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Speciation of cationic selenium compounds in *Brassica juncea* leaves by strong cation-exchange chromatography with inductively coupled plasma mass spectrometry

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Abstract

Strong cation-exchange chromatography (SCX-HPLC) was used in conjunction with inductively coupled plasma mass spectrometry (ICP-MS) to investigate cationic selenium species present in leaf extract of wild-type *Brassica juncea* supplemented with selenite. Total amount of Se accumulated by the leaves was found to be 352 $\mu g\, g^{-1}$. Cation-exchange solid-phase extraction (SCX-SPE) was used to pre-concentrate the cationic species present in the leaf extract. Methylselenomethionine (MeSeMet) and dimethylselenoniumproprionate (DMSeP) were synthesized and characterized by electrospray quadrupole time-of-flight MS (ESI-QTOF-MS). Laboratory synthesized and commercially available standards were used in chromatographic studies to identify the Se species in the leaf extract through retention time comparisons and standard addition method. Major cationic selenium species identified in the present study were MeSeMet and methylselenocysteine (MeSeCys) while selenomethionine (SeMet) was found in minor quantities.

Keywords: Se speciation; *Brassica juncea*; Strong cation-exchange chromatography-ICP MS; Cation-exchange solid-phase extraction

1. Introduction

Selenium is a naturally occurring element widely distributed in almost all natural forms on Earth [1]. Soil Se (usually as selenate) results from parent marine materials containing high amounts of Se [1]. Other sources of Se in the environment are due to oil refining [2] and mining [3] in the form of selenite (SeO_3^{2-}) and fossil fuel combustion in the form of elemental Se and selenium dioxide (SeO_2) [4]. Se levels above 2 ppm are found in seleniferous soils of western USA, Ireland, Australia, Israel and other countries [5]. Concern over Se as a contaminant in water and soils has increased in the past few years [6] requiring remedial actions at some point.

Phytoremediation is suggested as a low cost and environmentally friendly technology to clean up metal contaminated soil and water, using plants [7]. Plants that are suitable for Se phytoremediation must grow rapidly, tolerate salinity and other toxic conditions, produce high biomass with substantial uptake of metal for accumulation and volatilization of relatively large amounts of Se and ultimately produce forage for Se-deficient livestock [1]. *Brassica juncea* is one plant that has been identified for such characteristics and has been extensively studied for its phytoremediation [8] implications with fewer studies at the molecular level chemistry.

The amount of Se transported and/or localized in the plants depends on the chemical form of Se that the plant is exposed to [9]. In previous studies, when *Brassica* plants were treated with Se(VI), it was observed that the plant predominantly accumulated Se(VI). However, *Brassica* plants grown in a Se(IV) enriched medium, metabolized inorganic selenium to organic forms [10]. It would be of interest to know the nature of Se species accumulated in *Brassica* leaves as they are included in human diet [11] and also as fodder [7]. There is a very narrow range between Se as a nutrient and toxicant for human and animal species [12]. It is, therefore, important to know the total amount of Se accumulated and nature of various Se species

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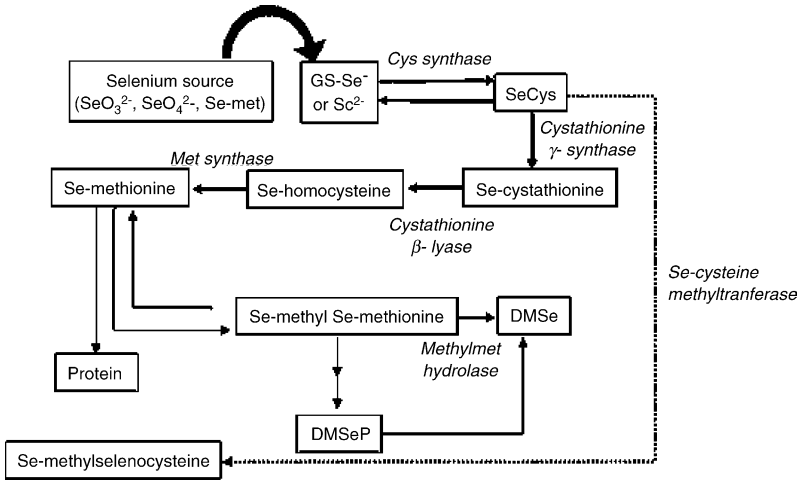

Fig. 1. Schematic diagram of possible Se cycle in plants [13]; the dotted line suggests an alternative pathway.

present in different parts of the plant that are used as Se supplement.

The pathway of Se biotransformation in plants is believed to be along the same lines as that of sulfur [9]. When plants are supplied with Se, most of the Se absorbed in the root is directly transported to the shoots and is converted to organic Se forms by enzymes present in chloroplast. Conversion of Se to selenocysteine (SeCys) is the first step in formation of organoselenium compounds. SeCys is quickly converted to a number of Se compounds such as the Se analog of glutathione, selenomethionine (SeMet), Se-cystathionine, Se-homocysteine to name a few (Fig. 1, shows a schematic diagram of possible Se cycle in plants [13] where the dotted line suggests an alternate pathway).

Although the entire biochemical pathway for volatilization of Se is not fully understood, it is believed that selenium species undergo transformation to form volatile dimethylselenide (DMSe) or dimethyldiselenide (DMDSe) [13]. One intermediate believed to be present in halophytes for the formation of the volatile Se species, DMSeP, is believed to act as an osmoprotectant [14]. A previous report [15] on possible presence of DMSeP in *B. juncea* leaves treated with Se(VI) was intriguing. It would be interesting to extend this finding to *B. juncea* plants supplemented with Se(IV).

The aim of the current study was identification and characterization of cationic selenium species in *Brassica* leaves when plants are grown hydroponically in a Se(IV) enriched medium. Various studies on speciation of Se in natural products and plants deal with liquid chromatography in conjunction with ICP-MS for sensitive and selective Se monitoring [13]. Previous studies relating separation of Se standards were performed using cation-exchange chromatography [16]. A good number of these standards are the species involved in Se metabolic pathway, hence cation-exchange chromatography was the preferred method for analysis of *B. juncea* leaf extract in the present study. The leaf extract was subjected to pre-concentration by SCX-SPE and later analyzed for cationic selenium species by SCX-HPLC coupled to ICP-MS. Since dimethylselenonium propionate and methylselenomethionine (MeSeMet) are two immediate precursors of volatile dimethylselenide, it would be worth investigating their possible presence in the leaves of *B. juncea*. These standards were synthesized in the laboratory and their molecular identity was confirmed by ESI-QTOF-MS. The two selenium compounds synthesized and other commercially available standards were then used for assignment of peaks observed in SCX-HPLC-ICP-MS chromatogram of leaf extract.

2. Experimental

2.1. Instrumentation

Chromatographic separations were carried out using an Agilent 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump, an autosampler, a vacuum degasser system, a thermostated column compartment and a diode array detector. The ICP-MS detector was an Agilent 7500ce (Agilent Technologies, Tokyo, Japan). The ICP-MS detector was equipped with an octopole reaction cell that can be operated with or without the reaction gas. A conventional Meinhard nebulizer, a Peltier-cooled spray chamber (2 °C) and a shielded torch constitute the sample introduction system under standard plasma conditions. The instrumental operating conditions are summarized in Table 1.

The chromatographic column (Phenosphere SCX, 125 mm × 4.0 mm I.D. with 5 μm particle size) used was strong cation-exchange column (Phenomenex, Torrance, CA, USA). The chromatographic runs were conducted using step elution program of pyridinium formate buffer, as outlined in Table 1.

ESI-MS used in the present study was a quadrupole time-of-flight (QTOF) system from Micromass (Platform, Micromass, Manchester, UK). The instrument was operated in positive ion mode. The applied voltage to the capillary was 30 V with N_2 as nebulizing gas. Operating parameters for ESI-MS are delineated in Table 1.

2.2. Chemicals and reagents

All commercial chemicals were of analytical grade and were used without further purification. All solutions were

Table 1
Instrumental conditions for ICP-MS, ESI-MS and SCX-HPLC

ICP-MS parameters	
Forward power	1300 W
External flow	15.0 L min^{-1}
Internal flow	1.0 L min^{-1}
Carrier gas flow	1.17 mL min^{-1}
Collision gas	4 mL min^{-1} H_2
Selected isotopes	^{77}Se, ^{78}Se, ^{80}Se
Dwell time	0.1 s per isotope
ESI-QTOF parameters	
Capillary voltage (kV)	3
Cone voltage (V)	30
Nebulizing gas	N_2
HPLC parameters	
Column	SCX (125 × 4.0 mm)
Mobile phase	(A) 0.75 mM pyridinium formate pH (3.0) and 3% MeOH (B) 8.0 mM pyridinium formate pH (3.2) and 3% MeOH
Gradient (step elution)	0–7 min 100% A 7–7.1 min 100% B 7.1–28 min 100% B 28–29 min 100% A 29–45 min 100% A
Injection volume	50 μl
Flow rate	1 mL min^{-1}

prepared in 18 MΩ cm doubly deionized water generated by a NanoPure treatment system (Barnstead, Boston, MA, USA). Selenomethionine, selenocystine ($SeCys_2$), methylselenocysteine (MeSeCys) were purchased from Sigma (Milwaukee, WI, USA). Formic acid was used as obtained from J.T. Baker (Phillipsburg, NJ, USA). Pyridine was obtained from Fischer Scientific (Fairlawn, NJ, USA). Dimethylselenide and 2-bromopropionic acid used in synthesis of dimethylselenoniumpropionate were obtained from Fisher Scientific and Sigma, respectively. Selenomethionine and methyl iodide involved in synthesis of MeSeMet were purchased from Sigma and Fisher Scientific, respectively. Syntheses were carried out as delineated in Ref. [17].

Individual stock solutions of concentration 10 μg mL^{-1} of synthesized and commercially available standards were prepared using doubly deionized water. Working standards were prepared by dilution of stock solution to the required concentration and used for chromatographic experiments.

Pyridinium formate 1 L solutions (0.75 and 8.0 mM) were prepared by dissolving the required amount of pyridine in 970 mL doubly deionized water and adjusting the pH using formic acid. The pH of the solutions were adjusted to 3.0 and 3.2, respectively, after which 30 mL of MeOH was added to the solutions and used as mobile phase in less than 2 weeks.

2.3. Sample plant growth

Wild type *B. juncea* seeds were surface sterilized by washing in 96% ethanol for 30 s, then in 0.65% sodium hypochlorite solution for 30 min, and finally, in sterile deionized water for 5–10 min, all on a rocking platform. Twenty-five sterilized seeds were sown in a grid pattern per Magenta box on half-strength MS medium (Sigma) with 10 g L^{-1} sucrose and 5 g L^{-1} phytagar (Sigma). After 2 days at 4 °C, the boxes were moved to a growth chamber kept at 25 °C under continuous light. On day 7, individual seedlings were transferred to 4-in. pots containing coarse sand and covered with a plastic dome. The pots were maintained in a greenhouse with controlled temperature (24 °C) and a long-day (16 h) photoperiod. The plants were watered twice a day, once with tap water and once with 1/2-strength Hoagland's solution. After 1 week in sand, the dome was removed. At the end of the second week, the plants were transferred to aerated Magenta boxes containing 1/2-strength Hoagland's solution. After 1 week in hydroponics, the nutrient solution was replaced by fresh solution containing 50 mM sodium selenite. After 1 week, the plants were harvested, separated to various plant components and immediately frozen in liquid N_2 and ground into a fine powder.

2.4. Sample preparation

2.4.1. Total Se analysis

Lyophilized leaf sample (50 mg) was treated with 1 mL of HNO_3 (Suprapure) 68% from Pharmaco (Hartford, CT, USA) and subjected to microwave digestion using CEM explorer-discover (Matthews, NC, USA) in septum sealed glass tubes. The digestion process was performed at 150 °C for 10 min by maintaining a pressure below 170 psi. The obtained clear solution was filtered and diluted to 36 mL and analyzed for total Se concentration by standard addition method using ICP-MS. This is the first nitric acid digestion on CEM explorer-discover unit, earlier applications of this unit were synthesis based.

2.4.2. Extraction of selenium species from Brassica

About 0.50 g of ground leaf was treated with 2 mL of 0.75 mM, aqueous pyridinium formate pH 3.0 solution and stirred at room temperature for 24 h. This heterogeneous solution was then centrifuged at 20 °C at a speed of 4000 rpm. The supernatant was filtered through 0.45 μm polyvinylidene fluoride (PVDF) filter (Alltech, Deerfield, IL, USA).

2.4.3. Strong cation-exchange solid-phase extraction (SCX-SPE)

2.4.3.1. SCX-SPE cartridge conditioning and extraction. SCX-SPE were performed using 12-port Visiprep vacuum manifold (Supelco, Bellefonte, PA, USA) under vacuum (<10 psi negative pressure). Stationary phase of SCX-SPE, strata SCX 200 mg/3 mL (Phenomenex, Torrance, CA, USA) was washed with MeOH followed by 0.75 mM pyridinium formate for 8 bed volumes each. About 1 mL of sample was introduced on SCX-SPE bed after conditioning and immediately washed with low concentration buffer for 8 bed volumes. The SCX-SPE bed was then allowed to completely dry after which about 15 bed volumes of 8.0 mM pyridinium formate was used to elute strongly retained cationic species.

2.4.3.2. SCX leaf extract. One millilitre of the supernatant obtained in *Brassica* leaf sample preparation step was passed through SCX-SPE cartridge. About 8 bed volumes of 0.75 mM pyridinium formate buffer were used to wash out neutral and anionic species from the cartridge. At this stage cationic species are strongly adhered to the stationary phase of SPE cartridge. To elute these cationic species 8 mM pyridinium formate was passed for 15 bed volumes. The effluent was then evaporated under a stream of nitrogen and the obtained residue was stored at −21 °C until analysis. At the time of analysis, 1 mL of 0.75 mM pyridinium formate solution was added to dissolve the residue and analyzed by SCX-HPLC-ICP-MS for selenium speciation.

2.4.3.3. SCX-SPE recovery studies. Stock solutions of 2.2 ppm concentration of commercially obtained standards, SeMet, $SeCys_2$ and MeSeCys were prepared individually in double distilled water. These stock solutions were diluted appropriately to three different concentrations of 2.2, 22 and 220 ppb for SPE recovery studies. Standards were acidified 1:1 with 0.75 mM pyridinium formate prior to SPE extraction. Similarly, a 1:1 mixture of standards and Brassica leaf extract in 0.75 mM pyridinium formate were prepared individually. Two sets of each of the standards and standards with Brassica leaf extract were prepared, among which one was subjected to SCX-SPE studies and the other was used to determine the total Se concentration. The Se concentration, which was determined using 10 ppb Y as internal standard, was compared to Se concentration of SCX-SPE extracts to determine recovery percentages of various selenium compounds and effect of sample matrix on solid phase extraction.

3. Results and discussion

3.1. Optimization of collision/reaction cell gas

The most abundant Se isotopes, ^{80}Se (49.7%) and ^{78}Se (23.6%) suffer strong interference from $^{40}Ar^{40}Ar$ and $^{38}Ar^{40}Ar$ in ICP-MS detection. To decrease the background for the above-mentioned selenium isotopes, collision/reaction cell was used. Argon and solvent-based interferences are eliminated, by using the collision cell, enabling detection of interfered analytes. When H_2 was used as collision gas, a maximum signal-to-noise ratio was observed for a flow rate of 4 mL min^{-1}, which did not improve with further increase in the flow. Hence H_2, under a flow rate of 4 mL min^{-1} was used as collision cell gas for all the experiments.

3.2. ESI-MS results

Two synthesized standards, MeSeMet and DMSeP were characterized for their molecular identity using ESI-QTOF-MS. ESI-QTOF-MS spectrum (Fig. 2a) of synthesized MeSeMet showed a molecular ion at *m/z* value of 212, which corresponds to M^+ ion of $(CH_3)_2SeCH_2CH_2CHNH_2COOH$. The characteristic isotopic pattern as exhibited by a single Se atom can be observed in the spectrum. The difference in experimental mass of the synthesized compound and that of calculated mass of MeSeMet was found to be −3.3 ppm. A 100 ppb sample solution of the synthesized standard on the SCX-HPLC-ICP-MS is about 18.2 min, as shown in Fig. 2b, which also shows a small peak at 14 min due to residual SeMet involved in the synthesis.

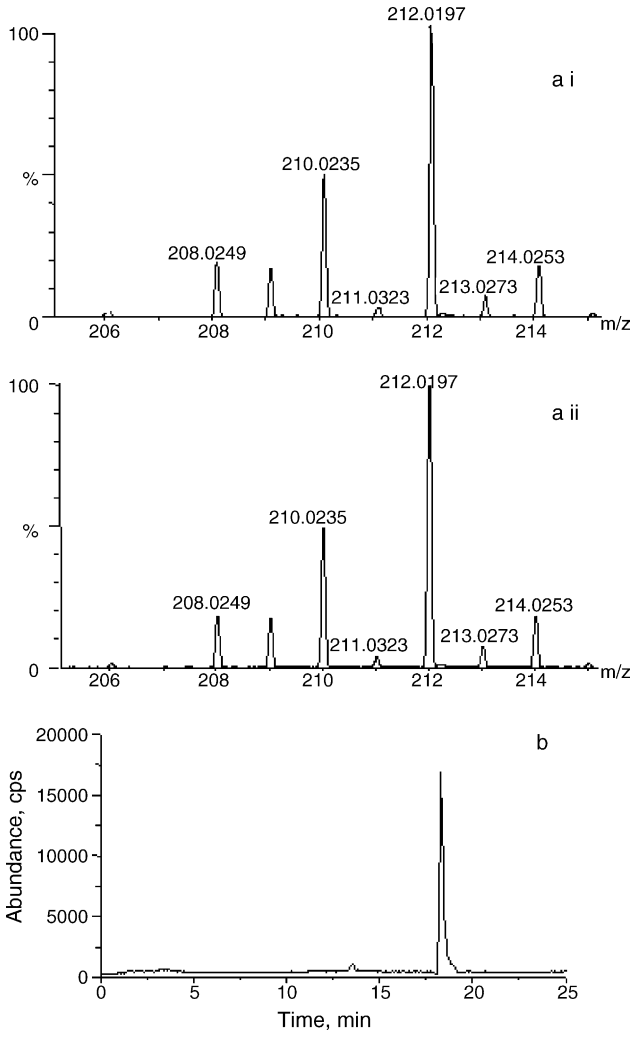

Fig. 2. (a) ESI-MS spectra of MeSeMet: (i) theoretical; (ii) experimental. (b) SCX-HPLC-ICP-MS of synthesized MeSeMet.

Another standard that was synthesized, DMSeP, showed most abundant peak in ESI-MS spectrum (Fig. 3a) at a nominal *m/z* 183, corresponding to the M^+ ion of $(CH_3)_2SeCH_2CH_2COOH$. A selenium pattern can also be observed in case of DMSeP. The difference in obtained mass for the synthesized standard versus the one proposed for the molecular formula of DMSeP was −2.7 ppm. A 100 ppb solution of the synthesized standard was subjected to SCX-HPLC-ICP-MS analysis and its retention time was found to be 17.6 min (Fig. 3b). A small peak at the dead volume of the chromatogram could be due to the unreacted residual DMeSe. High purity of the synthesized standard can be ascertained from the presence of a single cationic Se compound in the chromatogram (Fig. 3b).

The two synthesized compounds along with commercially available standards were used for identifying the Se species in *B. juncea* leaf extract by chromatographic experiments.

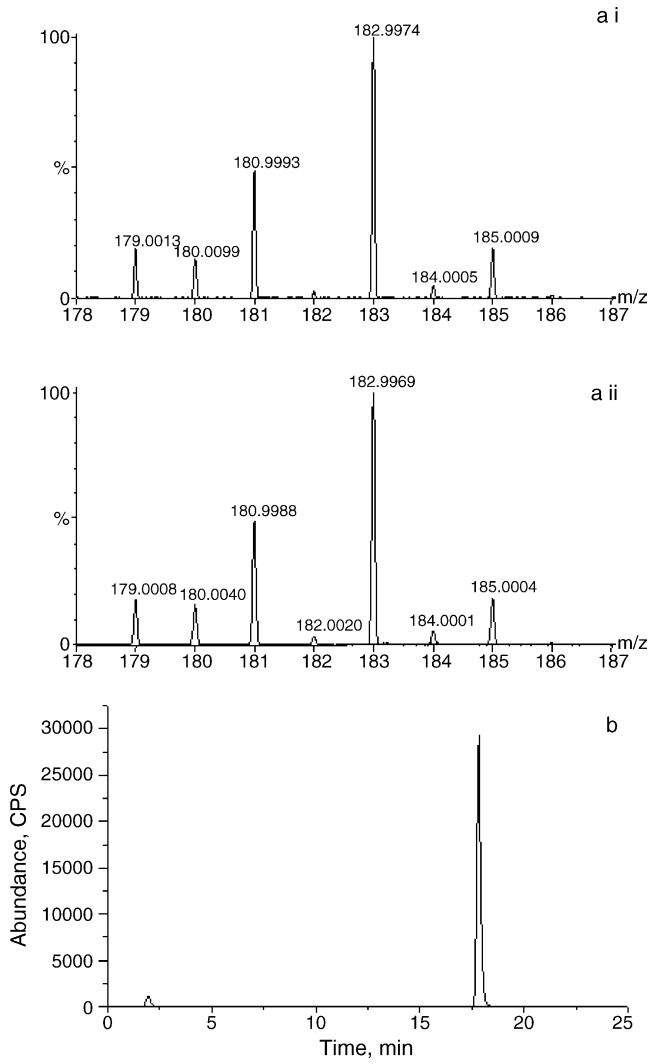

Fig. 3. (a) ESI-MS spectra of DMSeP: (i) theoretical; (ii) experimental. (b) SCX-HPLC-ICPMS of synthesized DMSeP.

3.3. Total Se concentration and speciation studies

After microwave digestion of *B. juncea* leaf, the total amount of Se was determined by the standard addition method. Sample was introduced to ICP-MS by continuous flow with peristaltic uptake of 1.2 mL min^{-1}. The concentration in dry leaf was found to be 352 μg Se g^{-1}.

Se is presumed to be metabolized in the plants by pathways similar to that of sulfur [18]. A pathway suggested for Se volatilization is given in Fig. 1 [13]. Se species involved in the pathway include low molecular weight weak acids, amino acids, and/or selenonium compounds. The charges on these compounds depend on their dissociation constants and the pH of the solution except for the selenonium groups that are positively charged even at higher pH. Among the compounds shown in Fig. 1, MeSeMet and DMSeP are positively charged regardless of the pH of the solution [16]. Similar dissociation constants of most of the remaining selenoamino acids requires use of gradient elution for ion-exchange separation [19]. Cation-exchange chromatography can be considered as a suitable technique to

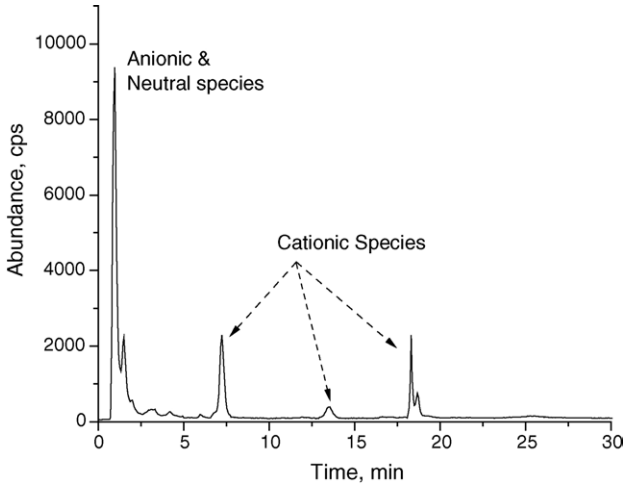

Fig. 4. SCX-HPLC-ICP-MS of wild type *Brassica juncea* leaf extract.

separate a mixture of various cationic species from anionic and zwitterionic compounds. Aqueous pyridine solution can be used as mobile phase as it is supposed to completely volatilize in the argon plasma and not clog the cones for long working periods [20]. As noted by other authors [21] 3% methanol was added to the mobile phase in order to enhance the sensitivity of ICP-MS for Se determination. Separation of cationic Se species by SCX-HPLC was conducted using the elution program adapted from Larsen et al. [16]. Fig. 4 shows the SCX-HPLC-ICP-MS chromatogram obtained for the leaf extract. Neutral and anionic Se species eluted at the void volume while four cationic species can be detected (Fig. 4). Since it is of interest to identify cationic Se species, SCX-SPE can be used to minimize the concentration of non-cationic species and also reduce matrix effects in leaf extract. To determine the amount of cationic selenium species recovered after solid phase extraction and account for any loss in sample during SCX-SPE, a set of commercially available standards was used. SeMet, $SeCys_2$ and MeSeCys solutions were prepared at 2.2, 22 and 220 ppb concentrations each and subjected to SCX-SPE extraction. The amount of Se present in these extracts was compared to standard solutions of same concentrations that were not subjected to SPE. Average recovery values for each species at all concentrations are given in Table 2. All species showed recovery above 70%. Five replicates of SCX-SPE for 22 ppb SeMet were conducted and results indicate a RSD of 4.3%.

Since a good recovery was observed for selenium standards, SCX-SPE was extended to study the effect of sample matrix on extraction of cationic selenium species. Leaf extract spiked with SeMet, SeCys2 and MeSeCys at 2.2, 22 and 220 ppb each were also subjected to extraction following the procedure mentioned

Table 2
SCX-SPE recovery study

Compound	%Recovery	%Recovery in matrix
SeMet	80.3	72.3
SeCys2	75.0	79.1
MeSeCys	70.5	76.7

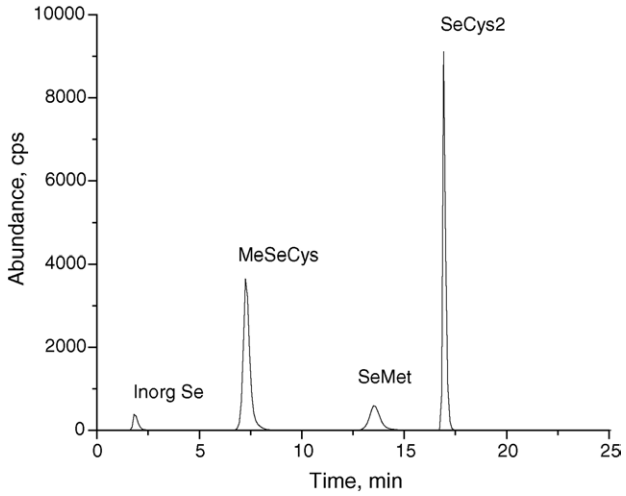

Fig. 5. SCX-HPLC separation of selenium standards. Concentration of each species is 10 ng mL^{-1} of Se.

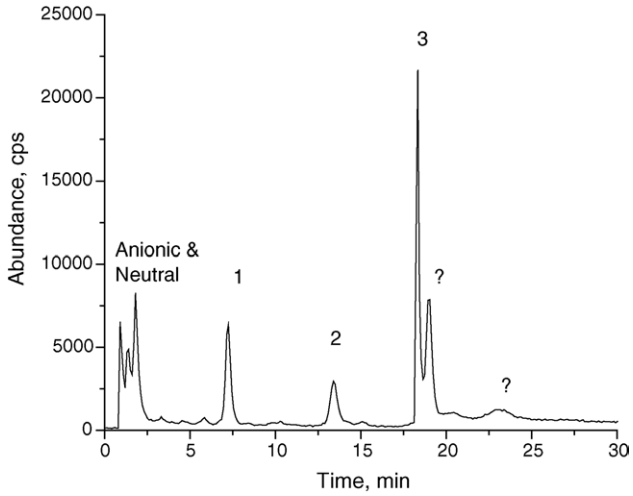

Fig. 6. SCX-SPE extract of Brassica leaf: (1) MeSeCys; (2) SeMet; (3) MeSeMet; (?) unknown.

in Experimental section. Amount of Se in Brassica leaf extract, and that of leaf extract subjected to SCX-SPE was determined. The recovery of Se seems to be in good agreement (RSD < 8%) with their corresponding Se species in non-matrix associated recoveries (Table 2).

The above study indicates that cationic selenium species can be effectively separated from neutral and anionic Se compounds by following gradient elution on SCX-SPE. Moreover, there is minimal effect of matrix on recovery of cationic selenium species and hence SCX-SPE can be used to pre-concentrate and reduce matrix effects in analysis of cationic species.

To establish the identity of Se species in the leaf extract, a mixture of commercially available Se standards was analyzed by SCX-HPLC-ICP-MS (Fig. 5) and the retention times (t_r) of these standards were observed as (a) inorganic selenium [Se (IV)] $t_r = 1.89$ min; (b) MeSeCys $t_r = 7.2$ min; (c) SeMet $t_r = 13.4$ min; and (d) $SeCys_2$ $t_r = 17.2$ min. Initial studies performed on pyridinium formate extract of the leaf showed some prominent cationic selenium species (Fig. 4). Since the primary goal of the present work was to identify cationic selenium species present in the leaf extract, SCX-SPE was employed to increase the relative concentration of cationic compounds. When leaf extract is loaded on SCX-SPE cartridge, phenylsulfonic acid groups of the SCX-SPE stationary phase form ion pairs with cationic species and result in their adsorption. Neutral and anionic compounds, which do not show any interaction with the stationary phase can be eliminated by washing the cartridge with low-ionic strength (0.75 mM pyridinium formate) solution. Strong cationic species are later released by ion-exchange elution using 8.0 mM pyridinium formate. The obtained cation rich leaf extract was lyophilized and subjected to SCX-HPLC-ICP-MS. An additional peak corresponding to cationic Se species that was not present in pyridinium formate extract (Fig. 4) appeared in the chromatogram with $t_r = 23.3$ min (Fig. 6). Such pre-concentration, therefore, makes it possible to visualize peaks that are non-distinguishable from baseline.

Among the five cationic peaks observed in the pre-concentrated leaf extract, identity of two major peaks could be established by retention time matching with available standards. The major cationic species identified in the original leaf extract were found to be MeSeCys and MeSeMet with ∼15% and ∼10% of the total ^{80}Se species, respectively. The other species that was identified in rather smaller amounts of ∼3.1% was SeMet, which exists as cationic species under experimental pH conditions, as earlier studies indicate zwitterionic existence of SeMet at pH ≥ 4.0 [22]. In the SCX-SPE leaf extract, peak at 19.5 min has almost the same abundance of MeSeCys, however, the identity of this compound could not be established.

Identification of SeMet in smaller quantities could be indicative of its methylation to MeSeMet, a precursor of volatile Se species, dimethyl selenide (DMeSe). Presence of DMeSe as the major volatile species in *B. juncea* plants was confirmed by other studies conducted in our laboratory [23]. To verify if the conversion of MeSeMet to DMeSe takes place with the involvement of DMSeP intermediate, SCX runs were conducted using an in-house synthesized standard of DMSeP. None of the peak retention times in the leaf extract matched with that of the DMSeP and hence no evidence of the presence of this intermediate was shown. This result falls in line with previous observations that DMSeP is an intermediate identified mostly in halophytes [14]. However, there was evidence in a previous report [15] of possibly finding DMSeP in wild type *B. juncea* leaf extract in minor amounts when *Brassica* plants were supplemented with Se(VI). Among a number of factors influencing the metabolic pathway of Se, the chemical form of Se species that the plant is supplemented with, determines its ultimate fate. Moreover, metabolic intermediates, mechanisms and enzymology involved in Se assimilation in plants is still a matter of speculation [14] and a number of studies must be performed before any definitive conclusions can be formulated. It is, therefore, not possible to rule out involvement of DMSeP as an intermediate in conversion of MeSeMet to DMeSe in non-halophytes.

MeSeCys is an organo-Se compound that is found as major organic Se species in plant residues that are available for decomposition [24]. Previous work in our laboratory by Grant et al.

[19] involved analysis of *B. juncea* roots that were supplemented with SeMet. These studies showed no evidence of MeSeCys. A possible explanation for this comes from the fact that there is higher volatilization of Se from the roots [19], which involves conversion of SeMet to MeSeMet. It is interesting to note in the same studies that roots did not show accumulation of MeSeCys even in plants genetically modified to over express selenocysteine methyltransferase, an enzyme involved in formation of MeSeCys. However, when there is lower rate of volatilization of Se, as in case of Se(IV) compared to SeMet [25], its conversion to other organic Se species [26] is possible. In the present study wild type *B. juncea* leaves, which were grown in Se(IV) rich environment, showed accumulation of MeSeCys in greater amounts than MeSeMet. Moreover, MeSeCys could undergo one-step cleavage to form methylselenol, suggested as a critical metabolite in Se chemoprevention [27], but not experimentally verified as of the time of this writing.

4. Conclusions

B. juncea accumulated a considerable amount of Se in the leaves, asserting its phytoremediative potential. Preconcentration of cationic species in leaf extract performed by SCX-SPE helped in detection of additional cationic selenium species. Three of the five cationic selenium species observed by SCX-HPLC-ICP-MS were identified by t_r matching and standard addition method (spiking). MeSeMet and MeSeCys were the major Se species observed. A higher amount of MeSeCys was found in leaves when compared to roots as observed in previous studies. Due to the presence of MeSeCys, *B. juncea* can be considered as a potential source of dietary Se supplement. SeMet was identified in minor quantities in the leaves. No evidence of DMSeP was observed under the current Se supplementation conditions for the wild type *Brassica* plants. Hence, in the leaves, MeSeMet is the likely precursor of volatile DMeSe when plants are supplemented with Se(IV). Finally, by establishing the presence the above mentioned selenium species in this study the suggested metabolic pathways are further substantiated [28].

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