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Relative Mass Defect Filtering of High-Resolution Mass Spectra for Exploring Minor Selenium Volatiles in Selenium-Enriched Green Onions

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In this study, the presence of minor Se-containing volatiles in Se-enriched green onions (*Allium fistulosum*) was investigated using the combination of high-resolution mass spectrometry, inductively coupled plasma mass spectrometry, and a simple relative mass defect-based algorithm to aid trace level analysis of unknown components. This confirmed the structures of volatiles reported previously, along with several unreported small molecular weight Se-containing volatiles from plants, such as MeSeSeSMe. This data analysis technique was also useful to link the results obtained from molecular and elemental mass spectrometry thus aiding in the search for new trace level Se-containing volatiles.

Ever since the Clark trial,[1] there has been increasing interest in understanding the selenium biochemistry within living organisms due to the suggested Se anticarcinogenic and antioxidant properties. Both volatile and nonvolatile forms of selenium have been explored within many biologically important systems in order to obtain as complete information as possible on the occurrence of selenium in its various forms. Plants from genus *Allium* represent one of the most widely studied groups as they are known to accumulate appreciable amounts of sulfur and selenium. Selenium occurs predominantly in *Allium* plants in the form of *Se*-methylselenocysteine (*Allium tricoccum*)[2] and γ-glutamyl-*Se*-methylselenocysteine (*Allium sativum* and *Allium fistulosum*)[3,4] and at intermediate levels as *Se*-methionine (*Allium cepa*).[3] These compounds are widely recognized for their selenium-related medicinal properties from epidemiological studies.[1] On the other hand, the information on Se-containing volatile constituents of *Allium* plants is restricted to only a few studies.[5,6] The volatile components of the *Allium* genus are released from their nonvolatile precursors, such as *Se*-methylselenocysteine-*Se*-oxide, by an enzyme mediated degradation, which takes place when the plants are crushed. While dimethylselenide is the major volatile in Se-accumulating plants and dimethyldiselenide is the major Se-volatile in Se-hyperaccululating plants, due to their high sulfur content, *Allium* plants contain considerable amounts of selenosulfenates as their major Se-containing volatiles. Alk(en)yl groups are mainly a combination of propyl, 1-propenyl, allyl, and methyl groups, depending on the plant species. (RS_nSeR': $n = 0-2$; R and R′ = methyl, allyl, 1-propenyl).[5,6]

Previously headspace gas chromatography with atomic emission detection has been employed to detect and identify various selenium volatiles released from cut *Allium* spp.[6] However, the unavailability of selenium-containing volatile standards has left many heavier selenium volatile compounds either unidentified or unconfirmed. The aim of present study is to explore the use of mathematics in conjunction with modern mass spectrometric techniques to improve the knowledge of minor selenium metabolites present in the *Allium* sp. Selenium-enriched green onions (*A. fistulosum*) were selected in this study. Here we present a simple mass defect-based algorithm in order to simplify high-resolution electron impact mass spectra. The spectra obtained thereby appeared similar to inductively coupled plasma mass spectra (element-specific). This data analysis technique was highly useful to relate the conclusions obtained from molecular and elemental mass spectrometry.

Recent developments in combined use of inductively coupled plasma mass spectrometry (ICPMS) (for element-specific detection within a molecule) and molecular mass spectrometry for corresponding structural characterization (a metallomics approach) have helped to confirm trace amounts of previously unidentified species in biological matrixes.[7] ICPMS offers very high sensitivity, multielemental detection capability, and low detection limits among other element-selective detectors when coupled to a GC system. Previously, the coupling of GC to ICPMS has been performed for detection of selenium and sulfur-containing plant volatiles.[8,9] Solid-phase microextraction (SPME) was used for selective extraction and preconcentration of the volatiles and

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for analysis, direct injection into the GC/ICPMS system, and gas chromatography/time-of-flight mass spectrometry (GC/TOF-MS). Extraction using SPME ensures preconcentration up to several orders of magnitude as compared to other time-consuming and tedious sample preparation methods. ICPMS, on the other hand, is also known for its high sensitivity and superior detection capabilities. So combined use of two highly sensitive techniques at the sample preparation and detection stages leads to detection of ultratrace species in complex biological samples. This approach is applied in this study for the determination of trace selenium containing volatiles present in the headspace. Complimentary with this, GC/TOF-MS is used as an additional technique for characterization and confirmation of novel selenium-containing volatiles, since the ability to do exact mass measurements is highly beneficial for studying the mass spectral fragmentation pathways of the selenium compounds.[10]

EXPERIMENTAL SECTION

Instrumentation. (a) Gas Chromatography. An Agilent Technologies (Agilent Technologies, Palo Alto, CA) model 6890 series GC was used for the separation of the species. Separation of selenium volatile compounds was performed on a DB-5 capillary column, 30 m × 0.320 mm i.d. × 0.25 μm (J&W Scientific, Folsom CA) in the case of ICPMS detection. Separation conditions are depicted in Table 1.

(b) ICPMS. An Agilent 7500ce ICPMS (Agilent Technologies, Tokyo, Japan) was used for the element-specific detection. The instrument is equipped with octopole collision/reaction cell and can be operated in both normal and collision cell modes. Hydrogen was used as a collision cell gas for interference-free detection of selenium at m/z 80 and 78. Introduction of collision cell gas not only leads to removal of isobaric interferences but also enables the detection of trace amount of selenium volatile species released due to low background noise at these masses. Sulfur isotope at m/z 34 was also monitored along with selenium for multielemental analysis. The GC was interfaced to ICPMS through a heated GC/ICPMS interface (Agilent Technologies).

(c) GC/TOF-MS. A Micromass GCT orthogonal time-of-flight mass spectrometer (Micromass, Manchester, U.K.) coupled to the Agilent 6890N GC was used for mass spectral characterization of the selenium volatiles released from the enriched green onions. Heptacosafluorotributylamine was used as a reference compound and as the lock mass compound (using the m/z 218.9856 ion) for mass calibration. High-mass measurement precision is achieved by constant leaking of a reference compound directly into the ionization source (internal standard), and ions arising from the reference compound are efficiently eliminated from the recorded mass spectra of any eluting species by simply subtracting the background information. Average mass accuracy of the ions arising from the internal standard usually was less than 0.001 Da. The instrument was recalibrated at any time the mass accuracy was determined to exceed this limit.

Reagents and Standards. All reagents were analytical grade and were used without purification. All solutions were prepared in 18 MΩ cm doubly deionized water generated by a NanoPure treatment system (Barnstead, Boston, MA). The following reagents were purchased from Fluka/Sigma-Aldrich (Milwaukee, WI): dimethyl selenide, dimethyl sulfide, dimethyl disulfide, dimethyl diselenide, diethyl disulfide, dimethyl trisulfide, and heptacosafluorotribuytlamine. Diethyl diselenide was purchased from Strem Chemicals (Newburyport, MA) Individual stock solutions of the standards were prepared by dissolution of chemicals in GC/MS grade hexane (Fisher Scientific, Fair Lawn, NJ). Standards were diluted in hexane as required. The 1000 μg mL^{-1} stock solutions of selenite were prepared by dissolving appropriate amount of Na_2SeO_3 (ICN Biomedicals, Aurora, OH) in doubly deionized distilled water. The stock solution was appropriately diluted for supplementation purposes.

Table 1. Instrumental Conditions.

ICPMS Parameters	
forward power	1150 W
plasma gas flow rate	15.0 L of Ar min^{-1}
auxiliary gas flow rate	0.87 L of Ar min^{-1}
carrier gas flow rate	1.0 L of Ar min^{-1}
sampling depth	6 mm
optional gas	Ar/N_2 (50:50), ~4% relative to carrier gas
Isotopes monitored	^{33}S, ^{34}S, ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se (100-ms dwell time)
collision cell gas	3.2 mL of H_2 min^{-1}
GC Parameters	
capillary column	DB-5 (5% phenyl−95% methylpolysiloxane)
GC carrier gas	2.4 mL of He min^{-1} (constant flow mode)
oven temperature	40 °C for 4 min 40−125 °C at 15 °C min^{-1}, hold 5 min 125−300 °C at 35 °C min^{-1}, hold 1 min
injection mode	pulsed splitless at 10 psi (0.5 min)
injection port temp	250 °C
ICP transfer line temp	250 °C
GC/TOF-MS Parameters	
capillary column	DB-5
GC carrier gas	2 mL of He min^{-1} (constant flow mode)
oven temp	50−220 °C at 10 °C min^{-1} hold 7.5 min
injection port temp	250 °C
MS transfer line temp	250 °C
ionization	electron impact: 70 eV at 180 °C
calibrant and lock mass	perfluorotributylamine, 218.9856 Da ($C_4F_9^+$) ion was used as a lock mass
EI+ temperature	180 °C
trap current	400 μA
m/z monitored	40−620
scan duration	0.9 s
interscan delay	0.1 s
GC re-entrant	250 °C
reference reservoir temp	80 °C
re-entrant temp	80 °C

Preparation of Selenium-Enriched Green Onions. The plant compartment selected for the characterization of volatile selenium in the present work was the leaves of the evergreen long white bunching onion, *A. fistulosum*. The seeds were purchased from Burpee (Warminster, PA). Cell packs containing three to five seeds and with four cells each of dimensions (0.13 m × 0.13 m × 0.10 m) were employed. Each cell contained Promix BX (Natorp landscape, Cincinnati, OH), which is a mixture of sphagnum, peat moss, perlite, vermiculite, dolomite, and calicitic lime stone. Cells were watered daily and fertilized with commercially available 15 N/30 P/15 K nutrient solution when needed.

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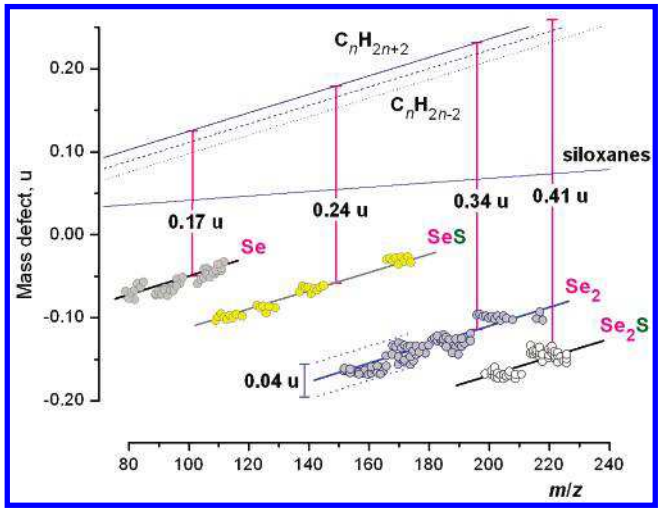


Figure 1. Differences in exact mass of aliphatic Se/S chalcogenides, hydrocarbons, and poly(dimethylsiloxanes) as a basis for the mass defect mask analysis.

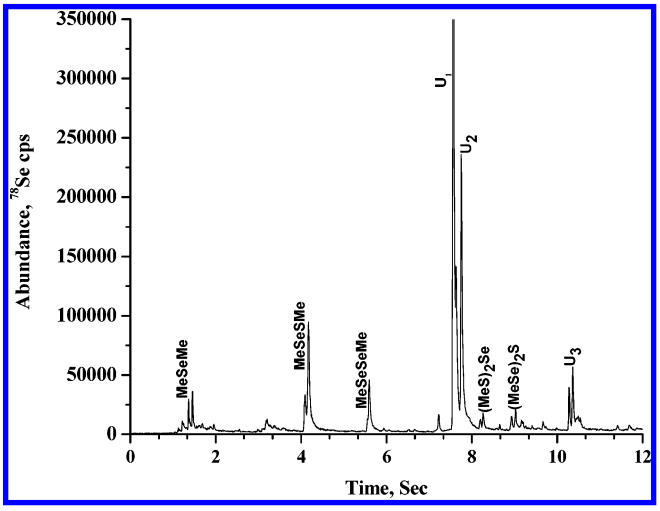
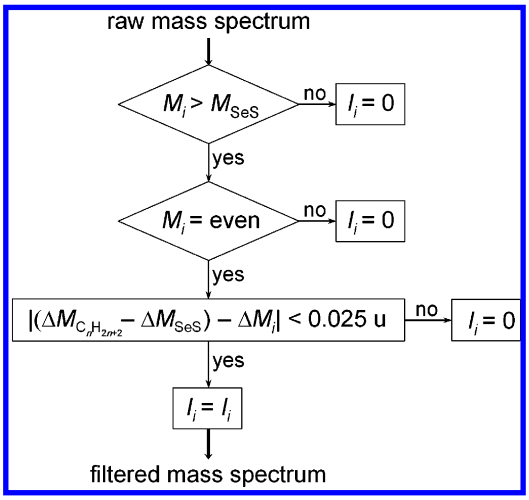


Figure 2. SPME-GC/ICPMS chromatogram of enriched green onion headspace.

Scheme 1. Algorithm for the Mass Defect Specific Filtering of High-Resolution Mass Spectra Using Contextual Information[a]



[a] In this example, volatile selenosulfenates (containing Se−S) are the targeted species.

Plants were grown in a greenhouse with temperatures between 16 and 21 °C. Before any selenium treatment, the cells were thinned to three plants per cell. One of the cell packs was not enriched with Se and was used as a control. Two months after seeding, the cells were treated with Na_2SeO_3 at the concentration 20 μg g^{-1}. To compensate for loss from run out, one week after the first treatment, the cells were treated with the same concentration of selenium as before. Plants were harvested 10 days after supplementation began. The green onions were washed with water and then were separated from the roots. Total selenium determination (ICPMS) after complete digestion of sample revealed that plants accumulated Se at the level of 200 μg of Se g^{-1} of fresh sample. No attempt was made to establish sterile conditions.

Sample Preparation. Enriched green onion plants were thoroughly washed and dried in air, then crushed using pestle and mortar, and quickly placed into the sample vial. The pestle and mortar and the sample vial were purged with N_2 prior to use to prevent the oxidation of selenium volatiles. The vial was closed with the Teflon septum, and the SPME fiber was exposed to the vial headspace for extraction of the volatiles.

Commercially available SPME fiber was used for extraction and preconcentration of volatile species. The fiber used was Carboxen/PDMS (film thickness 75 μm) obtained from the Supelco (Bellefonte, PA). The SPME fiber was exposed to the headspace of the crushed onion samples. After the extraction period of 30 min, the analytes were desorbed at 250 °C in the GC injection port using the 0.75-mm-i.d. inlet liner (Supelco). The fiber was held in the liner throughout the run to reduce any memory effects.

Synthesis of Matching Standards. Dimethyl selenosulfenate (MeSeSMe) and other selenium- or sulfur-containing volatiles were prepared in solution by mixing equal volumes of methanol or pentane solutions of dimethyl trisulfide and dimethyl diselenide (1000 ppm each) in a closed vial. The resulting solution was allowed to equilibrate at room temperature for a few hours and, after dilution with pentane, was subjected to chromatographic separation.[9]

RESULTS AND DISCUSSION

Since the volatile selenium constituents originate from their nonvolatile precursors, their identification provides indirect information on the selenium biochemistry and metabolism in the plants of interest. Many recent studies have been dedicated to identify the various selenium volatiles in biological systems. The analysis of selenium compounds in biological samples is not a trivial task since there are many techniques for the element-specific detection of Se species, such as ICPMS, but only few that offer molecular structure characterization of the detected compounds at the trace levels.[11] The valuable information obtained from the element-specific detection remains incomplete because of loss of structural information during ionization process. The species-specific characterization of selenium compounds by ICPMS is limited to only a few selenides and diselenides such as methyl and ethyl derivatives since these are commercially available, whereas, in complex biological matrixes a multitude of selenium-containing volatiles are possible and require identification.

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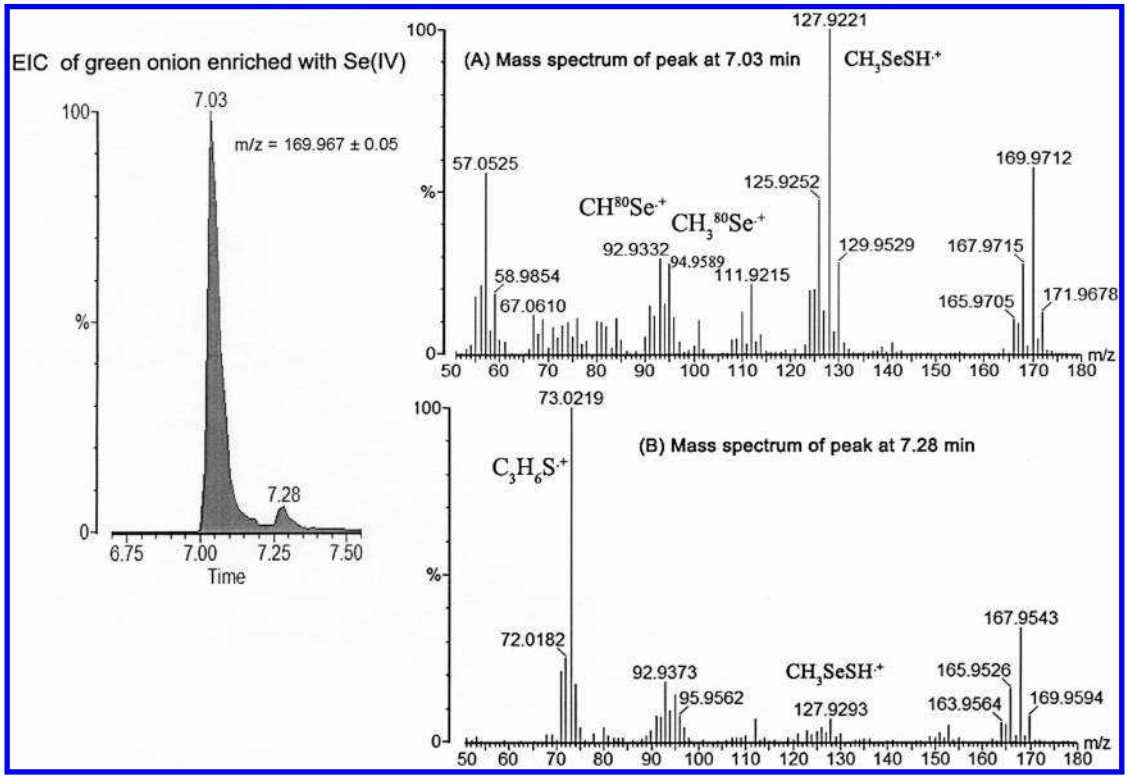

Figure 3. Extracted ion chromatogram from GC/TOF-MS of *m/z* 169.967 of green onions supplemented with Se(IV). Corresponding mass spectra (A) of peak at 7.03 min for PrSSeMe and (B) of peak at 7.28 min for MeSeSAll.

Unlike selenium-accumulating plants, such as *Brassica juncea*, *Allium* plant volatiles are mainly allyl groups containing selenides and diselenides. Mixed selenium- and sulfur-containing compounds have also been detected in the *Allium* plants.[6] The lack of commercially available standards requires in-house-synthesized standards and their subsequent characterization with the help of molecular mass spectrometry.[9] One of the main problems in trace element speciation is the misnmatched detection capabilities of ICPMS and molecular mass spectrometry techniques. Even though modern molecular spectrometry techniques, such as high-resolution time-of-flight mass spectrometry, offer comparably low detection levels with ICPMS, the complexity of the mass spectra sometimes prohibits direct identification of unknown species. The presence of the isotope pattern is helpful, although this is not a universal solution to the problem, since many elements (such as iodine or arsenic) are monoisotopic. Isotope pattern distortions due to the presence of isobaric interferences are also common.[10]

Analytical problems, such as lack of commercially available standards for direct analysis by element-specific detection via retention time matching and standard additions, require often complex molecular mass spectra for trace identification of the species of interest. For simplification purposes, a mathematical approach, such as mass defect analysis as a means of digital sample cleanup for molecular mass spectrometry, helps in interpretation. So, combined use of molecular mass spectrometry and mass defect analysis with element-specific detection has been employed to provide and complete the preliminary information on selenium volatiles obtained by ICPMS.

Mass Defect Mask Analysis. Due to the differences in the formation energy (stability) of various elements and the mass energy relationship ($\Delta E = \Delta mc^2$), exact masses of elements differ from the total mass of the constituent protons, neutrons, and electrons. For example, isobars such as $^{16}O_2$ and ^{32}S have different fractional masses (mass defect or the difference between the exact mass and the nearest integer mass). Since the mass defect of heavy atoms is larger than for lighter major elements, the exact mass of molecular interferences usually will be larger than that of single elements—a feature commonly utilized in high-resolution sector-field ICPMS. In this study, we reduce the complexity of mass spectra by tracking the selenium-containing species using the mass defect of the elements.[12] In this approach, a mass defect mask is applied to observed signals, and as a result, signals matching certain mass defects are visualized while others are suppressed. This is a postacquisition type of chemical noise suppression method (suppressing unwanted chemical signals) and such a visualization approach is well suited for elemental speciation of trace elements in complex biological systems because of the large differences in mass defects between trace elements of common interest (such as Se, As, Sn, and I) and the major elements H, C, N, O, S, and P.[13,14] Mathematical mass defect analysis is also used in peptide and protein identification[15] and in petroleum classification and characterization (Kendrick mass defect plots).[12]

The mass defect of molecules is a function of the molecular weight due to the contributions from other elements, such as hydrogen or oxygen. However, the mass difference between the

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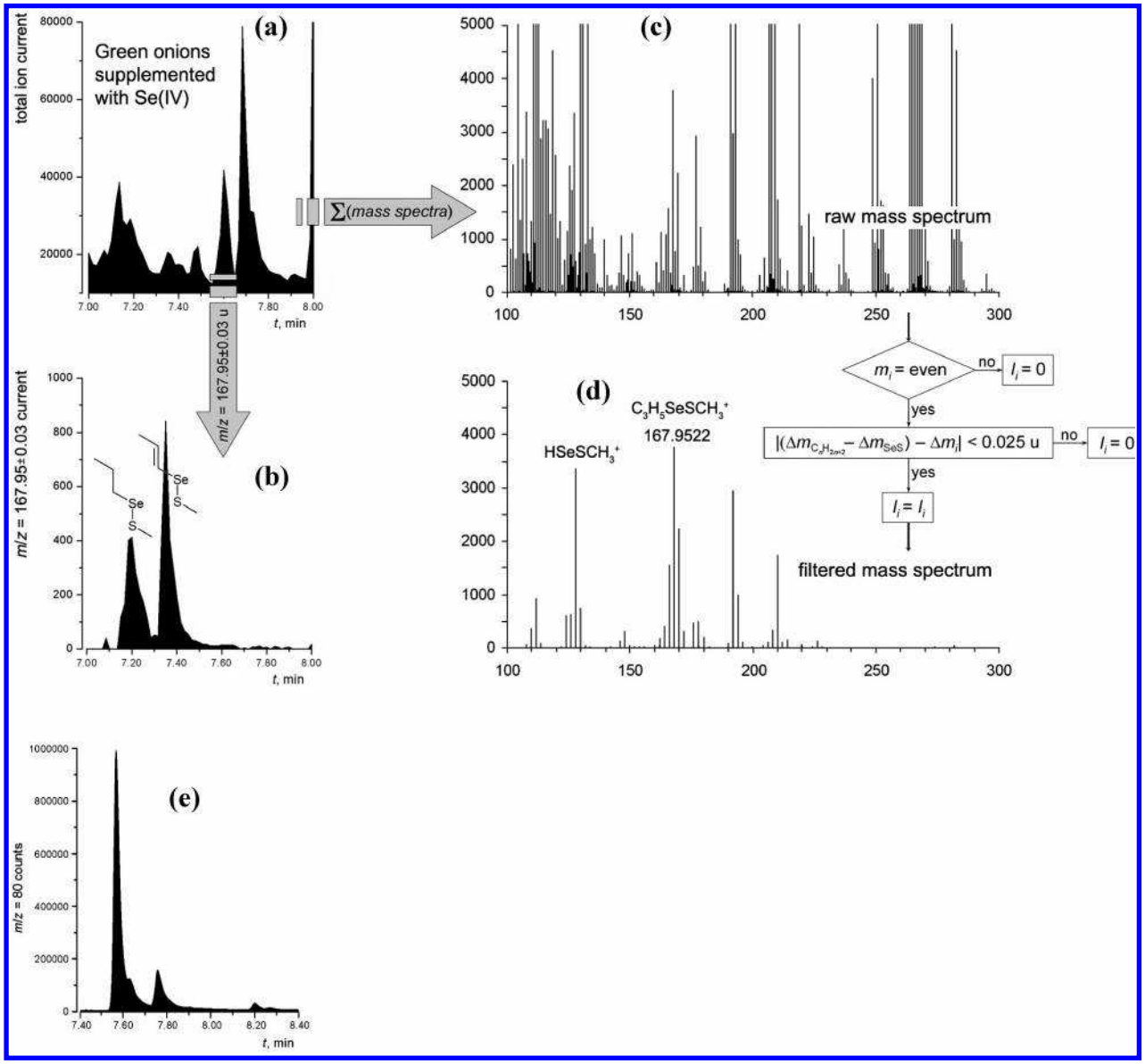

Figure 4. Search for selenosulfenates from the SPME-GC/TOF-MS data of Se(IV) supplemented green onions. (a) Total ion chromatogram, (b) Extracted ion chromatogram of *m/z* 167.95 ion, (c) raw mass spectrum merged within 7.0 and 8.0 min (d) filtered mass spectrum obtained after mass defect analysis, and (e) ICP-like chromatogram.

various aliphatic selenides and hydrocarbons remains constant as shown in Figure 1.

The mass defect of hydrocarbon C_nH_{2n+2} (with molecular weight M) can be calculated from the formula

$$\Delta m(C_nH_{2n+2}) = a(M + 12) \quad (1)$$

where $a = (M_H - 1)/(6 + M_H)$. Then, for any given mass spectral signal at mass M (for charge $z = 1$) one calculates the mass defect difference between the alkanes, $\Delta m(C_nH_{2n+2})$, alkyl chalcogenides, $\Delta m(X)$, and the signal of interest, Δm_i:

$$|\Delta m(C_nH_{2n+2}) - \Delta m(X) - \Delta m_i| \quad (2)$$

The value for the $\Delta m(X)$ is the relative mass defect (relative to the hydrocarbons) read directly from Figure 1, e.g., $\Delta m(Se) =$ 0.17 u. Gas chromatographic mass spectra usually contain large amounts of unwanted chemical information, which comes either from hydrocarbon or from poly(siloxane) fragment ions. These ions have odd mass whereas electron impact mass spectra of Se-containing volatiles produce abundant molecular ion clusters with even mass Se isotopes covering 92 and 86% of Se_1 and Se_2 isotope patterns, respectively. Mass parity thus can be effectively incorporated to remove any odd-mass ions. For example, ~80% of the ion current of a typical mass spectrum of a poly(dimethylsiloxane) capillary column bleed is of odd mass (data not shown). Overall, the element-specific noise filtering algorithm of high-resolution mass spectra is summarized in Scheme 1.

According to this scheme, every m/z channel at every scan is subjected to this logical test. The first step rejects all the ions with mass below the target substructure while the second step filters out all the odd-m/z values. The third step demands that the mass defect of the selected ion matches the theoretically

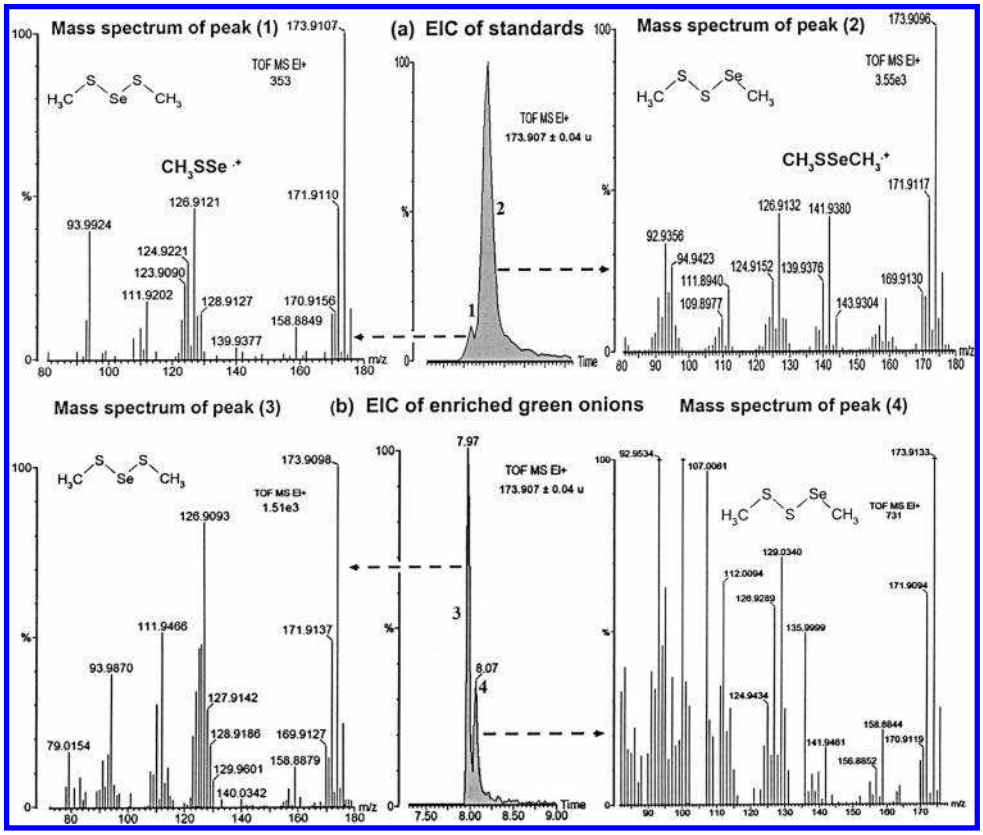

Figure 5. Extracted ion chromatogram (EIC) from GC/TOF-MS of *m*/*z* 173.907 of (a) standard mixture including $CH_3SSSeCH_3$ and $CH_3SSeSCH_3$ (b) and enriched green onion. Mass spectra of peak 1, $CH_3SSeSCH_3$ from standard; peak 2, $CH_3SSSeCH_3$ from standard; peak 3, $CH_3SSeSCH_3$ from sample; and peak 4. $CH_3SSSeCH_3$ from sample.

predicted mass defect of alkyl selenosulfenate at the *m*/*z* value of the selected ion as outlined above. As seen from Figure 1, the presence and absence of double bonds alter the mass defect of molecules; therefore, such variations must be incorporated into the matching tolerance for blind search of various molecules. In this example, the mass defect matching tolerance is set to ±0.025 Da.

Search for Trace Level Se Volatiles. The first approach toward the identification of minor selenium volatiles in enriched green onions was application of SPME-GC/ICPMS. Figure 2 depicts the SPME-GC/ICPMS chromatogram for the headspace analysis of green onion plants (supplemented with Se(IV)) after crushing to initiate the action of lyases and allinases in the plant tissue (a rapid acceleration of sensecense). As shown, several selenium-containing peaks are identified in the sample. Retention time matching with commercially available standards was performed, which revealed the presence of only a few earlier eluting commonly found selenium compounds such as dimethyl selenide (MeSeMe) and dimethyl diselenide (MeSeSeMe). In addition to these principle volatiles, several other mixed selenium sulfur-containing volatile species have also been detected in the headspace of green onions enriched with Se(IV) (Figure 2). Identification of these mixed selenium sulfur species was performed by an all-in-one laboratory-synthesized standard mixture obtained from the S/Se exchange reaction between the dimethyl diselenide, (MeSeSeMe) and dimethyl trisulfide (MeSSSMe). The chalcogen exchange reaction between the two species leads to formation of various selenosulfates and polychalcogenides such as dimethyl selenosulfenate (MeSeSMe), bis(methylthio) selenide ($(MeS)_2Se$), and bis(methylseleno) sulfide ($(MeSe)_2S$).[16] Retention time matching with the standards, also confirms the presence of MeSeSMe and trace levels of Me_2S_2Se and Me_2Se_2S in the sample. However, further characterization using molecular mass spectrometry was required to confirm the presence of these minor volatiles. The presence of $(MeSe)_2S$ from the plant samples has never been reported. The two isomers of Me_2S_2Se, MeSSeSMe, and MeSSSeMe exhibit slight difference in retention behavior on the GC column used; hence, their individual identification in the sample could also be performed using the current chromatographic method. From these two compounds, MeSeSSMe has been very recently reported in the headspace gases above genetically modified *Escherichia coli*.[17] Comparison of the mass spectra of synthetic MeSeSSMe and MeSSeSMe was also undertaken to confirm the identity of these species as described below.

The peaks labeled as U_1, U_2, and U_3 from GC/ICPMS could not be identified by retention time matching because we lacked laboratory-available standards. However, the multielemental detection capability of ICPMS helped to identify the presence of both S and Se isotopes in these three peaks. This was found to be useful in their identification through GC/TOF-MS.

GC/TOF-MS Analysis. GC/TOF-MS is a very common molecular mass spectrometric technique and is highly useful when used in conjunction with ICPMS because of their comparable detection limits. The high sensitivity of TOF-MS along with high resolution has made identification and confirmation of many

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species found in complex biological samples possible, especially for those that cannot be identified using element-specific detection due to lack of commercially available standards. In such cases, ICPMS helps to selectively screen for the presence of unknown selenium or sulfur compounds, while TOF-MS helps in their identification by its ability to do exact mass measurements. The benefits of exact mass measurements become more important for accurate assignment of polyselenide molecular ions because of skewed isotopic distribution patterns. In this study, the complimentary use of GC/TOF-MS has helped to characterize the unknown peaks and also confirm the novel selenium species from senescing plants, such as MeSSeSMe, MeSSSeMe, and MeSeSeSMe in the green onion samples supplemented with Se(IV). A close look at the time-of-flight mass spectra also helped to elucidate other later eluting selenium compounds, the presence of which could only be speculated in the previous studies.[6]

As similar column and chromatographic conditions were used for both GC/ICPMS and GC/TOF-MS experiments, similar elution order of the selenium compounds was expected. Based on the relative elution profile of the commercially avilable selenium standards on GC/ICPMS and GC/TOF-MS systems, the retention times of known and the unknown on the two systems were correlated and assigned accordingly. The peaks at 7.03 and 7.28 min in Figure 3 were assigned as the peaks labeled as U_1 and U_2. These unknown species were identified as methylpropyl selenosulfenate (PrSSeMe) and methyl-1-propene selenosulfenate (MeSeSAll), respectively. The corresponding molecular ions at m/z 169.9712 and 167.9543 were identified in the EI^+ spectra. A close look at the fragment ions reveal the presence of ion cluster at m/z 92.9332 and 94.9589 (corresponding to ions $CH^{80}Se^{\cdot+}$ and $CH_3{}^{80}Se^{\cdot+}$) in both cases and at m/z 73.0219 (corresponding to $C_3H_6S^{\bullet+}$ ion) in allylmethyl selenosulfenate spectra leading to the above structures. The relative abundance of the fragment ions in the case of allylmethyl selenosulfenate is similar to that described by Cai et al. for this molecule.[5]

Identity of these two species in the sample was also confirmed through mass defect mask analysis as described earlier in the text. Using the above signal filtering strategy, we were able to easily locate Se-containing compounds of interest (in this example, alkyl selenosulfenates—containing the −Se−S− group) from the complex GC/TOF-MS mass spectra. Figure 4 shows the strategy of such search. All the mass spectra were merged together from the chromatographic window of interest yielding a raw mass spectrum, which then was subjected to the SeS-specific search as shown in Scheme 1. This resulted in a very simple spectrum where the presence of the Se-containing isotope pattern at m/z 168 was evident. Using this information, one can now obtain the conventional individual ion chromatogram of the identified mass (167.95 u), which resulted in two compounds: methylpropyl and allylmethyl selenosulfenate (Figure 4). Note that the two molecular ions of these species are overlapping, thus distorting the Se isotope pattern. It is interesting to see that such a strategy basically allows us to generate ICP-like element-specific chromatograms from the TOF-MS data as shown in Figure 4.

Accurate mass measurements and the correct isotope pattern were also used to confirm other species such as MeSeMe, MeSeSeMe, MeSeSMe, Me_2S_2Se, and MeSeSeSMe in the sample. Also, the presence of two structural isomers of MeS_2Se, such as

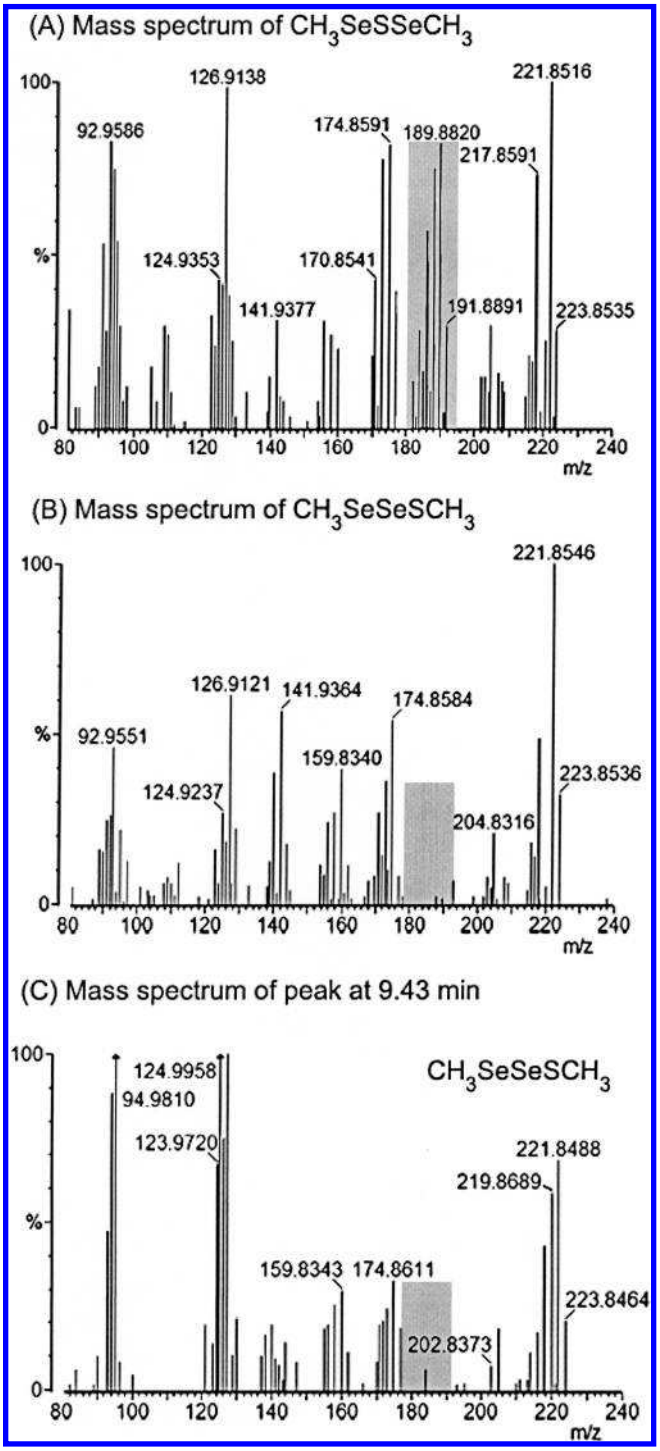

Figure 6. Mass spectra of $CH_3SeSeSCH_3$ from the Se-enriched onions compared to the standard spectra of both $C_2H_6Se_2S$ isomers, $CH_3SeSeSCH_3$ and $CH_3SeSSeCH_3$.

MeSSeSMe and MeSeSSMe, were also identified for the first time in plant samples. The distinction between the symmetric and the asymmetric form was made from their differences in retention times and EI+ fragmentation patterns. The symmetric form (MeSSeSMe) eluted earlier than the asymmetric form (MeSSSeMe). As shown in Figure 5, the selenium-containing ion cluster at m/z 141.938 (C_2H_6SeS, formed as chalcogen exchange product[16]) is more pronounced in the species MeSeSSMe in the EI+ mass spectra. This is expected due to presence of MeSe- and MeS-containing group in the compound. Mass spectra of these two

Table 2. Summary of the Se-Containing Volatiles Identified from Se-Enriched Green Onions

structure[a]	abundance[b]	method of identification[c]
CH_3**Se**CH_3 (110 Da)	0.3	RT, MS, EC
CH_3**Se**SCH_3 (142 Da)	1.7	RT, MS, EC
CH_3**SeSe**CH_3 (190 Da)	1	RT, MS, EC
$CH_3CH_2CH_2SSeCH_3$ (170 Da)	14.2	RT, EC
$CH_3CH{=}CHSSeCH_3$ (168 Da)	3.8	RT, EC
$CH_3SSeSCH_3$ (174 Da)	<0.3	RT, MS, EC
$CH_3SSSeCH_3$ (174 Da)	<0.3	RT, MS, EC
$CH_3SSeSeCH_3$ (222 Da)	<0.3	RT, MS, EC
$CH_3CH_2CH_2SSeSCH_3$ (202 Da)		EC
$CH_3CH_2CH_2SSeSCH_2CH_2CH_3$ (230 Da)		EC

[a] Monoisotopic molecular weight based on ^{80}Se. [b] Relative abundance from all the Se-containing volatiles as compared to CH_3**SeSe**CH_3 determined with GC/ICPMS. $CH_3SeSeCH_3$ headspace concentration was found to be at ~0.45 ng μL^{-1} [c] RT: retention time matching with standards as performed on a 30- and 60-m DB-5 (0.25 and 1 μm, respectively) capillary columns. MS: electron impact mass spectra compared to mass spectra of the standards. EC: elemental composition and isotope patterns verified with high-resolution GC/TOF-MS.

isomers also differ by the presence of fragment ion at *m/z* 126.9093 ($MeSSe^+$), in the case of MeSSeSMe. This ion is of at relatively small abundance in the case of MeSSSeMe. Both the isomers were identified in the sample (Figure 5b).

In the case of $C_2H_6Se_2S$, only one isomer could be detected from Se-enriched onions. In order to confirm the presence of this species, comparison of the mass spectra of synthetic MeSeSSeMe and MeSeSeSMe species with the mass spectra from the sample was undertaken. The two isomers display different fragmentation patterns. As shown in Figure 6, the mass spectrum of MeSeSSeMe exhibits very pronounced selenium containing cluster at *m/z* 189.8820. On the other hand, MeSeSeSMe is characterized by the presence of two selenium-containing ion clusters at *m/z* 159.834 and at *m/z* 141.936 corresponding to Se_2^+ and $MeSeSMe^+$, respectively. As described earlier, the ion at *m/z* 141.936 is due to the presence of the MeS and Me−Se groups in the molecule. These two species also exhibit different retention times on the column. The mass spectrum of peak at 9.43 min from the sample is in agreement with the synthetic MeSeSeSMe with the characteristic presence of fragment ion *m/z* 159.834 cluster (with its characteristic Se_2 isotope pattern) and absence of ion cluster at *m/z* 189.8820.

Hence, only the asymmetric form of Me_2Se_2S could be identified in the Se-enriched green onions. The presence of this species was also confirmed through the above-mentioned filter strategy. In this case, the volatile species with the substructure Se_2S were the target species. The relative mass defect in this case was set to 0.41 u (Scheme 1).

A close look at the TOF mass spectra also helped to elucidate the presence of other later eluting selenium compounds, which could only be suggested in the previous studies. One of such compounds eluted at 11.05 min in GC/TOF-MS spectrum with the exact mass of 201.9413 Da and elemental composition $C_4H_{10}S_2Se$ (data not shown). Accurate molecular weight measurement and the correct isotope pattern corresponds to the species PrSSeSMe. This species was suggested to be present in the onion oil by the Cai et al.[6] However, lack of an available standard and its presence at very trace levels left its identification unconfirmed. PrSSeSPr (with the corresponding molecular ion at 229.9678 Da) was also found to be present in the sample. The presence of this species in plant samples has never been reported.

It is interesting to note that a wide variety of methyl- and propyl-containing selenosulfenates have been identified to be released from the allium plant investigated in this study. The result obtained is in contrast to the selenium-accumulating plants where the major selenium-containing volatile released is dimethyl selenide. Of all the selenium volatiles present, methylpropyl selenosulfenate is found to be most abundant in the headspace of Se-supplemented green onions (Table 2). The relative abundance of all the Se-containing volatiles is calculated by comparing the GC/ICPMS peak areas of the compounds with the 1 ppm $CH_3SeSeCH_3$ standard injected similarly to sample. Also, there are wide varieties of propyl group-containing sulfur volatiles present in the sample such as methyl propyl disulfide, dipropyl disulfide, propyl propenyl disulfide, propyl trisulfide, etc. (data not shown). Dipropyl disulfide is the most abundant peak in the GC/TOF-MS chromatogram. Since propyl group-containing disulfides are the most abundant sulfur volatiles in onions, this might suggest that methylpropyl selenosulfenate might be an S/Se exchange product between the major propyl group-containing disulfides and dimethyl diselenide.

CONCLUSIONS

A study of the minor Se-containing volatiles in senescing Se-enriched green onions led to the identification of previously unreported selenium species such as MeSSSeMe and MeSeSeSMe. Both isomers of MeS_2SeMe (MeSSeSMe and MeSSSeMe) have been detected, while only one Me_2Se_2S isomer, MeSeSeSMe, was found to be present in the samples. A variety of propyl group-containing selenosulfenates was also identified in the sample. The beneficial complementary use of both atomic and molecular spectrometry to approach the trace level Se volatiles has been demonstrated.

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