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Speciation analysis of selenium in rice samples by using capillary electrophoresis-inductively coupled plasma mass spectrometry

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ABSTRACT

An enzyme-assisted extraction used to extract all species of selenium in rice sample and a sensitive analytical method for the determination of ultratrace Se(VI), Se(IV), $SeCys_2$ (selenocystine) and SeMet (selenomethionine) with capillary electrophoresis-inductively coupled plasma mass spectrometry were firstly described in this study. The extraction method is simple, effective and can be used to extract trace selenium compounds in rice with high extraction efficiency and no altering its species. The analytical method has a detection limit of 0.1–0.9 ng Se/mL, and can be used to determine trace Se(VI), Se(IV), $SeCys_2$ and SeMet in rice directly without any derivatization and pre-concentration. With the help of above methods, we have successfully determined Se(VI), Se(IV), $SeCys_2$ and SeMet in selenium-enriched rice within 18 min with a recovery of 90–103% and a RSD (relative standard deviation, $n = 6$) of 3–7%. Our results indicated that selenium-enriched rice contained only one species of selenium, SeMet, and its concentration is in range of 0.136–0.143 μg Se/g dried weight. The proposed method providing a realistic approach for the nutritional and toxical evaluation of different selenium compounds in nutritional supplements.

1. Introduction

Selenium is an essential trace element for human health and has received considerable attention for its possible role as an effective, naturally occurring anticarcinogenic agent [1]. Many studies revealed that Se is essential for the efficient and effective operation of many aspects of the immune system in both animals and humans [2], and epidemiological studies showed that Se intake correlates inversely with death from various types of cancer [3]. To date, Se deficiency is still a very serious nutritional and health problem in China, and the appearance of selenium-enriched foods (e.g. selenium-enriched rice) to be an effective way for providing selenium for humans [4]. However, the concentration and the species of selenium in different foods may be variable and their bio-availability is quite different. Therefore, it is very important to develop a sensitive and accurate analytical method for the quantification of different selenium compounds in selenium-enriched foods or nutritional supplements to ensure human health.

So far, the main techniques employed to the speciation analysis of selenium are based on the combination of separation techniques such as liquid chromatography (HPLC) or gas chromatography (GC) and element-selective detectors including atomic fluorescence spectrometry (AFS) [5–8], atomic absorption spectrometry (AAS) [9–11], inductively coupled plasma atomic emission spectrometry (ICP-AES) [12–14], and inductively coupled plasma-mass spectrometry (ICP-MS) etc. [15–22]. However, GC-based techniques require a previous derivatization step due to the low volatility of selenium compounds, and the derivatization is one of the most critical steps in the speciation analysis of selenium. Low yield as well as degradation phenomena in derivatization can heavily affect the quality of the results [21]. HPLC-based methods do not require previous derivatization, however, HPLC-based techniques often suffer from one or more of the following deficiencies: lower resolution, poor sensitivity (combined with UV, AES, AAS and so on), inadequate stability due to much organic solvent (combined with ICP-MS) and so on. In addition, chromatographic separations provide interactions of species between stationary and mobile phase, probably resulting in the destruction of complexes [23,24].

In comparison with chromatographic techniques, capillary electrophoresis (CE) has several advantages such as higher separation efficiency, small sample volume requirement, minimal reagent consumption, various separation modes and low operating cost etc. [25]. On the other hand, ICP-MS possesses several inherent advantages such as extremely high sensitivity for most metallic ions, wide

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linear dynamic range, high speed analysis and the ability to perform isotopic analysis [26]. Therefore, the coupling of CE and ICP-MS promises a powerful tool for elemental speciation and has been used in the speciation analysis of some elements such as arsenic [25,27] and selenium etc. [27–29]. However, the speciation analysis of selenium in rice sample by using CE–ICP-MS has not been reported since its complicated matrix. The main aim of the present study is to develop a novel method for the simultaneous determination of ultratrace level of Se(VI), Se(IV), $SeCys_2$ and SeMet in food samples such as rice etc. by using CE–ICP-MS, in hope of providing a realistic approach for the nutritional and toxical evaluation of different selenium compounds in nutritional supplements.

2. Experimental

2.1. Chemicals and reagents

The analytical grade of selenic acid, SeMet and $SeCys_2$ were purchased from Shenzhen Meryer Chemical Technology Co., Ltd. (Shenzhen, China). The analytical grade of sodium selenite was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The 1000 μg/mL stock standard solution of $SeCys_2$ was prepared by dissolving above $SeCys_2$ solid in 0.2% NaOH solution, and the 1000 μg/mL stock standard solutions of Se(IV), Se(VI) and SeMet were prepared by dissolving above standard matters in Milli-Qwater. All the stock standard solutions were stored at 4 °C, and the running standard solutions were prepared by diluting stock standard solutions to the desired concentration with running buffer solution. The super-pure cetyltrimethylammonium bromide (CTAB) was obtained from Sigma company (USA), analytical grade of sodium tetraborate ($Na_2B_4O_7$ $10H_2O$) and sodium dihydrogenphosphate (NaH_2PO_4 $2H_2O$) were purchased from Shanghai Reagents Co. Ltd. (Shanghai, China). The running buffer solution of 20 mmol/L of NaH_2PO_4–10 mmol/L of $Na_2B_4O_7$–0.2 mmol/L CTAB (pH 8.60) was prepared by dissolving above reagents in Milli-Q water. All solutions were treated by ultrasonic agitation and filtered through a 0.22 μm membrane filter before use. All experiments were performed at room in which the temperature was regulated in 25–27 °C by an air conditioner, and water used in this experiment is Milli-Q water (18.2 MΩ/cm) prepared by a Milli-Q equipment (Millipore, Bedford, USA), the super-pure grade of HNO_3, CH_3OH and NaOH were purchased from Shanghai Reagents Co., Ltd. (Shanghai, China). Selenium-enriched rice samples were purchased from BeiJing TaiJi TongSheng Co., Ltd. (BeiJing, China).

2.2. CE–ICP-MS system

The CE–ICP-MS system used in this study is the same as that reported in our previous paper, which consists of a home-made CE unit and an Agilent 7500ce ICP-MS system (Agilent Technologies, USA) [25]. The home-made CE was coupled to ICP-MS with a home made interface [25]. Sample solution was injected into CE–ICP-MS for determination with electro-migration injection. The CE capillary was conditioned daily by purging with Milli-Q water for 10 min, 0.1 mol/L NaOH solution for 10 min, Milli-Q water for 10 min and running buffer solution for 10 min, respectively. Between each run, the CE capillary was flushed with Milli-Q water and running buffer solution for 2 min, respectively.

2.3. Determination of Se(VI), Se(IV), $SeCys_2$ and SeMet in selenium-enriched rice

All species of selenium in selenium-enriched rice was extracted with the modified method of Huerta et al. [30]. Firstly, about 0.5 g of selenium-enriched rice, which was crashed with a muller previously, was accurately weighed and put into a 15 mL polyethylene centrifuge tubes, and then 40 mg protease, 20 mg lipase and 5.0 mL of Milli-Q water was added into it. Then, the whole was incubated for 16 h at 37 °C and was then centrifuged for 10 min at 5000 rpm to obtain supernatants. The supernatants was further filtered through a 0.22 μm membrane filter and diluted to desired volume with milli-Q water (according to the selenium content in the sample), and the final solution was used for CE–ICP-MS determination under continuous sample-introduction mode.

3. Results and discussion

3.1. Optimization of CE–ICP-MS conditions for the analysis of Se(VI), Se(IV), $SeCys_2$ and SeMet

It was well known, selenium has six isotopes, and ^{78}Se and ^{80}Se are two most abundant isotopes. Their abundances are 23.8% and 46.9%, respectively. Therefore, the m/z of 78 and 80 were monitored in the ICP-MS measurement of selenium in order to obtain higher sensitivity. However, the determination of ^{78}Se and ^{80}Se by quadrupole ICP-MS is associated with isobaric interferences of $^{40}Ar^{38}Ar^+$ and $^{40}Ar^{40}Ar^+$. In order to eliminate the isobaric interferences, H_2 gas dynamic reaction cell (DRC) technique was used in this study, and the experimental results showed that the isobaric interferences of $^{40}Ar^{38}Ar^+$ and $^{40}Ar^{40}Ar^+$ can be completely eliminated under H_2 gas DRC mode when the flow rate of H_2 reached 3.0 mL/min. It was also reported that only 30% of selenium was ionized to form univalent ion in ICP since selenium has a high ionization potential of 940.7 kJ/mol [31]. So the sensitivity of selenium detected with ICP-MS is relatively poor in comparison with other elements such as arsenic etc. It was reported that the addition of organic solvent such as methanol in sample solution would lead to an increased population of C^+ and/or carbon-containing polyatomic ions and a subsequent electron transfer from the analyte ion to the carbon-containing ion, which brought higher signal intensity [32–34]. In this study, the effect of methanol's concentration on the selenium sensitivity was studied by adding different concentration of methanol (2%, 3%, 4%, 5% and 6%) into sample solution. The experimental results indicated that selenium has a highest sensitivity and stability in the solution containing 5% methanol. Therefore, the 5% was selected as the optimum methanol concentration in our work.

In CE separation, the migration of ions generally occurs under the combined action of electrophoretic flow and electroosmotic flow (EOF). Applying positive voltage on the injection side, EOF is oriented toward the cathode, while the electrophoretic mobility of anions, such as SeO_4^{2-} (Se^{6+}) and SeO_3^{2-} (Se^{4+}), is in opposite direction. If the magnitude of the EOF is higher than electrophoretic mobilities, Se(IV) and Se(VI) will consequently move towards the cathode. This is usually the case for Se(IV). However, Se(VI) was not detected at all even under high EOF conditions because of its high electrophoretic mobility. Therefore, it is necessary to suppress or reverse the direction of EOF in order to separate Se(IV) and Se(VI) [35]. In this study, a cationic surfactant, CTAB, was added into the NaH_2PO_4–$Na_2B_4O_7$ buffer solution (2:1, mole concentration) to modify capillary surface, which led to positive-fixed charges and reversed EOF. The experimental results indicated that Se(VI), Se(IV), $SeCys_2$ and SeMet can be baseline separated by using above reverse CE mode. The effect of CTAB concentration on the separation of above four selenium compounds was studied by adding different concentration of CTAB (0.1, 0.2, 0.3, 0.4, 0.5 mmol/L) into running buffer solution, the results showed that the CE current was unstable when the concentration of CTAB was lower than 0.2 mmol/L. Whereas, when the concentration of CTAB was higher than 0.3 mmol/L, the separation efficiency of four selenium compounds became worse. Considering the separation efficiency and

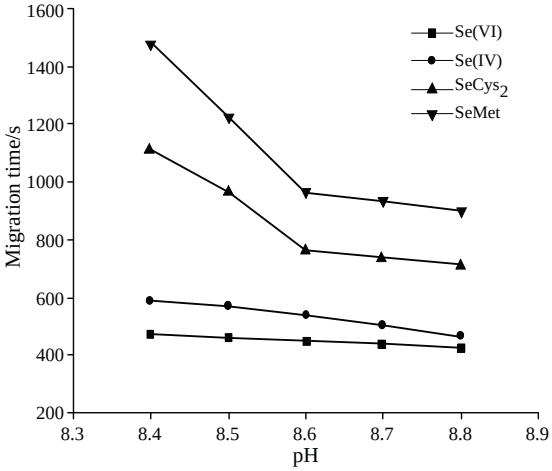

Fig. 1. The effect of pH on the migration time of Se(VI), Se(IV), $SeCys_2$ and SeMet. The data was obtained by determining a 200 ng/mL mixed solution of Se(VI), Se(IV), $SeCys_2$ and SeMet with CE–ICP-MS under conditions of Table 1 except the pH of buffer solution.

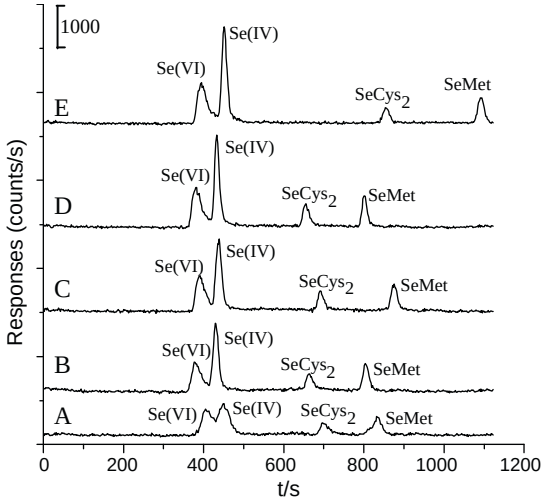

Fig. 2. The electropherograms of Se(VI), Se(IV), $SeCys_2$ and SeMet under different concentrations of phosphate-borate buffer solution. The data was obtained by determining a 200 ng/mL mixed solution of Se(VI), Se(IV), $SeCys_2$ and SeMet with CE–ICP-MS under conditions of Table 1 except buffer solution concentration. (A) 10 mmol/L NaH_2PO_4–5.0 mmol/L $Na_2B_4O_7$–0.2 mmol/L CTAB; (B) 15 mmol/L NaH_2PO_4–7.5 mmol/L $Na_2B_4O_7$–0.2 mmol/L CTAB; (C) 20 mmol/L NaH_2PO_4–10 mmol/L $Na_2B_4O_7$–0.2 mmol/L CTAB; (D) 25 mmol/L NaH_2PO_4–12.5 mmol/L $Na_2B_4O_7$–0.2 mmol/L CTAB; and (E) 30 mmol/L NaH_2PO_4–15 mmol/L $Na_2B_4O_7$–0.2 mmol/L CTAB.

reproducibility, 0.2 mmol/L was selected as the optimum concentration of CTAB in this study.

The pH and concentration of buffer solution greatly affects the separation of analytes by affecting the electroosmosis flow (EOF). The effect of pH on the separation was investigated in detail in the range of 8.40–8.80 and the results showed that lower pH is favorable to prolong the migration time and improve the electrophoretic resolution (see Fig. 1). From Fig. 1, we found that Se(VI) and Se(IV) cannot be completely separated when pH 8.8, and all of Se(VI), Se(IV), $SeCys_2$ and SeMet can be baseline separated when pH is lower than 8.70. When the pH is lower than 8.5, the analytical time was greatly extended. Considering the analytical time and electrophoretic resolution, we selected pH 8.60 as the optimum pH for the separation of Se(VI), Se(IV), $SeCys_2$ and SeMet.

The effect of the buffer concentration on the separation was also studied by using different concentrations of phosphate-borate buffer solution (phosphate/borate = 10/5.0, 15/7.5, 20/10, 25/2.5 and 30/15 mmol/L) at pH 8.60, and the results are shown in Fig. 2. The results showed that the higher concentration of buffer solution is favorable to improve the resolution and prolong the migration times. Considering the analytical time and electrophoretic resolution, 20 mmol/L NaH_2PO_4–10 mmol/L$Na_2B_4O_7$ containing 0.2 mmol/L CTAB (pH 8.60) was selected as the running buffer solution.

The effect of the separation voltage on the migration time and electrophoretic resolution was investigated in the range of −18 to −14 kV, and the results are shown in Fig. 3. The results showed that higher voltage was favorable to shorten migration time and improve the sensitivities. However, higher voltage led to worse electrophoretic resolution and poorer reproducibility due to Joule heating effect. Considering the reproducibility, analytical time and resolution, −16 kV was selected as the separation voltage.

Different injection times (5, 10, 15, 20 and 25 s) were tested in this experiment, and the results showed that the sensitivities of four selenium compounds become higher with the increase of injection time. However, longer injection time will degrade electrophoretic resolution and broaden the peaks. Considering both sensitivity and separation efficiency, we selected 10 s as sample's injection time. As we mentioned in our previous paper [25], the flow rate of pump1,which was used to transport the analyte solution eluted out from the CE capillary to three-way PEEK and introduce a tiny sheath flow into the outlet of CE capillary, will influence the peak shape and the sensitivity. By change the rate of pump 1 from 1.5 rpm (3 μL/min) to 7.5 rpm (15 μL/min), we found that 6.0 rpm of pump 1 (12 μL/min) provide a better peak shapes and sensitivity. The optimum rate of pump 2, which was used to pump Milli-Q water to ICP-MS nebulizer in order to achieve stable atomization and ionization efficiency, was selected by change the rate from 0 rpm (0 μL/min) to 10.0 rpm (400 μL/min), and the results showed that the flow rate of pump 2 = 5.0 rpm (200 μL/min) is the best choice.

At above optimum conditions (see Table 1), four species of selenium compounds were baseline separated within 18 min under continuous sample-introduction mode (see Fig. 4).

3.2. Reproducibility, linear relationship and detection limits

At the optimal CE–ICP-MS conditions shown in Table 1, the same experiment was repeated for six times in order to investigate the

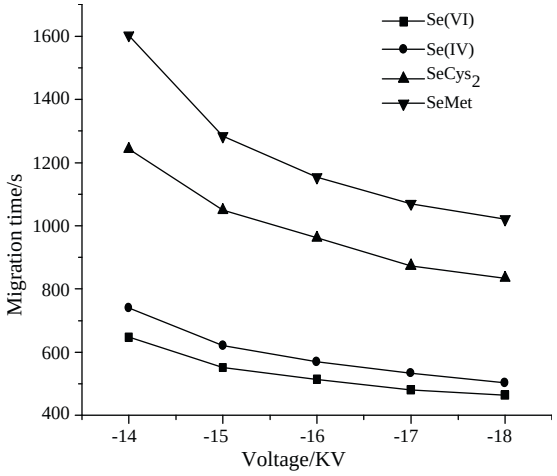

Fig. 3. The effect of separation voltage on the migration time of Se(VI), Se(IV), $SeCys_2$ and SeMet. The data was obtained by determining a 200 ng/mL mixed solution of Se(VI), Se(IV), $SeCys_2$ and SeMet with CE–ICP-MS under conditions of Table 1 except separation voltage.

Table 1
Optimal Running Parameters of CE–ICP-MS.

Parameters	Value
CE voltage	−16 kV
Sampling time	10 s
CE capillary	i.d. 75 μm; o.d. 365 μm; 80 cm long
Lab temperature	23–25 °C
Running buffer solution	20 mmol/L NaH_2PO_4–10 mmol/L $Na_2B_4O_7$–0.2 mmol/L CTAB (pH 8.6)
Velocity of pump 1	12 μL/min
Velocity of pump 2	200 μL/min
RF power	1300 W
Outer plasma gas	15 L/min
Intermediate plasma gas	0.90 L/min
Carrier gas	0.75 L/min
Makeup gas	0.30 L/min
DRC reaction gas	H_2
Reaction gas flow rate	3.0 mL/min
Monitored isotope (m/z)	^{78}Se, ^{80}Se
Nebulizer type	MCN (optimum flow is 50–200 μL/min)

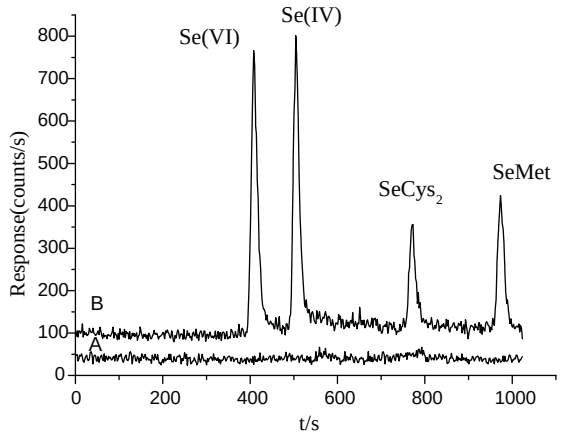

Fig. 4. The electropherograms of Se(VI), Se(IV), $SeCys_2$ and SeMet under the optimal CE–ICP-MS conditions shown in Table 1. The data was obtained by determining a 200 ng/mL mixed solution of Se(VI), Se(IV), $SeCys_2$ and SeMet and a reagent blank with CE–ICP-MS, respectively. (A) Blank, (B) mixed standard solution.

reproducibility of our method. The RSD (relative standard deviation, $n = 6$) of migration times was calculated to be 5%, 5%, 4% and 4% for Se(VI), Se(IV), $SeCys_2$ and SeMet respectively, and that of peak areas is 3%, 3%, 5% and 4% for Se(VI), Se(IV), $SeCys_2$ and SeMet respectively. A series of different concentrations of mixed standard solutions (10, 20, 50, 100, 200, and 400 ng/mL) were determined in order to obtain calibration curve. The linear correlation coefficients between counts (peak area) and concentrations were better than 0.990 for all Se(VI), Se(IV), $SeCys_2$ and SeMet in the concentration range of 10–400 ng/mL (see Table 2). The instrument detection limits of CE–ICP-MS ($3\sigma/S$, the concentration necessary to yield a net signal equal to three times the standard deviation of the background) were calculated to be 0.2, 0.1, 0.90 and 0.50 ng/mL for Se(VI), Se(IV), $SeCys_2$ and SeMet respectively under continuous sample-introduction mode.

As we mentioned above, GC-ICP-MS and HPLC-ICP-MS can also been used to the speciation analysis of selenium. However, GC-ICP-MS can only be used for the analysis of volatile selenium compounds or requires a complicated derivatization step in order to determine non-volatile selenium compounds [8,15,21,22]. HPLC-ICP-MS does not require previous derivatization, however, it suffer from inadequate stability due to much organic solvent [16–18]. In addition, GC or HPLC separations provide interactions of species between stationary and mobile phase, which probably resulting in the destruction of complexes [23,24]. In comparison with GC-ICP-MS and HPLC-ICP-MS, CE–ICP-MS has obvious analytical advantage, such as similar sensitivity, higher separation efficiency, much less sample and reagent consumption, low operating cost, water-phase separation system that is suitable for biological sample and moderate separation conditions that is favorable to prevent the change of selenium species during separation process.

3.3. The extraction of selenium compounds in rice sample

To perform the speciation analysis of selenium compounds in selenium-enriched rice, the extraction of each selenium compound is another key point. The extraction method must be capable of quantitatively extracting each selenium species from samples without altering the individual selenium species. So far, various methods including traditional solid-liquid extraction, sequential extraction and microwave-assisted extraction etc. have been developed to extract different selenium compounds in various samples [22,27,29]. However, these methods can only be used to the samples, which has relatively simple matrix, such as yeast, coal fly ash and so on. For rice sample, which has abundant protein and more complicated matrix, above methods could not give a satisfactory extracting efficiency. Huerta et al. reported that enzyme-assisted extraction, which used protease and lipase as assisted enzyme, should be the most efficient approach for quantitative extraction of selenium compounds from biological samples with no degradation of selenoamino acids [30]. In this study, the enzyme-assisted extraction, which used protease and lipase as assisted enzyme, was used to extract selenium compounds in selenium-enriched rice sample, and the optimal extracting conditions including the mass ratio of rice sample to protease and lipase, extracting temperature and extracting time were investigated in detail. The experimental results showed that the optimal amount of protease and lipase are 40 mg and 20 mg respectively for 0.5 g of rice sample (in 5.0 mL water) and the best extracting temperature is 37 °C. More amount of protease and lipase will increase the background of reagent blank and harm the detection limit of the method, whereas, less amount of protease and lipase will decrease the extracting efficiency of selenium compounds. Under above optimal extracting temperature and the ratio of rice sample to protease and lipase, all selenium compounds in rice samples can be completely extracted within 16 h.

In summary, the optimal conditions of enzyme-assisted extraction for rice sample are: two twenty-fifths protease, twenty-fifth of lipase, ten times of water, the extracting temperature of 37 °C and the extracting time of 16 h. Under above optimal conditions, all

Table 2
Linear relationship and detection limit of the method.

Aanlyte	Regression equation[a]	Correlation coefficient	Liner range (ng/mL)	Detection limit[b] (ng/mL)
Se(VI)	$y = 987x + 105$	0.990	10–400	0.2
Se(IV)	$y = 1091x + 556$	0.998	10–400	0.1
$SeCys_2$	$y = 255x - 245$	0.992	10–400	0.9
SeMet	$y = 462x + 379$	0.992	10–400	0.5

[a] x = concentration (ng/mL), y = counts.
[b] Instrument detection limit.

Table 3
The analytical results of selenium-enriched rice samples.

[a]Total Con	Se-enriched rice											
	Sample 1				Sample 2				Sample 3			
	0.150 ± 0.001 μg Se/g				0.145 ± 0.001 μg Se/g				0.142 ± 0.001 μg Se/g			
	[b]Added Con.	[b]Detected Con.	RSD (n = 6)	Rec. (%)	[b]Added Con.	[b]Detected Con.	RSD (n = 6)	Rec. (%)	[b]Added Con.	[b]Detected Con.	RSD (n = 6)	Rec. (%)
Se (VI)	0	<DL			0	<DL			0	<DL		
	1.00	0.911	3%	91%	0.50	0.463	4%	93%	0.20	0.205	6%	103%
Se (IV)	0	<DL			0	<DL			0	<DL		
	1.00	0.902	3%	90%	0.50	0.481	5%	96%	0.20	0.182	7%	91%
$SeCys_2$	0	<DL			0	<DL			0	<DL		
	1.00	0.951	5%	95%	0.50	0.460	5%	92%	0.20	0.183	6%	92%
SeMet	0	0.143	3%		0	0.139	4%		0	0.136	4%	
	1.00	1.162	4%	102%	0.50	0.612	3%	95%	0.20	0.341	6%	102%

[a] The concentrations determined with ICP-MS after samples were completely decomposed with 7 mol/L HNO_3, unit is μg Se/g dried weight.
[b] Unit is μg Se/g dried weight.

selenium compounds in rice samples can be completely extracted without altering the species.

3.4. Determination of Se(VI), Se(IV), $SeCys_2$ and SeMet in selenium-enriched rice sample

In order to verify the reliability of our methods, the Se(VI), Se(IV), $SeCys_2$ and SeMet in selenium-enriched rice were extracted by the method described above and their concentrations were determined with CE–ICP-MS under the conditions of Table 1. The analytical results were shown in Table 3 and their electropherograms were shown in Fig. 5. From Fig. 5, only SeMet was detected in selenium-enriched rice and its concentration is in the range of 0.136–0.143 μg Se/g dried weight. The recoveries were also obtained by determining selenium-enriched rice sample spiked with different concentrations of Se(VI), Se(IV), $SeCys_2$ and SeMet. The recoveries were also shown in Table 3 together with RSD. From Table 3, we found that the recovery was 90–103% and RSD (n = 6) was smaller than 7% for all four selenium compounds.

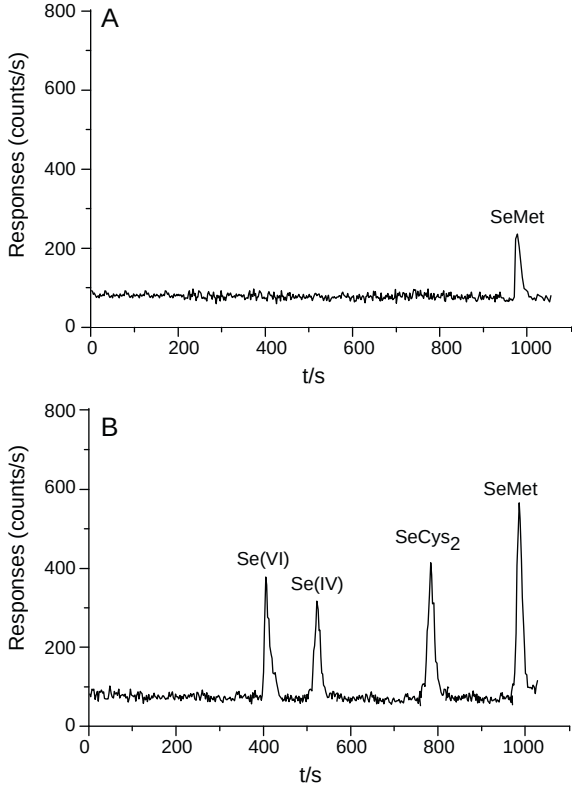

Fig. 5. The electropherograms of Se(VI), Se(IV), $SeCys_2$ and SeMet under the optimal CE–ICP-MS conditions shown in Table 1. (A) selenium-enriched rice; (B) selenium-enriched rice spiked with 1.0 μg/g of Se(VI), Se(IV), $SeCys_2$ and SeMet.

It was well known, if all selenium compounds in samples were completely extracted and if each selenium species keep no change during extraction process are two key points to the speciation analysis of selenium. From the results shown in Table 3, we found that the sum of the concentrations of each selenium species was consistent with the total concentration of selenium (the concentration determined with ICP-MS after sample was completely decomposed with 7 mol/L HNO_3), indicating that all selenium had been completely extracted by using our method. The approximately 100% of recovery for all Se(VI), Se(IV), $SeCys_2$ and SeMet obviously indicated that each selenium species kept no change during extracting and analytical process, since the recovery of at least one selenium species should excessively deviated from 100% if any selenium species was changed during extracting and analytical process. The method detection limits of each selenium compound in rice samples can be calculated according to the detection limits of instrument and the mass ratio of final solution used for CE–ICP-MS analysis to sample. In the case of selenium-enriched rice, 1 mL extracting solution of 0.5 g sample can be directly analyzed by using our method without any pretreatment, therefore, the method detection limits of each selenium compound in selenium-enriched rice were calculated to be 0.4, 0.2, 1.8 and 1.0 ng Se/g dried weight for Se(VI), Se(IV), SeCys2 and SeMet, respectively.

4. Conclusion

We herein reported a novel sensitive analytical method for the determination of ultratrace Se(VI), Se(IV), $SeCys_2$ and SeMet with CE–ICP-MS and a enzyme-assisted extraction used to extract all species of selenium in rice sample. The extraction method is simple, effective and can be used to extract trace selenium compounds in rice with a satisfactory recovery and no altering selenium species. The analytical method has a detection limit as low as 0.1–0.9 ng Se/mL, and can be used to determine trace Se(VI), Se(IV), $SeCys_2$ and SeMet in rice directly without any derivatization and pre-concentration. Using above methods, we have successfully determined Se(VI), Se(IV), $SeCys_2$ and SeMet in selenium-enriched rice within 18 min with a recovery of a recovery of 90–103% and a RSD of 3–7%. Our results indicated that selenium-enriched rice contained only one species of selenium, SeMet, with a concentration in the range of 0.136–0.143 μg Se/g dried weight. The success of this study provided a realistic approach for the nutritional and

toxical evaluation of different selenium compounds in nutritional supplements.

Acknowledgements

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