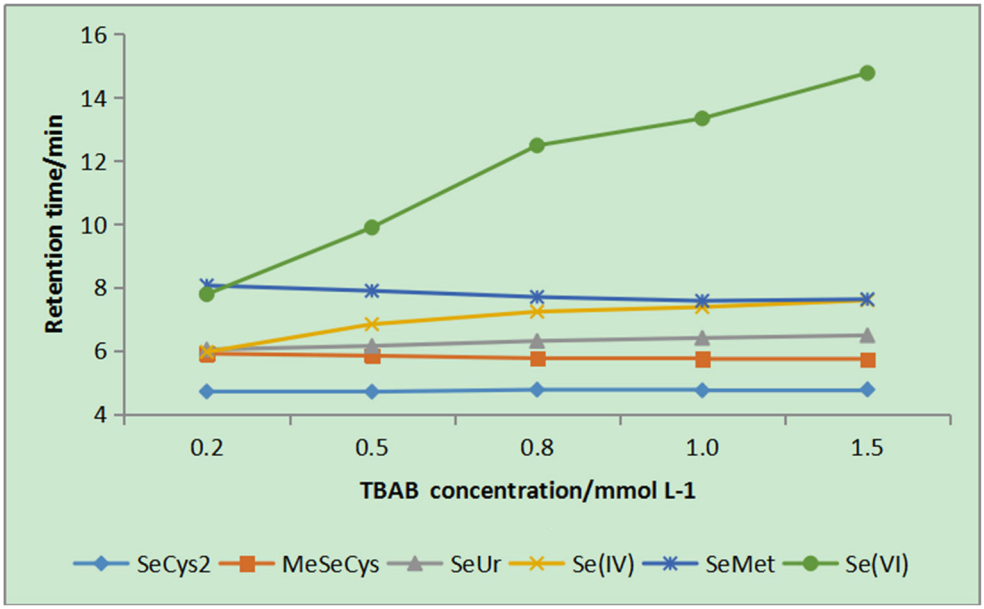

Fig. 2 Influence of TBAB concentrations on retention times of Se species (error bars mean standard deviation, as follows)

to avoid. The low flow rate of the mobile phase improved the resolutions, while retention times of Se species were increased and accompanied by the sensitivity decreasing. On the other hand, peaks of Se species (MeSeCys, SeUr, and Se(IV)) could be overlapped with the high flow rate of the mobile phase. Finally, the flow rate of the mobile phase was set at 0.6 mL·min^{-1}.

In conclusion, a reversed-phased C18 column and the mobile phase consisted of a 25 mmol·L^{-1} phosphate buffer solution and 5 mmol·L^{-1} TBAB and 5% (v/v) methanol (pH 6.0) with IP-RP-HPLC system was adopted for determining six Se species (Fig. 1). The column temperature was set at 35 °C, and the flow rate of the mobile phase was 0.6 mL·min^{-1}. Figure 1 also showed the chromatogram of five Se species ($SeCys_2$, SeMeCys, Se(IV), SeMet, and Se(VI)) with AE-HPLC system by using an anion-exchange column with 40 mmol·L^{-1} phosphate buffer and 5% (v/v) methanol (pH 6.0). It was obviously visible that the IP-RP-HPLC system had more advantages than that of the AE-HPLC system for Se species determination.

Optimization of UV-HG-AFS Conditions

Online ultraviolet (UV) irradiation was applied to transform organic Se species into Se(VI) in this study. When the UV lamp was turned off, the only peak (Se(IV)) can be found;

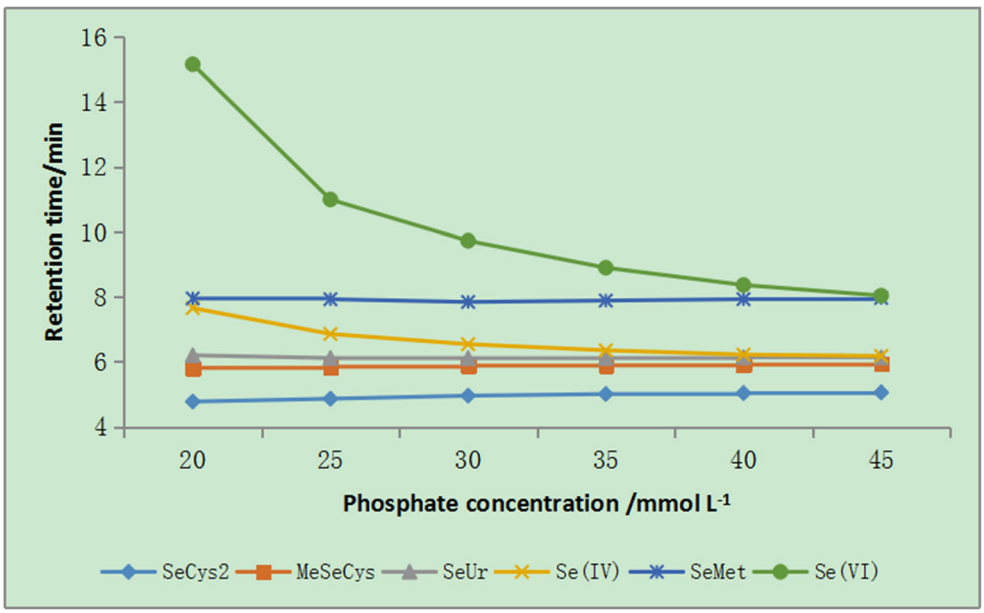

Fig. 3 Influence of phosphate concentrations on retention times of Se species

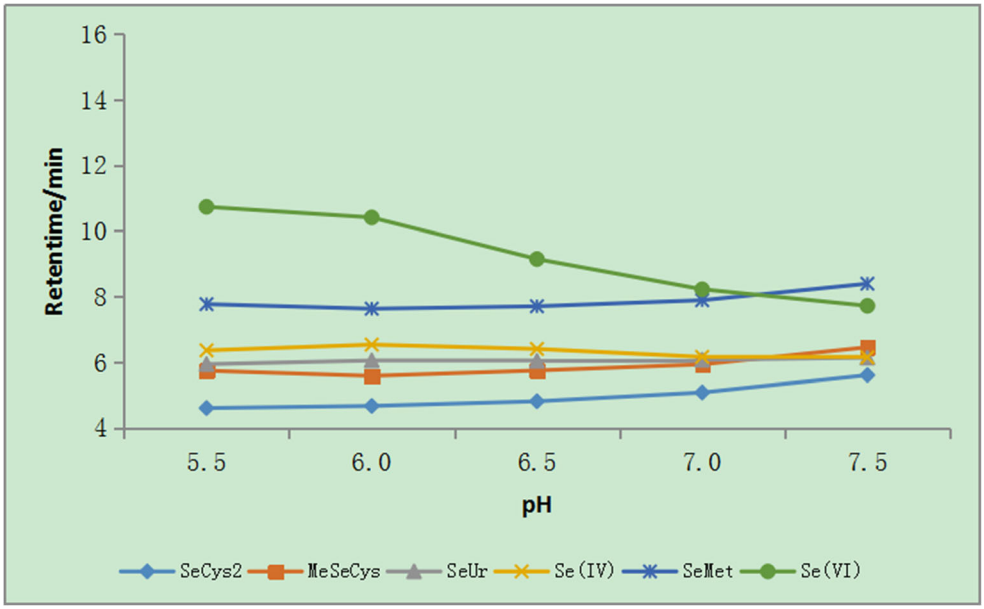

Fig. 4 Influence of pH on retention times of Se species

other peaks of Se species were almost invisible. The UV lamp was therefore kept on throughout the analysis process. In order to improve the reduction efficiencies of Se species, KI concentrations were optimized. The results showed that the peak areas of organic Se species and Se(VI) were lower than Se(IV). Even though the online UV irradiation and reductant of KI were applied, the organic Se species and Se(VI) were still partially transformed into the hydride generation.

Owing to the significant influences on the signal intensities of Se species in HG-AFS, concentrations of KBH_4 and HCl were studied. As shown in Fig. 6, the concentrations of KBH_4 had a strong influence on the peak areas for Se species. The signal of Se species increased dramatically with increasing of KBH_4 concentration and accompanied by the signal-to-noise ratio increasing. Appropriate sensitivities of Se species were obtained with 2.0% (w/v) KBH_4 (Fig. 6). When the concentration of KI was set at 0.5% (w/v), most Se species (especially Se(VI)) reached the maximum of peak areas (Fig. 7). The concentrations of HCl were ranged between from 5 to 25% (v/v). The signal of most Se species reached the maximum values at 10% (v/v) concentration of HCl (Fig. 8). The concentrations of KBH_4 had the most obvious impact on the signal intensities of Se species in HG-AFS, followed by KI concentrations, and finally HCl concentrations.

The influences of flow rates of carrier gas and shielding gas, voltage, and current of hollow cathode lamp of Se on the

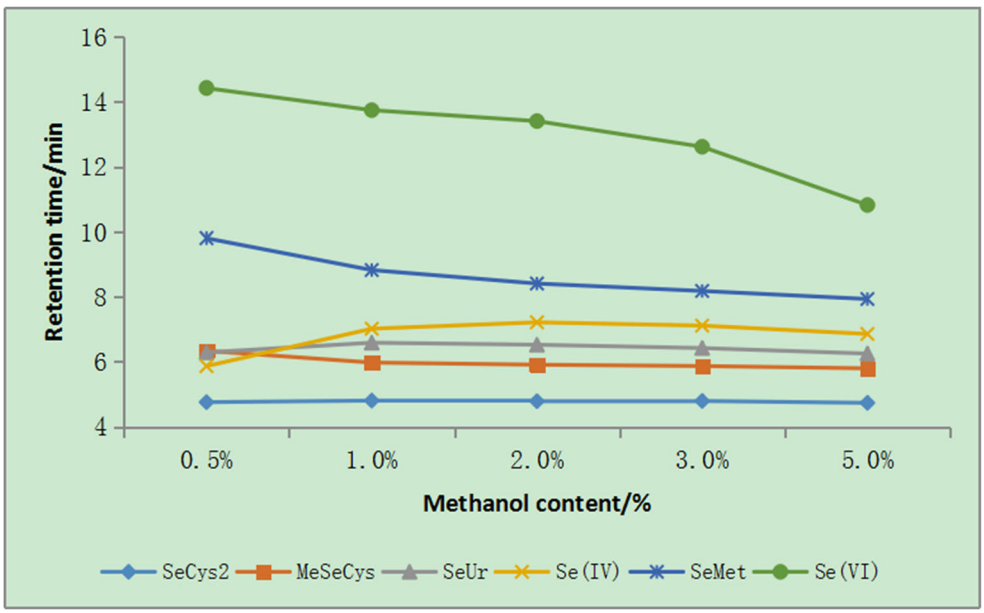

Fig. 5 Influences of methanol contents on retention times of Se species

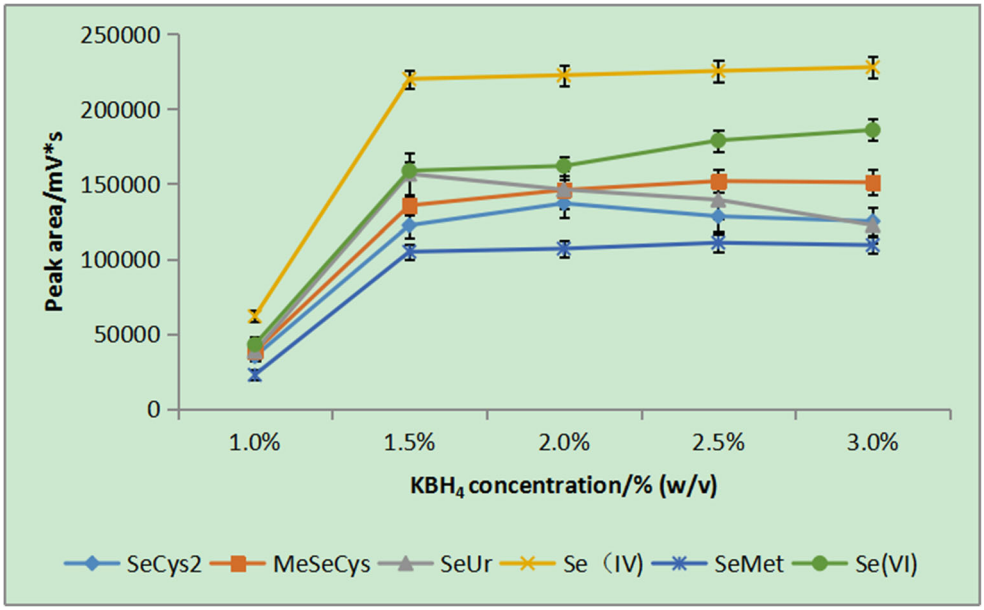

Fig. 6 Influence of KBH_4 concentrations on peak areas of Se species

signals of Se(IV) were also optimized. When the corresponding values were at 300 mL·min^{-1}, 800 mL·min^{-1}, 310 V, and 110 mA, respectively, the optimal signal-to-noise ratio was got. The working conditions, such as voltage and current of hollow cathode lamp of Se, concentrations of KBH_4, and HCl, were also suitable for total Se determination.

Optimization of Sample Pretreatment

Owing to the low content of Se and low extraction efficiency from the sample matrix, the determination of Se species contents in food samples may remain a challenge. Organic Se, such as selenoamino acids and selenoproteins, has covalent bonds with proteins and peptides. It is difficult to be extracted by water, acids (e.g., HCl), and alkali solutions (e.g., KOH). Enzymatic hydrolysis was often applied to extract elementary species in food samples (Reyes et al. 2009; Xiao et al. 2017). The extraction efficiency mainly depends on the nature of the sample, the extraction mode, and condition.

Microwaves would fragment cells and organic Se species would be released efficiently from cells and their components. A small number of previous pieces of literature reported using the microwave-assisted technique for extraction of elementary species, such as for As species (Saucedo-Velez et al. 2017),

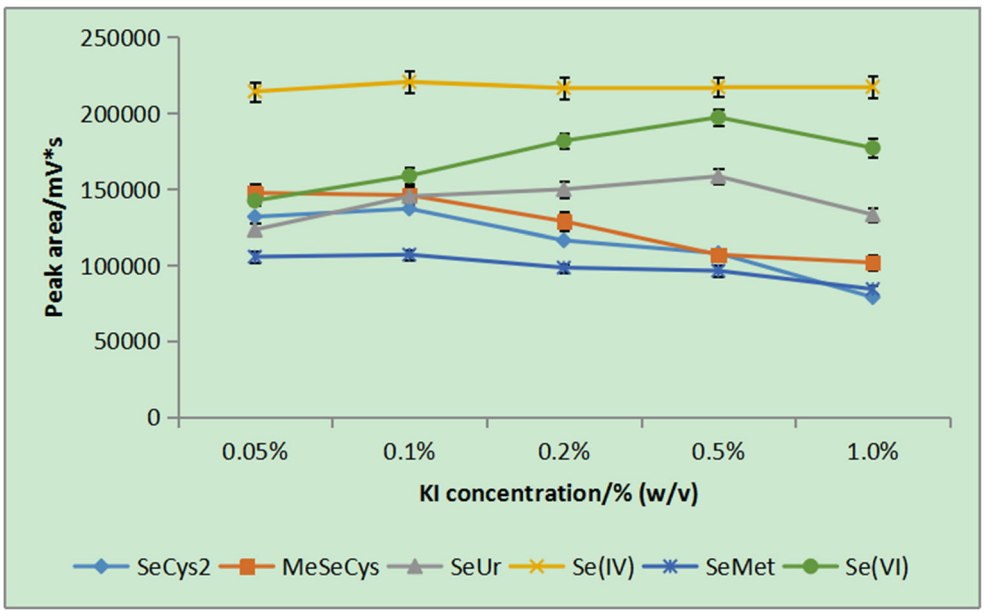

Fig. 7 Influence of KI concentrations on peak areas of Se species

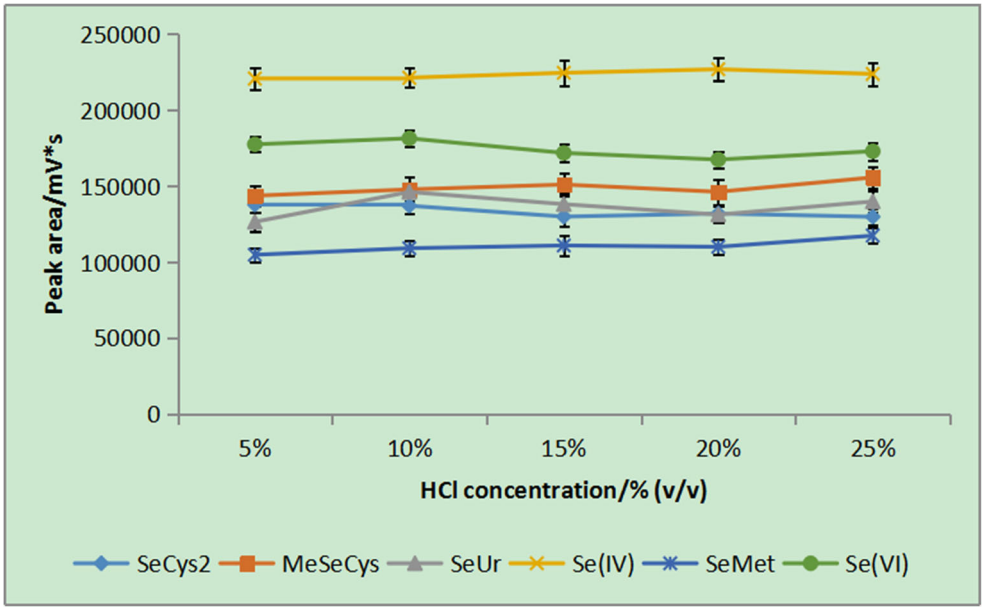

Fig. 8 Influence of HCl concentrations on peak areas of Se species

for Se species (Moreda-Piñeiro et al. 2018), and for Sb species (Quiroz et al. 2016). In this study, the types and dosage of proteinases with microwave-assisted extraction technology were detailed studied.

The stability of Se species was firstly to be investigated at first. A microwave-assisted extraction procedure for all Se species standard solutions was carried out. By the comparison of original standard solutions of Se species, microwave-assisted enzymolysis does not provide any apparent Se species degradation.

In view of the protein and fat content of soybean is higher than rice and sweet potato, the matrix effect of soybean may be serious. So, the extraction efficiencies of Se species were mainly researched on soybean samples. Different types and dosages of proteinases were investigated to achieve high extraction efficiencies. Eight enzyme treatments, such as a single enzyme (K, XIV, lipase, pepsin, trypsin) and mixed enzyme (K + XIV, pepsin + trypsin, trypsin + K + XIV, lipase + XIV), were researched. The amount of each enzyme was 20 mg. As shown in Fig. 9, the enzyme treatments of K, K + XIV, and trypsin + K + XIV had much higher extraction efficiencies that ranged from 90~97%, followed by the enzyme treatments of XIV and lipase + XIV; the extraction efficiencies were both about 70%. The enzyme treatments of pepsin, trypsin, and pepsin + trypsin were bad; the extraction efficiencies were lower than 40%. In view of high extraction efficiency and simplicity, only proteinase K was used for extraction of Se species in Se-enriched grain crop samples in the end. Because the cost of proteinase K was relatively expensive. The cost of the reagent also needed to take into consideration. The result showed that 20 mg proteinase K was a suitable dosage for enzymolysis a sample (Fig. 10). To sum up, the optimal pretreatment conditions of samples were 0.25-g sample with 5 mL water was broke cell wall of grain crops with ultrasonic for 3 min, and then, 20 mg proteinase K was added and extracted by microwave irradiation for 30 min at 55 °C.

Method Validation

Method Accuracy

Recovery experiments were used to confirm the proposed method's accuracy and precision. The low total Se content of each grain crop sample was individually spiked with Se species solution (80 $\mu g \cdot kg^{-1}$) before digestion. Table 2 showed the accuracy and precision of the method for Se species analysis in three-grain crop samples. The recoveries of Se species were ranged from 82 to 97%, and the relative standard deviations (RSDs) were below 10% for all cases (n = 6). The reproducibilities were calculated ranged from 6.0–15.0% (n = 6) after three batch analysis performed during non-consecutive days.

A certified reference material (SELM-1) and extraction efficiencies of Se species in three-grain crop samples were also used to validate the accuracy of the method. The extraction efficiency was equal to the sum of all Se species contents of the sample divided by the total Se content. The measured value of SeMet in the SELM-1 was 3128 ± 97.7 $mg \cdot kg^{-1}$, and the certificate value of SeMet was 3190 ± 148 $mg \cdot kg^{-1}$.

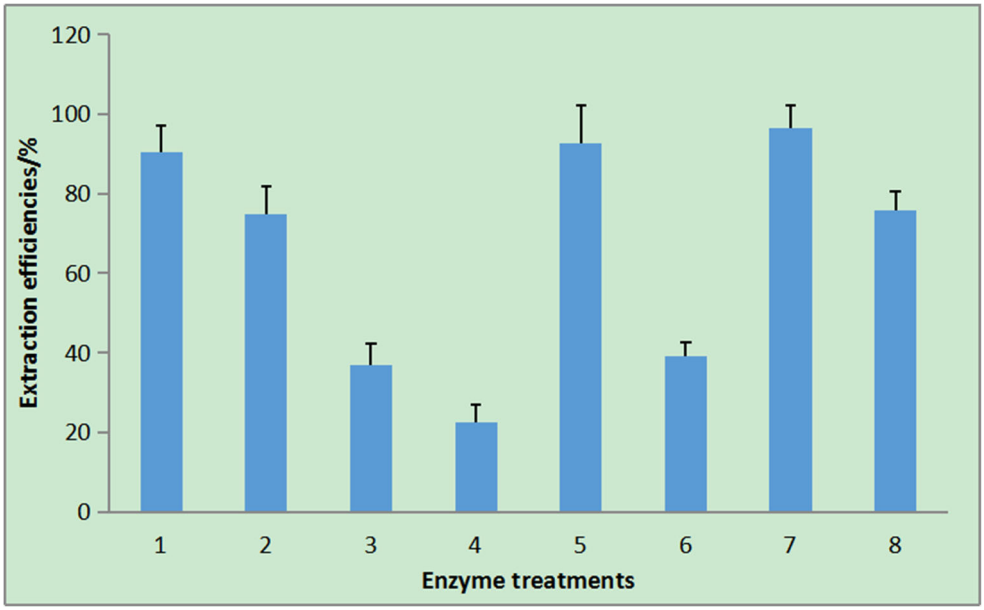

Fig. 9 Extraction efficiencies of different enzyme treatments (1-K, 2-XIV, 3-pepsin, 4-trypsin, 5-K + XIV, 6-pepsin + trypsin, 7-trypsin + K + XIV, 8-lipase + XIV) on Se-enriched soybean samples

There was no significant difference between measured values and certificate values (Sig < 0.05). As shown in Fig. 11, SeMet is the main Se species in SELM-1, but it also existed small amount of other Se species, such as $SeCys_2$, MeSeCys, Se(IV), Se(VI), and some unknown Se species. The mean extraction efficiency of Se species in three-grain crop samples was almost 90%.

Recovery experiments and a certified reference material, rice flour (GBW10045), were also carried out to validate the accuracy of total Se determination. The recoveries of total Se in samples were ranged from 91 to 95%, and the RSDs (intra-day) were below 6%. The measured values of total Se in the rice flour (GBW(E) 080684a) were 0.078 ± 0.05 $mg{\cdot}kg^{-1}$, and the certificate value was 0.081 ± 0.011$mg{\cdot}kg^{-1}$.

Analytical Parameters

Calibration curves were made by plotting concentrations against peak areas for each Se species. Good linear regression with values (R) between 0.998 and 0.999 was obtained. A low added concentration (40 $\mu g{\cdot}kg^{-1}$) of six Se species was determined by the proposed method at 11 times, the detection

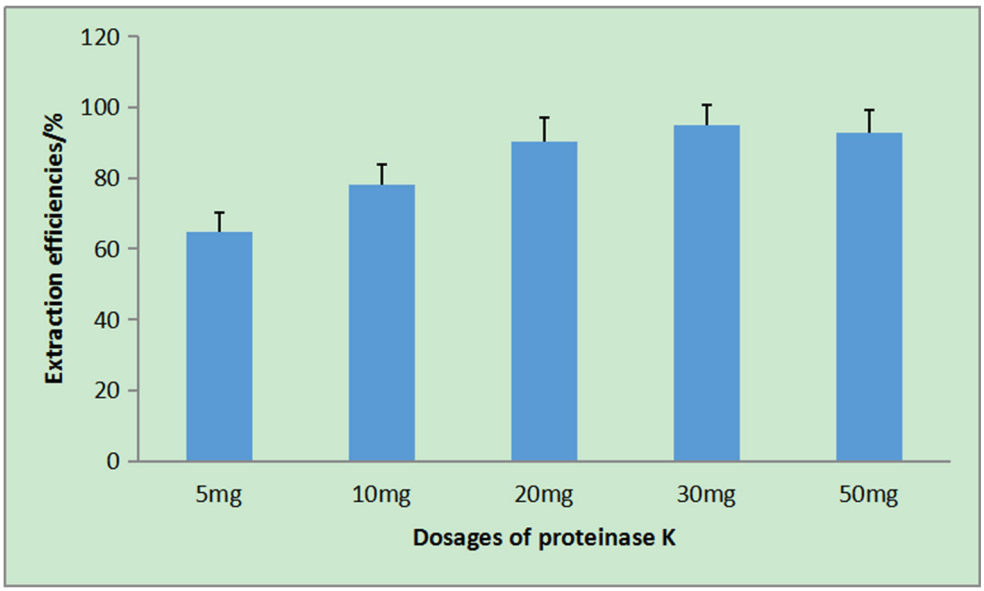

Fig. 10 Extraction efficiencies of different dosages of proteinase K on Se-enriched soybean samples

Table 2 Accuracy and precision of the method for determining Se species

Se species	Spiked level/ μg·kg^{-1}	Rice		Soybean		Sweet potato	
		Recovery/ %	RSD/ %	Recovery/ %	RSD/ %	Recovery/ %	RSD/ %
SeCys2	80	87	7.9	82	6.5	84	6.8
MeSeCys	80	95	3.1	97	4.5	92	2.6
SeUr	80	86	5.3	84	4.0	88	3.1
SeMet	80	93	4.2	94	2.8	96	3.7
Se(VI)	80	90	4.6	86	4.9	93	2.1
Total Se	80	95	3.4	92	2.7	91	5.6

limits (LODs) were calculated as three times as the standard deviation/slop, and the quantitation limits (LOQs) were calculated as ten times as the standard deviation/slop. The LODs of $SeCys_2$, MeSeCys, SeUr, Se(IV), SeMet, and Se(VI) were 1.1, 1.0, 1.7, 0.77, 1.3, and 1.2 μg·L^{-1}, respectively. The LOQs for six Se species were between 2.5 and 5.6 μg·L^{-1}. The method of detection limits (MDLs) was equal to LODs*solution volume (5.0 mL)/sample weight (0.25 g). The MDLs of $SeCys_2$, MeSeCys, SeUr, Se(IV), SeMet, and Se(VI) were 22, 20, 34, 15.4, 26, and 24 μg·kg^{-1}, respectively.

The LODs were compared with the previous pieces of literature (Table 3). It showed that the LODs of the proposed method were near the previous references, except that Zhang et al. (2020). However, the proposed method was slightly more convenient. The relatively sensitivies of Se(IV) by the AFS system were lower than that by the ETAAS system.

Determination of Se Species Contents in Se-Enriched Gain Crop Samples

The total Se content of Se-enriched rice, soybean, and sweet potato samples was 0.53, 0.72, and 0.18 mg·kg^{-1}, respectively. The total Se contents of non-Se-enriched rice, soybean, and sweet potato control samples were 0.051, 0.063, and 0.021 mg·kg^{-1}, respectively. Obviously, the accumulating ability of Se in soybean and rice was greater than that in sweet potato.

As shown in Table 4 and in Fig. 12, SeMet was found in all Se-enriched grain crop samples, and $SeCys_2$ was found in Se-enriched rice. The major Se species in Se-enriched grain crop samples by foliar spraying with sodium selenite solution was SeMet, which represented the total Se contents 77%–90%. No inorganic Se species was found in all Se-enriched grain crop samples. It was indicated that application of selenium fertilizer

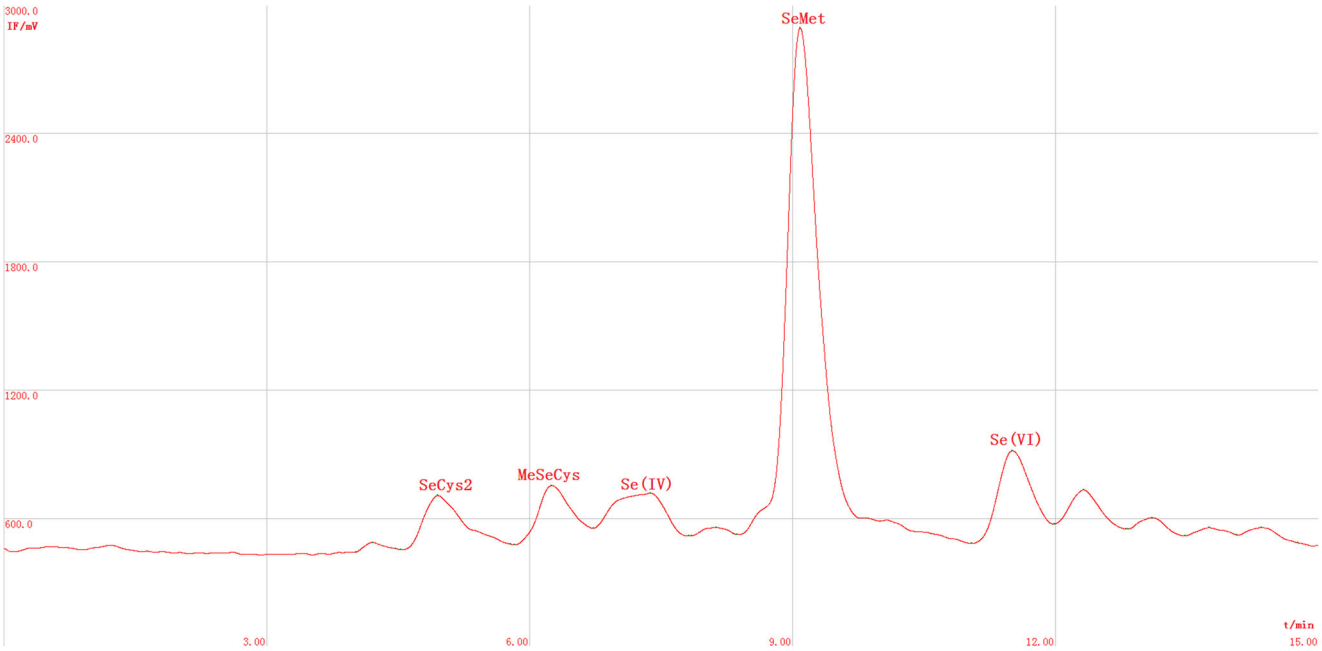

Fig. 11 Chromatogram of Se species in SELM-1

Table 3 Limits of detection (LODs) of Se species by the proposed method, compared to the previous reported

Main instrument	LODs/μg·L^{-1}						Ref.
	$SeCys_2$	MeSeCys	SeUr	Se(IV)	SeMet	Se(VI)	
RP-HPLC-UV-HG-AFS	1.1	1.0	1.7	0.77	1.3	1.2	This study
AE-HPLC-ICP-MS	1.4	2.5	-	6.0	3.2	1.6	Moreda-Piñeiro et al. (2018)
AE-HPLC-ICP-MS	0.01	0.009	0.01	-	0.009	-	Zhang et al. (2020)
RP-AF-HPLC-UV-HG-AFS	0.35	-	-	0.40	0.54	1.7	Viñas et al. (2005)
HPLC-TR-HG-AFS	0.40	2.8	-	1.0	4.6	3.3	Sanchez-Rodas et al. (2013)
HPLC-ESI-MS-MS	0.92	-	-	-	0.95	-	Gong et al. (2012)
ETAAS	-	-	-	0.15	-	-	Eağda and Tüzen (2017)

-, not reported

to grain crops leaf, inorganic Se can be absorbed and transformed into organic Se.

Similar conclusions were found in plants. For example, SeMet was detected to be the main Se species in heart of palm and golden berries, and no inorganic Se was found (Moreda-Piñeiro et al. 2018); organic Se species (SeMet and MeSeCys) contents in Se-enriched kale seedlings were almost 80% (Maneetong et al. 2013); $SeCys_2$ and SeMet in Brazil nut were the primary Se species (Silva et al. 2013). But, there were several different conclusions. Tie et al. (2015) found that MeSeCys represented 66.4% of the total Se content in the soluble protein of Se-enriched soybean which was obtained by root application of sodium selenite with manure. In our study, Se-enriched soybean was produced by foliar application of sodium selenite, and only SeMet was detected. The reason for the difference may be applicated selenium fertilizer to different parts of soybean. Mazej et al. (2006) found that Se(VI) (64% of total Se) was the main Se species in chicory leaves that were cultivated in an aeroponic system. It was indicated that the different application method of selenium fertilizer into different plants, the types of Se species in plants may be different.

Conclusion

Six Se species, such as $SeCys_2$, MeSeCys, SeUr, Se(IV), SeMet, and Se(VI), were successfully determined by using the IP-RP-HPLC-UV-HG-AFS system. And, a quick and easy pretreatment method was developed to extract Se species from Se-enriched grain crop samples based on enzymatic hydrolysis with microwave assisted. The proposed method exhibited rapid, good analytical characteristics, wide linearity, and low detection limits. Most importantly, the experiment cost, including instrument cost and operation cost, and regent cost were low. Obviously, it was a potential approach for the determination of Se species contents in grain crop.

In this study, Se-enriched rice, soybean, and sweet potato were produced by foliar application with sodium selenite solution. But, the capability of Se enrichment was relatively differences in three-grain crops. The highest enrichment capacity of Se was soybean, secondly rice, and the lowest was sweet potato. The three-grain crops can be absorbed and transformed exogenous Se(IV) into organic Se (mainly as SeMet). There was no disputing the fact that Se-enriched grain crops, especially soybean and rice could be produced in this way.

Table 4 Se species contents and total Se contents in Se-enriched crop samples (n = 3)

Samples	Se species/mg·kg^{-1}						Total Se/mg·kg^{-1}
	$SeCys_2$	MeSeCys	SeUr	Se(IV)	SeMet	Se(VI)	
Rice	0.051 ± 0.013	n.d.	n.d.	n.d.	0.41 ± 0.017	n.d.	0.53 ± 0.033
Soybean	n.d.	n.d.	n.d.	n.d.	0.65 ± 0.032	n.d.	0.72 ± 0.028
Sweet potato	n.d.	n.d.	n.d.	n.d.	0.16 ± 0.014	n.d.	0.18 ± 0.014

n.d., not detected. Results expressed as mean ± standard deviation

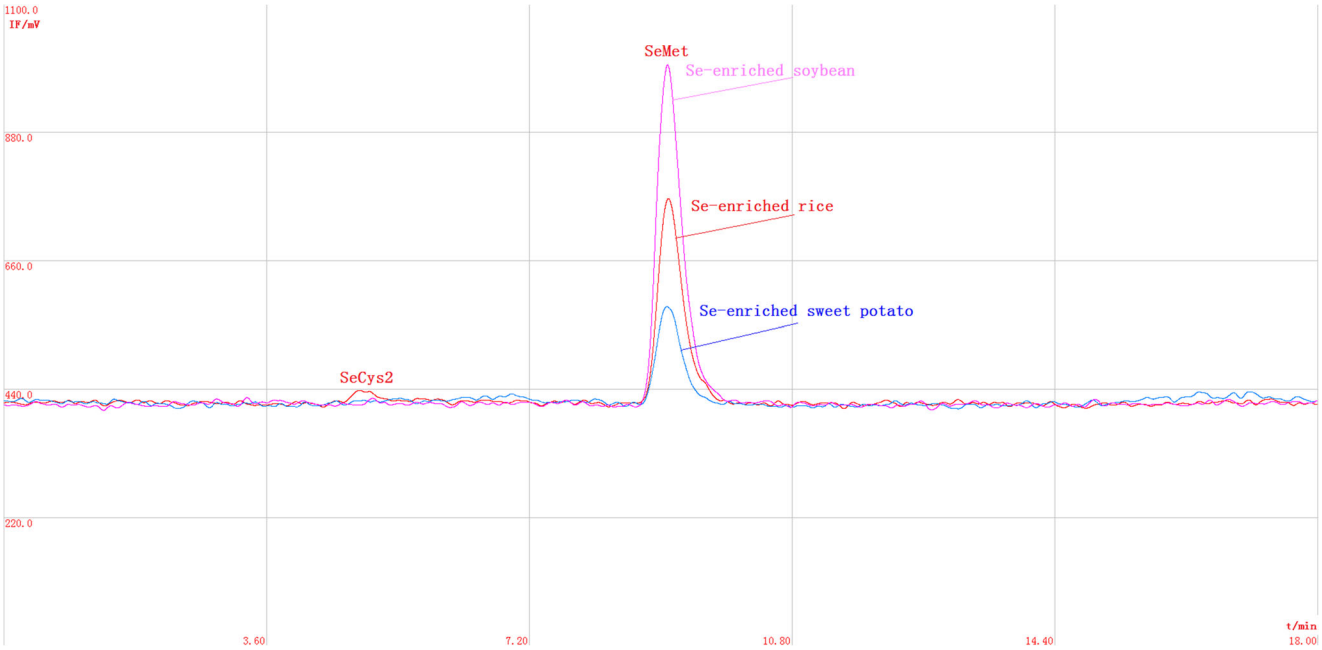

Fig. 12 Chromatogram of Se species in three-grain crops

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Declarations

Ethics Approval and Consent to Participate This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

Conflict of Interest Yihua Wei declares no conflict of interest. Jinyan Zhang declares no conflict of interest. Shuyan Qiu declares no conflict of interest. Qingqing Huang declares no conflict of interest. Linfeng Yuan declares no conflict of interest. Liumeng Chen declares no conflict of interest. Tingcan Dai declares no conflict of interest. Tianhua Tu declares no conflict of interest. Biaojin Zhang declares no conflict of interest. Han Yan declares no conflict of interest. Weihong Li declares no conflict of interest.

References

Acikkapi AN, Tuzen M, Hazer B (2019) A newly synthesized graft co-polymer for magnetic solid phase microextraction of total selenium and its electrothermal atomic absorption spectrometric determination in food and water samples. Food Chem 284:1–7. https://doi.org/10.1016/j.foodchem.2019.01.091

Cao Y, Yan L, Huang H, Deng B (2016) Selenium speciation in radix puerariae using ultrasonic assisted extraction combined with reversed phase high performance liquid chromato- graphy-inductively coupled plasma-mass spectrometry after magnetic solid-phase extraction with 5-sulfosalicylic acid functionalized magnetic nanoparticles. Spectrochim Acta B 122:172–177. https://doi.org/10.1016/j.sab.2016.07.003

Combs GF (2001) Se in global food systems. Br J Nutr 85:517–547. https://doi.org/10.1079/BJN200028

Dai X, Song R, Xiong Y (2017) The expression of ERK and JNK in patients with an endemic osteochondropathy, Kashin-Beck disease. Exp Cell Res 359:337–341. https://doi.org/10.1016/j.yexcr.2017.08.015

Dai Z, Imtiaz M, Rizwan M, Huang H, Tu S (2019) Dynamics of Selenium uptake, speciation, and antioxidant response in rice at different panicle initiation stages. Sci Total Environ 691:827–834. https://doi.org/10.1016/j.scitotenv.2019.07.186

Dinh QT, Cui Z, Huang J, Tran TAT, Wang D, Yang W, Zhou F, Wang M, Yu D, Liang D (2018) Selenium distribution in the Chinese environment and its relationship with human health: a review. Environ Int 112:294–309. https://doi.org/10.1016/j.envint.2017.12.035

Dumont E, Ogra Y, Vanhaecke F, Suzuki KT, Cornelis R (2006) Liquid chromatography-mass spectrometry (LC-MS): a powerful combination for selenium speciation in garlic (Allium sativum). Anal Bioanal Chem 384:1196–1206. https://doi.org/10.1007/s00216-005-0272-6

Eağda E, Tüzen M (2017) A simple and sensitive vortex-assisted ionic liquid-dispersive microextraction and spectrophotometric determination of selenium in food samples. Food Chem 232:98–104. https://doi.org/10.1016/j.foodchem.2017.03.104

Eiche E, Bardelli F, Nothstein AK, Charlet L, Göttlicher J, Steininger R, Dhillon KS, Sadana US (2015) Selenium distribution and speciation in plant parts of wheat (*Triticum aestivum*) and Indian mustard (*Brassica juncea*) from a seleniferous area of Punjab, India. Sci Total Environ 505:952–961. https://doi.org/10.1016/j.scitotenv.2014.10.080

Gao Q, Liu Z, Wu J, Geng Y, Zhang Q, Tie M, Gu X, Tanokur M, Xue Y (2019) Foliar application is an effective method for incorporating selenium into peanut leaf proteins with antioxidant activities. Food Res Int 126:108617. https://doi.org/10.1016/j.foodres.2019.108617

Gladyshev VN, Hatfield DL (1999) Selenocysteine-containing proteins in mammals. J Biomed Sci 6:151–160. https://doi.org/10.1159/000025383

Gong L, Xu Q, Lee C, Zhang H (2012) Selenium speciation analysis of Misgurnus anguillicaudatus selenoprotein by HPLC-ICP-MS and HPLC-ESI-MS/MS. Eur Food Res Technol 235:169–176. https://doi.org/10.1007/s00217-012-1747-2

Gopalakrishna R, Gundimeda U, Zhou S, Zhou S, Bui H (2018) Redox regulation of protein kinase C by selenometabolites and selenoprotein thioredoxin reductase limits cancer prevention by selenium. Free Radic Biol Med 127:55–61. https://doi.org/10.1016/j.freeradbiomed.2018.05.062

Hadrup N, Ravn-Haren G (2020) Acute human toxicity and mortality after selenium ingestion: a review. J Trace Elem Med Biol 58:126435. https://doi.org/10.1016/j.jtemb.2019.126435

Jitaru P, Goenaga-Infante H, Vaslin-Reimann S, Fisicaro P (2010) A systematic approach to the accurate quantification of selenium in serum selenoalbumin by HPLC-ICP-MS. Anal Chim Acta 657:100–107. https://doi.org/10.1016/j.aca.2009.10.037

Ju W, Li X, Li Z, Wu GR, Fu XF, Yang XM, Zhang XQ, Gao XB (2017) The effect of selenium supplementation on coronary heart disease: a systematic review and meta-analysis of randomized controlled trials. J Trace Elem Med Biol 44:8–16. https://doi.org/10.1016/j.jtemb.2017.04.009

Lenz M, Lens PNL (2009) The essential toxin: the changing perception of Se in environmental sciences. Sci Total Environ 407:3620–3633. https://doi.org/10.1016/j.scitotenv.2008.07.056

Lima Barros SÉ, Silva Dias TM, Moura MSB, Moura MSB, Soares NRM, Pierote NRA, Araújo COD, Maia CSC, Henriques GSH, Barrors VCB, Neto JMM, Parente JML, Marreiro DN, Nogueira NN (2020) Relationship between selenium status and biomarkers of oxidative stress in Crohn disease. Nutrition 74:110762. https://doi.org/10.1016/j.nut.2020.110762

Maneetong S, Chookhampaeng S, Chantiratikul A, Chinrasri O, Thosaikham W, Sittipout R, Chantiratikul P (2013) Hydroponic cultivation of selenium-enriched kale (Brassica oleracea var. alboglabra L.) seedling and speciation of selenium with HPLC-ICP-MS. Microchem J 108:87–91. https://doi.org/10.1016/j.microc.2013.01.003

Mazej D, Falnoga I, Veber M, Stibilj V (2006) Determination of selenium species in plant leaves by HPLC-UV-HG-AFS. Talanta 68:558–568. https://doi.org/10.1016/j.talanta.2005.04.056

Moreda-Piñeiro J, Sánchez-Piñero J, Mañana-López A, Turnes-Carou I, Alonso-Rodrígue E, López-Mahí P, Muniategui-Lorenzo S (2018) Selenium species determination in foods harvested in seleniferous soils by HPLC-ICP-MS after enzymatic hydrolysis assisted by pressurization and microwave energy. Food Res Int 111:621–630. https://doi.org/10.1016/j.foodres.2018.06.003

Natasha SM, Niazi NK, Khalid S, Murtaza B, Bibi I, Rashid MI (2018) A critical review of selenium biogeochemical behavior in soil-plant system with an inference to human health. Environ Pollut 234:915–934. https://doi.org/10.1016/j.envpol.2017.12.019

NSBC - National bureau of statistics of China (2020) China Statistical Yearbook. http://www.stats.gov.cn/tjsj/ndsj/2020/indexch.htm. Accessed Dec 2020

Ochsenkühn-Petropoulou M, Michalke B, Kavouras D, Schramel P (2003) Selenium speciation analysis in a sediment using strong anion exchange and reversed phase chromatography coupled with inductively coupled plasma-mass spectrometry. Anal Chim Acta 478:219–227. https://doi.org/10.1016/S0003-2670(02)01518-0

Oliveira AF, Landero J, Kubachka K, Nogueira ARA, Zanettie MA, Caruso J (2016) Development and application of a selenium speciation method in cattle feed and beef samples using HPLC-ICP-MS: evaluating the selenium metabolic process in cattle. J Anal At Spectrom 31:1034–1040. https://doi.org/10.1039/c5ja00330j

Panhwar AH, Tuzen M, Kaz TG (2017) Ultrasonic assisted dispersive liquid-liquid microextraction method based on deep eutectic solvent for speciation, preconcentration and determination of selenium species (IV) and (VI) in water and food samples. Talanta 175:352–358. https://doi.org/10.1016/j.talanta.2017.07.063

Pirola I, Rotondi M, Cristiano A, Maffezzoni F, Pasquali D, Marini F, Coperchini F, Paganelli M, Apostoli P, Chiovato L, Ferlin A, Cappellia C (2020) Selenium supplementation in patients with subclinical hypothyroidism affected by autoimmune thyroiditis: results of the SETI study Suplementos de selenio en pacientes con hipotiroidismo subclínico afectados por tiroiditis autoinmune: resultados del estudio SETI. Endocrinol Diabetes Nutr 67:28–35. https://doi.org/10.1016/j.endien.2019.12.002

Polona S, Vekoslava S, Ivan K, Emese K (2006) Selenium species determination in selenium-enriched pumpkin (*Cucurbita pepo L.*) seeds by HPLC-UV-HG-AFS. Anal Sci 21:1501–1410. https://doi.org/10.2116/analsci.21.1501

Quiroz W, Astudillo F, Bravo M, Cereceda-Balic F, Vidal V, Palomo-Marín MR, Rueda-Holgado F, Pinilla-Gil E (2016) Antimony speciation in soils, sediments and volcanic ashes by microwave extraction and HPLC-HG-AFS detection. Microchem J 129:111–116. https://doi.org/10.1016/j.microc.2016.06.016

Reyes LH, Mar JLG, Rahman GMM, Seybert B, Fahrenholz T, Kingston HMS (2009) Simultaneous determination of arsenic and selenium species in fish tissues using microwave-assisted enzymatic extraction and ion chromatography–inductively coupled plasma mass spectrometry. Talanta 78:983–990. https://doi.org/10.1016/j.talanta.2009.01.003

Sanchez-Rodas D, Mellano F, Morales E, Giraldez I (2013) A simplified method for inorganic selenium and selenoaminoacids speciation based on HPLC-TR-HG-AFS. Talanta 106:298–304. https://doi.org/10.1016/j.talanta.2012.11.005

Sánchez-Rodas D, Mellanoc F, Martinez F, Palenciae P, Giraldeza I, Morales E (2016) Speciation analysis of Se-enriched strawberries (*Fragaria ananassa Duch*) cultivated on hydroponics by HPLC-TR-HG-AFS. Microchem J 127:120–124. https://doi.org/10.1016/j.microc.2016.02.017

Saucedo-Velez AA, Hinojosa-Reyes L, Villanueva-Rodriguez M, Caballero-Quintero A, Hernandez-Ramirez A, Guzman-Mar JL (2017) Speciation analysis of organo arsenic compounds in livestock feed by microwave-assisted extraction and high performance liquid chromatography coupled to atomic fluorescence spectrometry. Food Chem 232:493–500. https://doi.org/10.1016/j.foodchem.2017.04.012

Shah M, Kannamkumarath SS, Wuilloud JCA, Wuilloud RG, Caruso J (2004) Identification and characterization of selenium species in enriched green onion (*Allium fistulosum*) by HPLC-ICP-MS and ESI-ITMS. https://doi.org/10.1039/b312320k

Silva EG, Mataveli LRV, Arruda MAZ (2013) Speciation analysis of selenium in plankton, Brazil nut and human urine samples by HPLC-ICP-MS. Talanta 110:53–57. https://doi.org/10.1016/j.talanta.2013.02.014

Thiry C, Ruttens A, Temmerman LD, Schneider Y, Pussemiera L (2012) Current knowledge in species-related bioavailability of selenium in food. Food Chem 130:767–784. https://doi.org/10.1016/j.foodchem.2011.07.102

Thosaikham W, Jitmanee K, Sittipout R, Maneetong S, Chantiratikul A, Chantiratikul P (2014) Evaluation of selenium species in selenium-enriched pakchoi (Brassica chinensis Jusl var parachinensis (Bailey) Tsen & Lee) using mixed ion-pair reversed phase HPLC–ICP-MS. Food Chem 145:736–742. https://doi.org/10.1016/j.foodchem.2013.08.116

Tie M, Li B, Zhuang X, Han J, Liu L, Hu Y, Li H (2015) Selenium speciation in soybean by high performance liquid chromatography coupled to electrospray ionization-tandem mass spectrometry

(HPLC-ESI-MS/MS). Microchem J 123:70–75. https://doi.org/10.1016/j.microc.2015.05.017

Tinggi U (2003) Essentiality and toxicity of selenium and its status in Australia: a review. Toxicol Lett 137:103–110. https://doi.org/10.1016/S0378-4274(02)00384-3

Viñas P, López-García I, Merino-Meroño B, Campillo N, Hernández-Córdoba M (2005) Determination of selenium species in infant formulas and dietetic supplements using liquid chromatography-hydride generation atomic fluorescence spectrometry. Anal Chim Acta 535:49–56. https://doi.org/10.1016/j.aca.2004.11.068

Wang J, Wang Z, Mao H, Zhao H, Huang D (2013) Increasing Se concentration in maize grain with soil or foliar-applied selenite on the Loess Plateau in China. Field Crop Res 150:83–90. https://doi.org/10.1016/j.fcr.2013.06.010

WHO - World Health Organization (1996) Trace Elements in human nutrition and health. Geneva, pp 112–119. http://www.who.int/nutrition/publications/micronutrients/9241561734/en/index.html

Xiao Z, Li S, Guo J, Rao Z, Liu C, Jia Z, Suo D, Wang S, Li Y, Fan X (2017) Application of enzymatic probe sonication for selenium speciation in animal feeds. J Chromatogr A 1530:51–58. https://doi.org/10.1016/j.chroma.2017.11.026

Yang L, Mester Z, Sturgeon RE (2004) Determination of methionine and selenomethionine in yeast by species-specific isotope dilution GC/MS. Anal Chem 76:5149–5156. https://doi.org/10.1021/ac049475p

Yin H, Qi Z, Li M, Ahammed GL, Chu X, Zhou J (2019) Selenium forms and methods of application differentially modulate plant growth, photosynthesis, stress tolerance, selenium content and speciation in Oryza sativa L. Ecotox Environ Saf 169:911–917. https://doi.org/10.1016/j.ecoenv.2018.11.080

Zhang H, Zhao Z, Zhang X, Zhang W, Huang L, Zhang Z, Yuan L, Liu X (2019) Effects of foliar application of selenate and selenite at different growth stages on selenium accumulation and speciation in potato (*Solanum tuberosum L.*). Food Chem 286:550–556. https://doi.org/10.1016/j.foodchem.2019.01.185

Zhang K, Guo X, Zhao Q, Han Y, Zhan T, Li Y, Tang C, Zhang J (2020) Development and application of a HPLC-ICP-MS method to determine selenium speciation in muscle of pigs treated with different selenium supplements. Food Chem 302:12573. https://doi.org/10.1016/j.foodchem.2019.125371