Anal. Chem. **2002,** *74,* 5837−5844

Simultaneous Monitoring of Volatile Selenium and Sulfur Species from Se Accumulating Plants (Wild Type and Genetically Modified) by GC/MS and GC/ICPMS Using Solid-Phase Microextraction for Sample Introduction

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A sensitive method for determining ultratrace volatile Se species produced from *Brassica juncea* seedlings is described. The use of a new commercially available GC/ICPMS interface in conjunction with solid-phase microextraction is a promising way to perform these studies. The addition of optional gases (O_2 and N_2) to the argon discharge proved to increase the sensitivity for Se and S as well as for Xe, which as a trace contaminant gas, was used for ICPMS optimization studies. However, the optimization parameters differ when an optional gas is added. In the best conditions, limits of detection ranging from 1 to 10 ppt can be obtained depending on the Se compound and 30 to 300 ppt for the volatile S species. The use of GC/MS with similar sample introduction permits the characterization of several unknown species produced as artifacts from the standards. The method allows the virtually simultaneous monitoring of S and Se species from the headspace of several plants (e.g., onions, garlic, etc.) although the present work is focused on the *B. juncea* seedlings grown in closed vials and treated with Se. Dimethyl selenide and dimethyl diselenide were detected as the primary volatile Se components in the headspace. Sulfur species also were present as allyl (2-propenyl) isothiocyanate and 3-butenyl isothiocyanate as characterized by GC/MS.

The emission of various volatile sulfur and selenium species from plants is important since certain plants can take up these elements from the growth medium and, through several biochemical steps, transform them to volatile species.[1−4] This phenomenon is called phytovolatilization and may be a part of the mechanism governing phytoremediation, important for its potential in cleaning polluted sites. Understanding various biotransformation processes for sulfur and selenium in plants is useful in attempting to make the phytoremediation techniques more effective. Selenium toxicity is encountered in arid and semiarid regions of the world that have seleniferous alkaline soils, and it is also released into the environment by industrial activities such as oil refining. Moreover, the fundamental importance of selenium in human health results in the growing interest in the biochemistry of this element[5,6] and hence the analytical methodology to meet these increasing needs.

The biogenic volatilization of selenium from soil and plant tissues is recognized as an important self-detoxification process that is closely related to the microbial activity.[7] Several studies have also shown that the ability of plants to volatilize Se is influenced by the concentration of Se in the root medium and by the chemical form of Se supplied.[1,4,8] Another important factor is the concentration of sulfate in comparison to selenate in the substrate, since they can compete for the enzymes responsible for the volatilization process.[4] Therefore, the development of enhanced analytical methodologies for the simultaneous monitoring of S and Se volatile species in plants represents an important challenge to the analytical chemist.

Several instrumental methods are used for the detection and determination of volatile Se and S compounds and rely primarily on gas chromatographic (GC) techniques for separation utilizing different detectors; usually gas chromatography with atomic emission detection (GC−AED),[9,10] mass spectrometric detection (GC/MS),[11−16] and flame photometric detection (GC−FPD).[17] GC-AED has been used for the detection of natural-abundance

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10.1021/ac020285t CCC: $22.00
Published on Web 10/01/2002

organoselenium compounds in gaseous samples collected in Tedlar bags from human breath.[9] Similarly, sulfur-containing compounds produced in broccoli seedlings stored in anaerobic conditions have been monitored by GC−FPD when headspace sampling (HS) is used.[17] HS-GC is also a suitable method for the direct analysis of plant volatile species. Where the identification can be made using GC/MS, it also yields results that are almost free from interferences.[12] A newer possibility for element-specific GC detection that has exhibited very promising results is inductively coupled plasma mass spectrometry (ICPMS).[18] ICPMS shows high sensitivity and very good selectivity for diverse sample types.[19] The present work addresses the use of this new instrumental approach in conjunction with an attractive sample introduction method for volatile species, solid-phase microextraction (SPME). Headspace SPME sampling techniques are often the method of choice for volatile compounds, particularly to enhance simplicity and sensitivity for GC separations.[20−22] Both advantageous characteristics will be useful for the extraction and further characterization of Se and S volatile species formed in several plants (e.g., garlic, onion, etc.). However, the main goal of this work is to illustrate the applicability of the developed methodology for the analysis of volatile Se and S species from *Brassica juncea* seedlings. GC/ICPMS and GC/MS, used as complementary techniques with SPME for sample introduction, allow identification of the species and quantification at ultratrace levels. The investigation of several parameters, such as additional gases in the Ar plasma, will be studied to optimize the overall detection and sensitivity characteristics.

EXPERIMENTAL SECTION

An Agilent Technologies (Agilent Technologies, Palo Alto, CA) model HP 6890 series GC was used for the separation of the species. The GC was interfaced to an Agilent 7500s ICPMS (Agilent Technologies; Tokyo, Japan) through a GC/ICPMS interface (Agilent Technologies; Tokyo, Japan). Selenium species were separated 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. For GC/MS, the column was a RTX-5 SILMS, 15 m × 0.25 mm i.d. × 0.25 μm (Restek Corp.; Bellefonte, PA). Control and operation of the system was achieved using the Agilent 7500 ICPMS ChemStation software. Instrumental operating parameters for GC/MS and ICPMS are summarized in Table 1.

Table 1. Operating Conditions for GC/ICPMS and GC/MS

	GC
model	Agilent 6890A
column	capillary column DB-5 (5% dimethylpolysiloxane)
split ratio	5:1
column size	30 m × 0.320 mm × 0.25 μm
inlet liner	SPME injection sleeve, 0.75 mm i.d.
carrier gas	He, 2.4 mL min^{-1}
injector port temperature	200 °C
column temperature	35 °C, 4 min isothermal; then 15 °C min^{-1} to 125 °C; then 5 min isothermal
	ICPMS
model	Agilent 7500s
optional gas	5% N_2 or O_2
rf power	1100 W
sampling cone	nickel (5.5 mm sampling depth)
Ar plasma gas flow rate	15 L min^{-1}
Ar carrier gas flow rate	0.50 L min^{-1}
Ar auxiliary gas flow rate	1.00 L min^{-1}
Isotope monitored	^{77}Se, ^{78}Se; ^{33}S, ^{34}S, and ^{131}Xe (0.10-s dwell-time)
	Interface
	uncoated, deactivated, ∼1 m, 0.320-mm i.d., in stainless steel tube surrounded by Ar/N_2 gas flow. heating via two auxiliary ports at 200 °C
	GC/MS
model	HP 6890
column	RTX-5 SILMS capillary column
split ratio	10:1
column size	15 m × 0.250 mm × 0.25 μm
inlet liner	SPME injection sleeve, 0.75-mm i.d.
carrier gas	He, 1 mL min^{-1}
injector port temperature	200 °C
column temperature	35 °C, 4 min isothermal; then 15 °C min^{-1} to 125 °C then 30 °C min^{-1} to 250 °C
transfer line	280 °C

Headspace solid-phase microextraction (HS-SPME) sampling of volatiles was done using 75-μm Carboxen poly(dimethylsiloxane) (PDMS) SPME fiber and a sampling assembly obtained from Supelco (Bellefonte, PA).

Chemicals and Reagents. All reagents were of analytical grade and were used without any further purification. Dimethyl selenide (DMeSe), dimethyl sulfide (DMeS), and dimethyl disulfide (DMeDS) were purchased from Fluka (Milwaukee, WI). Dimethyl diselenide (DMeDSe) and diethyl disulfide (DEtDS) were purchased from Sigma-Aldrich (Milwaukee, WI). Diethyl diselenide (DEtDSe) was purchased from Strem Chemicals (Newburyport, MA). The stock solutions of 1000 ppm were prepared by dilution of 2.5 μL of the compound with 2500 μL of HPLC grade methanol (Fisher Scientific; Fair Lawn, NJ).

The liquid Ar used to run the ICPMS and the gases evaluated as optional gases (O_2 and N_2) were obtained from Wright Brothers (Cincinnati, OH).

System Description and Procedure. Optional gas is initially mixed with the Ar carrier gas flow. Inside of the oven, the chromatographic column is connected to a fused-silica capillary through a glass connector. The fused-silica capillary is inserted into a stainless steel tube (1-mm i.d.) through a metallic T piece (Swagelok). The carrier gas (Ar + optional gas) then surrounds the fused-silica capillary inside of the stainless steel tube. Heating

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tape/heating packing material to keep the transfer line at a sufficient temperature is wrapped onto stainless steel tubing from the top of the oven to the torch connection of the interface and is electrically heated and controlled by the GC. This device uses a demountable torch with a central channel that consists of a rigid metallic assembly heated by means of a second electric heater (although some inductive heating may occur as well). The operating conditions for the GC/ICPMS and GC/MS are summarized in Table 1. For initial optimization studies, 1 μL of 1 ppm analyte solution in pentane (split 5:1) was carried out.

Sample Treatment. This study was carried out with two different *B. juncea* lines: a transgenic line transformed with tDNA including the Se-cysteine methyl transferase gene and wild-type plants. Homozygous Se-cysteine methyl transferase and wild-type seeds were sterilized by rinsing them 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. Sterilized seeds were sown in a grid pattern in each 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, they were gently placed in 20-mL vials (∼5 cm high × 2 cm diameter) with half strength hydroponic solution and left to stand for 1 day. The composition of the hydroponic solution has been described elsewhere.[23] The next day, Se-containing solutions were added as Na_2SeO_3, Na_2SeO_4, Se-methionine, and KSeCN to the final concentration of 200 μmol of Se L^{-1}, and a poly(tetrafluoroethylene) gray butyl septum was placed on the top and capped to seal (see Figure 3). Control plants received the same treatment, but no Se was spiked in that case. The vials were placed under constant light in a controlled environment room maintained at 25 °C for 1 week. Afterward, the SPME fiber was exposed for 10 min to the vial headspace. The fiber was then immediately inserted into the GC injection port and left to stand for 3 min to thermally desorb the analytes. To ensure that there was no memory effect from previous extractions or condensation of larger analytes inside the pores of the Carboxen phase,[24,25] the SPME fiber was conditioned between runs for ∼5 min in a hot injector port.

RESULTS AND DISCUSSION

Optimization of Instrumental Parameters: Optional Gases in the Discharge. The study of different optional gases mixed with the Ar carrier gas flow stemmed from the observation that the O_2, initially added in order to eliminate the carbon deposits on the cones due to the organic solvents, produced an increase in sensitivity for Se. The possibility was also extended to the use of N_2. Therefore, the study of the different parameters affecting the discharge (rf power or carrier gas flow) in the presence of mixing gases was required to fully characterize the system. For this purpose, two different signals were monitored: the area of DMeDSe (one of the target analytes) and the continuous signal of ^{131}Xe (commonly used for optimization studies and present as a contaminant in the Ar supply gas).

When no optional gas was added, higher rf power yielded an almost exponential decrease in sensitivity of GC/ICPMS for both ^{131}Xe and DMeDSe under constant carrier gas flow with a maximum signal at 500 W. This is in agreement with the report of Tao et al.[26] when a prototype interface similar to the one reported in this work was used. However, the low rf power causes high instability and may even extinguish plasma and, therefore, is not suitable for practical considerations. The addition of oxygen causes an increase of analytical signal as the power is increased, and the addition of nitrogen gives an effect similar to that of oxygen, exhibiting a maximum response at about 1000−1100 W in both cases. The best sensitivity is, overall, achieved working at 1100 W with 5% N_2 as optional gas. This can be explained as due to the relatively high radiative loss for O_2 and N_2 in comparison with the argon, and therefore, the introduction of these gases has a cooling effect on plasma and higher rf power is needed to compensate and achieve the maximum sensitivity.[27,28] No significant changes of the background signal (for ^{77}Se) were observed when the optional gases were added.

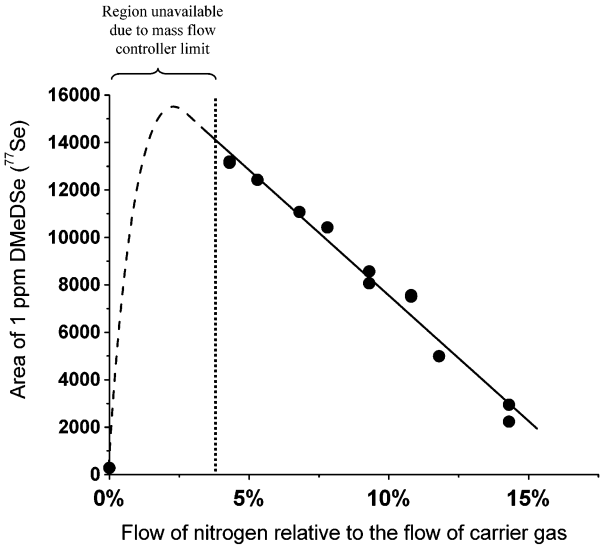

Figure 1. Effect of the percentage of nitrogen as optional gas on the ^{77}Se response (DMeDSe as peak area).

The carrier gas flow rate was also optimized to show that the increase in sensitivity from optional gases was not an effect of increasing the flow rate. No major changes were observed by increasing the carrier gas flow when no optional gas was added or N_2 optional gas was added. However, in the case of O_2 addition, a large increase in sensitivity is observed at ∼0.7 L min^{-1}. Since the presence of Xe was observed with both optional gases (N_2 and O_2), the optimization was performed by monitoring of DMeDSe and DMeSe peak areas. Higher carrier gas flow resulted not only in changes of peak area but also in broadening of the DMeSe peak whereas the width of DMeDSe peak was unaffected. The effect of optional gas amounts relative to the carrier gas was studied using nitrogen. It was observed that increasing above 5% N_2 (since 5% is the minimum amount that the mass flow controller allows) shows a corresponding linear decrease in Se response (see Figure 1).

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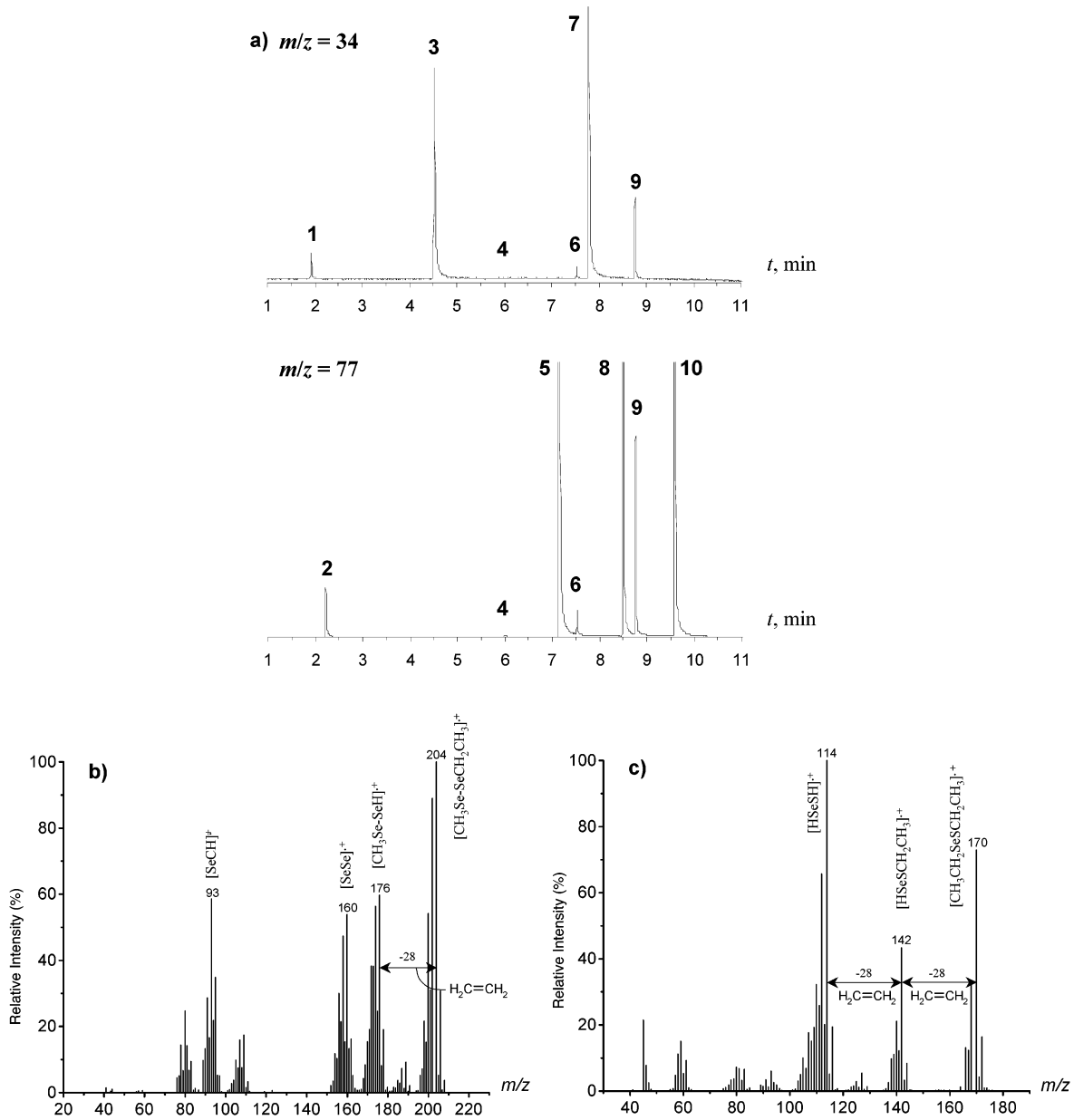

Figure 2. (a) GC/ICPMS chromatogram of mixed volatile selenium- and sulfur-containing 100 ppb of DMeS, DMeDS, DEtDS, and their selenium analogues using the HS-SPME injection technique: (1) DMeS; (2) DMeSe; (3) DMeDS; (4) Me−Se−S−Me; (5) DMeDSe; (6) Me−Se−S−Et and Et−Se−S−Me; (7) DEtDS; (8) Me−Se−Se−Et; (9) Et−Se−S−Et; (10) DEtDSe. Chromatograms are scaled to show all the minor peaks. GC/MS identification of two byproducts. (b) Mass spectrum of ethylmethyl diselenide (8, Me−Se−Se−Et). (c) Ethyl selenoethylsulfenate (9, Et−Se−S−Et).

Separation and Identification of the Se Compounds and Stability of the Different Species. Initial studies on the separation conditions for GC/ICPMS were accomplished by direct injection of a daily prepared solution of the standards (DMeSe, DMeDSe, DEtDSe) in methanol and pentane. The final optimum separation conditions are summarized in Table 1. Solutions several days old (stored in the refrigerator) showed the presence of few unknown species that indicated further investigation was necessary. Figure 2a shows the GC/ICPMS chromatogram obtained from a 1-week-old solution containing 100 ppb (as the individual compounds) of each—DMeS, DMeDS, and DEtDS—and the corresponding Se analogues (DMeSe, DMeDSe, DEtDSe). However, seven different species can be observed (in the Se trace), one of them with an intensity comparable to the standards. Therefore, more concentrated solutions of the same standards were prepared and stored for 1 week for further characterization of the unknown compounds by GC/MS. Identities of all the species shown in Figure 2a were elucidated using GC/MS. Panels b and c in Figure 2 show the mass spectra of the two unknown species.

The compound eluting at ~8.5 min corresponds to the ethylmethyl diselenide (EtMeDSe) formed from the cross-interaction of DMeDSe and DEtDSe. This was observed in aqueous and pentane or methanol solutions. The Se isotopic pattern for a molecule containing two selenium atoms is observed in Figure 2b. Additionally, the formation of selenosulfenates has been also documented (Me−Se−S−Me) due to the interaction of MeSeH and MeSH[29] or DMeDS and DMeDSe.[30] The other minor

Table 2. Analytical Characteristics for Determination of DMeSe, DMeDSe, DEtDSe and DMeS, DMeDS, and DEtDS

compound	detection limit, ppt	retention time, min	peak width at half-height, s	method precision,[b] % RSD	precision of the isotope ratio,[c] %
DMeS	300	1.94 ± 0.02	1.8	7	2.1
DMeDS	80	4.56 ± 0.02	2.3	12	1.7
DEtDS	25	7.78 ± 0.02	1.7	10	2.2
DMeSe	7 (65 ppb)[a]	2.21 ± 0.01	2.4	7	1.1
DMeDSe	1 (7 ppb)[a]	7.13 ± 0.01	2.0	8	2.3
DEtDSe	1 (7 ppb)[a]	9.58 ± 0.01	1.6	13	2.2

[a] DL for direct injection (1-μL solutions in pentane). [b] Evaluated at the level of 100DL ($n = 4$). [c] 34/33 for S and 78/77 for Se.

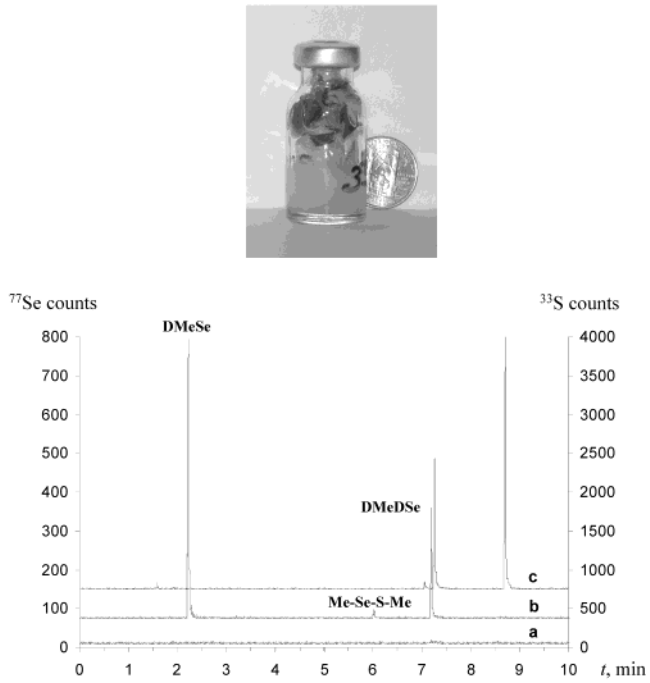

Figure 3. Photograph of the plant in the glass vial and analysis of volatile selenium and sulfur species from genetically modified *B. juncea* treated with SeO_3^{2-}. (a) Se channel from control plant (no Se added); Se (b) and S (c) channels from plant (grown in the presence of SeO_3^{2-}). All chromatograms are offset.

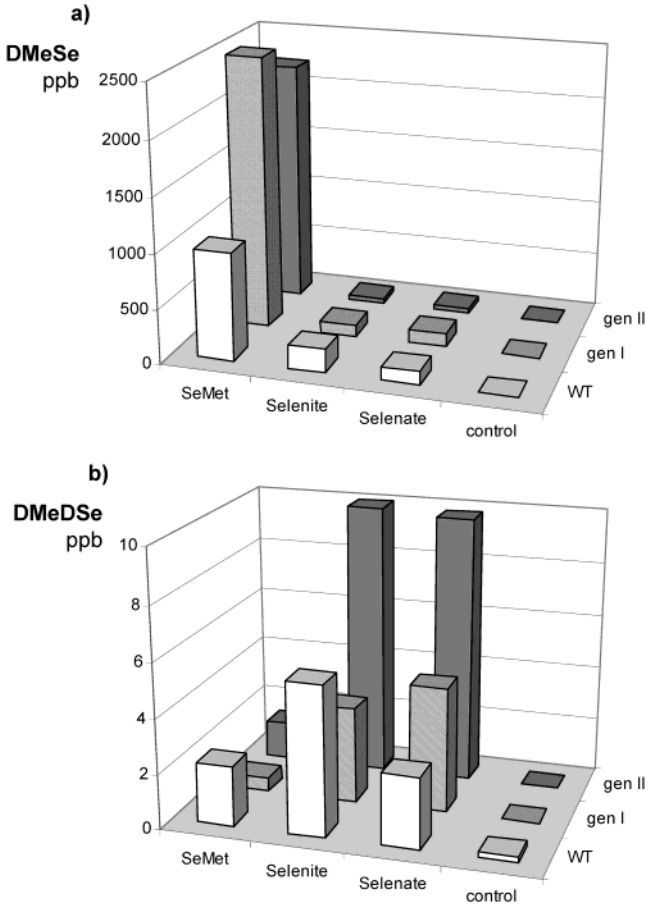

Figure 4. Headspace concentration of DMeSe (a) and DMeDSe (b) from *B. juncea* plants grown with different Se sources. Comparison of wild-type plants (WT) with the genetically modified (gen I and gen II).

unknown species eluting at 8.8 min is ethyl selenoethylsulfenate (Et−Se−S−Et), obtained by cross-interaction of DEtDSe and diethyl disulfide (DEtDS), which is found to be a minor contamination of commercially available DEtDSe. The mass spectrum of Et−Se−S−Et can be observed in Figure 2c. Mass spectrum of Et−Se−S−Et can be easily misinterpreted as that of diethyl selenone, Et−Se(O_2)−Et (as observed in an earlier work[30] with Me−Se−S−Me and dimethyl selenone). However, the simultaneous capability of ICPMS to selectively monitor S and Se signals allows the confirmation of the compound (as can be observed in the chromatogram of Figure 2a where both elements, S and Se, can be observed). Moreover, in the mass spectra of Et−Se−S−Et, the abundance of molecular ion ($m/z = 170$) is significantly higher than that of diethyl selenone.[31] The fact that alkyl selenones are not volatile is often forgotten and thus may lead to such confusion with selenosulfenates. Other cross-products have been also observed when S and Se volatile species are mixed together for a certain period of time, namely, Me−S−Se−Me, Et−S−S−Me, Et−S−Se−Me, and Me−S−Se−Et. The last two compounds cannot be resolved chromatographically either on DB-1 or DB-5 capillary columns.

Analytical Figures of Merit. Detection limits, precision for five manual injections, and linearity up to 0.5 ppm have been evaluated for DMeS, DMeDS, DEtDS, and the corresponding Se analogues; these parameters were evaluated using both direct injection and SPME headspace sampling. Detection limits (DL) were calculated using the formula (1) modified from Brushwyler et al.:[32]

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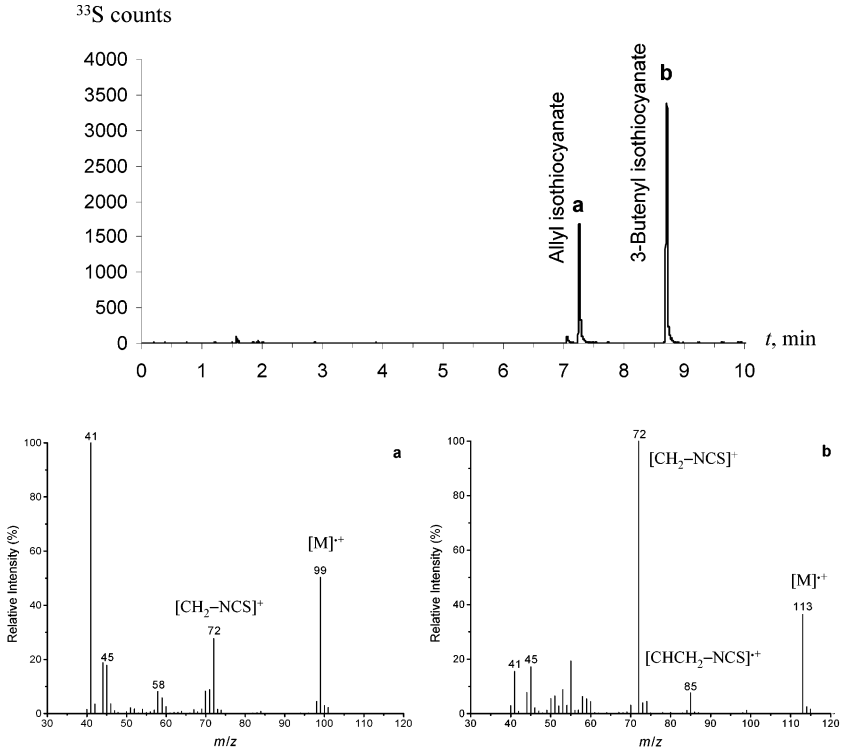

Figure 5. Analysis of sulfur-containing volatile species in wild-type *B. juncea* by HS-SPME-GC/ICPMS and GC-mass spectra of two major headspace volatiles: (a) allyl isothiocyanate and (b) 3-butenyl isothiocyanate.

$$\mathrm{DL} = \lim_{\phi_i \to \mathrm{DL}} \left\{ \phi_i \frac{3 s_b}{A_i} \sqrt{n} \right\} \qquad (1)$$

Here ϕ_i is volume fraction of analyte (ppt, ppb), s_b is the average standard deviation of background near the peak of analyte (∼200 data points), A_i is the peak area of analyte corrected for the background signal, and n is the number of data points in the analyte peak (usually $n = 20-100$). The results can be observed in Table 2 and they were obtained for optimal conditions of rf power and carrier gas flow using 5% N_2 as the optional gas. The optimum exposure time for SPME fiber to the headspace of the standards was 10 min, and only Carboxen PDMS fiber was tested. Quantitation studies were done with aqueous solutions of standards placed in similar vials. No sample shaking, stirring, or heating was performed in order to have a realistic idea of the volatilized species under those conditions (mimicking the situation on the plant seedlings). This table illustrates also retention time, peak width at half-height, and precision of the isotope ratio (78/77 for Se and 34/33 for S). As can be observed in the table, the increase in detection capability for all the species, when SPME is used as sample introduction is dramatic (∼3 orders of magnitude). This shows high promise for detection of volatile Se species at subpicogram levels and S species at picogram levels and drastically improves those presented on the most recent publications obtained for sulfur.[33] The precision in both cases (five manual injections and fiber exposure to five different vials) is adequate for this approach (see Table 2). The responses indicate linearity of up to 4 ppm in both cases (maximum concentration assayed). It should be noted that due to the traces of ^{82}Kr present in the N_2, ^{82}Se could not be monitored, and the results expressed in the table correspond to the ^{77}Se isotope (although ^{78}Se was always simultaneously monitored to be sure of the presence of Se). The reported isotope ratios are slightly higher than expected probably due to the presence of $^{38}Ar^{40}Ar^{+}$.

Detection of Volatile Species in *B. juncea*. *B. juncea* plants were grown hydroponically in nutrient solutions inside a glass vial with a septum cover through which the SPME needle was pierced. Previous studies have shown that the Se volatilization rate is dependent on the form of Se supplied and the uptake of Se is strongly influenced by the presence of S in the growing medium.[8] In the present study, the only S source is the sulfate present in the nutrient solution (∼0.5 mol L^{-1}) and remains constant plant to plant; therefore, no variation in the S species produced can be expected. However, monitoring both Se and S in the headspace of the plant was necessary. As an example, Figure 3 shows the chromatograms corresponding to S and Se present in the headspace of one of the plants enriched with Na_2SeO_3. As can be observed, two main selenium compounds are present from *B. juncea*: DMeSe and DMeDSe. Due to the ultratrace detection limits available with this instrumentation (GC/ICPMS), it was possible to observe some other Se-containing species that could not yet be identified due to the poorer sensitivity of GC/MS and no further attempts at preconcentration were made. Our studies were extended to the samples enriched with different Se sources: Na_2SeO_3, Na_2SeO_4, Se-methionine, and KSeCN. The different enrichment procedures did not provide any significant difference in terms of the species released by the plants (mainly DMeSe and DMeDSe), but important differences were observed in the concentration of these species. Previous studies in this field have shown that wild-type Indian mustard treated with selenate

(33) Rodriguez-Fernandez, J.; Montes-Bayón, M.; Garcia, R. P.; Sanz-Medel, A. *J. Anal. At. Spectrom.* **2001**, *16*, 1051−1056.

accumulates Se mainly as inorganic forms in root and shoot tissues whereas selenite and Se-Met-treated plants accumulate Se in the form of Se-methylselenocysteine.[8,34] Se-methionine is volatilized much more readily than selenate or selenite to form mainly DMeSe through an intermediate selenonium compound (Se-methylselenomethionine). On the other hand, DMeDSe could be produced through Se-methylselenocysteine but in much lesser extent than DMeSe in *Brassica* tissues and very little literature exits describing the presence of this species. This could be ascribed to the lack of highly sensitive/selective techniques for the determination of this species at very low levels.

Parts a and b of Figure 4 show the results obtained for the analysis of the *B. juncea* volatilized DMeSe and DMeDSe, respectively, in the plants treated with different Se sources. The results plotted in the graph correspond to the average of three plants in each case, and the variation plant to plant is within 30%. According to the results previously published, the production of DMeSe is dramatically increased when the Se source is Se-methionine, as described above. An important difference in intensity is observed when comparing the wild type with the genetically modified plants in terms of the production of DMeSe and DMeDSe. This fact is extremely interesting since the DNA modification to overexpress the gene to produce Se-cysteine methyl transferase would only affect the production of Se-methylselenocysteine in the plant, which is not directly correlated to the production of DMeSe. On the other hand, the production of DMeDSe is directly related to the amount of Se-methylselenocysteine through Se-methylselenocysteine−Se-oxide. Results shown in Figure 4b proved such theory, since noticeably higher amounts of DMeDSe are produced in the genetically modified plants.

Enrichment with $SeCN^-$ did not produce any species significantly different from those obtained with any of the other Se enrichments (mainly DMeSe and minimum amount of DMeDSe). The volatilization rate was, in this case, similar to that treated with selenate, which is in agreement with recently published results by De Souza et al.[35]

In terms of the volatile S species detected in the plants, some highly abundant volatile sulfur species were detected by GC/ICPMS (see Figure 5): one of them eluting very close to DMeDSe (7.3 min) and the other one at ~8.7 min. Further identification studies by GC/MS indicated that the main volatile species present in the *B. juncea* headspace are allyl isothiocyanate and 3-butenyl isothiocyanate (found in ratio of 2:1). Mass spectra for these compounds also are given in Figure 5. In both spectra, a stable pattern at $m/z = 72$ corresponds to the $[CH_2{-}N{=}C{=}S]^+$ ion. The mass spectrum of allyl isothiocyanate agrees with the one documented in the literature.[36] Allyl isothiocyanate is the volatile sulfur compound released during the decomposition of leaf tissues of *B. juncea*. These compounds have been observed in each one of the plants analyzed, and their levels have proved to be constant despite treatment or modification used, and therefore, these species could be used as an in vivo internal standard for normalization of the Se signal for run-to-run variations. They have been detected by Bending and Lincoln[37] and are generally

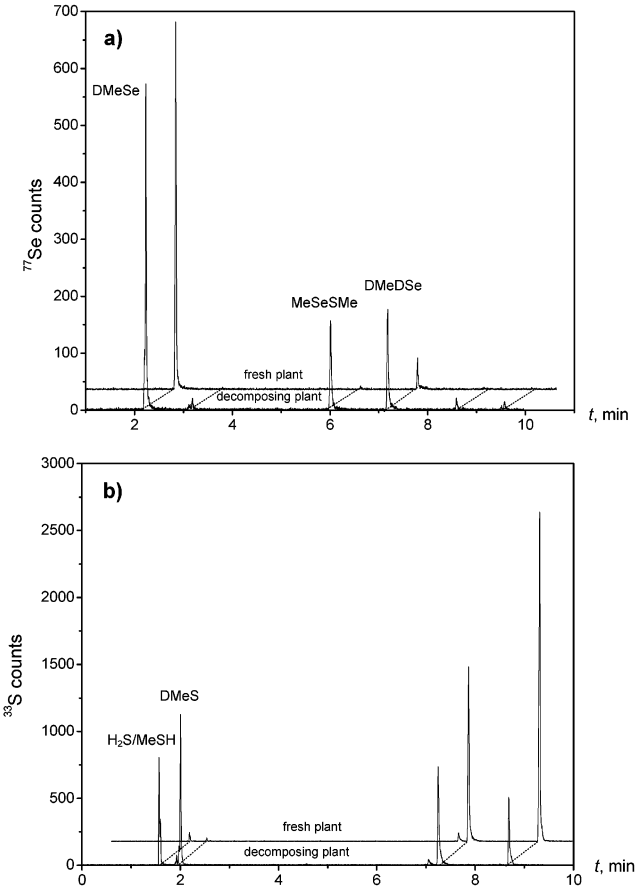

Figure 6. Analysis of sulfur- and selenium-containing volatile species in wild-type *B. juncea* treated with $SeCN^-$. Comparison of selenium (a) and sulfur volatiles (b) in fresh plant versus the decomposing plant.

recognized as decomposition products of the plant glucosinolate sinigrin. One of the species, 3-butenyl isothiocyanate, is formed from the glucosinolate gluconapin.[38] The isothiocyanates are believed to act as a plant defense mechanism during invasion by pathogens or insect pests. They are also speculated to enter the S assimilation pathway to produce other volatile S gases (e.g., DMeS); however, the other volatile S species detected in the plants analyzed in the present study were significantly smaller (see Figure 5).

However, when the plants are left out of the refrigerator and their natural decomposition starts to take place, the production of H_2S and DMeS seems to increase. Figure 6 shows the comparison of the Se and S traces in the fresh plant and in the old one. It is likely possible that the peak assigned to the H_2S is overlapping with MeSH (or is MeSH), as reported for the DB-5 column previously,[33] therefore explaining the eventual increase in Me−Se−S−Me (appears at ~6.0 min) from cross-interaction between DMeDS or MeSH and DMeDSe. The lack of a MeSH standard avoids checking this hypothesis. It should be noted that the abundance of $CH_3{-}Se{-}S{-}CH_3$ is really small in the Se channel, and therefore, the presence of DMeDS is probably not observed on the S trace due to sensitivity problems. Two minor

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(35) De Souza, M. P.; Pickering, I. J.; Walla, M.; Terry, N. *Plant Physiol.* **2002**, *128*, 625−633.
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(37) Bending, G. D.; Lincoln, S. D. *Soil Biol. Biochem.* **1999**, *31*, 695−703.
(38) Chen, S.; Andreasson, E. *Plant Physiol. Biochem.* **2001**, *39*, 743−758.

selenium-containing species from an old plant treated with $SeCN^-$ matches in retention time with MeEtDSe (8.50 min) and DEtDSe (9.58 min).

CONCLUSIONS

The coupled technique of HS-SPME/GC/ICPMS has proven suitable for the speciation of volatile selenium species in plants. The ultratrace detection limits achieved permit the speciation of these compounds at very low levels in biological samples such as plants and require minimal sample treatment.

Use of mixing gases such as oxygen and nitrogen increases the sensitivity of GC/ICPMS for Se. Maximum sensitivity for volatile selenium species can be achieved by mixing 5% nitrogen with the argon makeup gas. Although the use of oxygen or nitrogen leads to spectral interferences, the ^{77}Se minor isotope is not affected and, therefore, can be used for quantification. Monitoring xenon, which is a common impurity in the argon plasma gas, may be used for ICPMS optimization studies, as the behavior of ^{131}Xe in the plasma is similar to that of ^{77}Se.

The use of GC/MS in combination with SPME allows the identification of several unknown species found as decomposition products in the standards and also as volatilization products from the *Brassica* seedlings.

ACKNOWLEDGMENT

The authors thank Agilent Technologies for loan of the 6980 GC system and 7500s ICPMS. We are also grateful to NIEHS under Grant ES04908 for partial support. Presented in part at the 2002 Winter Conference on Plasma Spectrochemistry. Scottsdale, AZ, January 2002.

Received for review April 30, 2002. Accepted August 8, 2002.

AC020285T