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Speciation of Volatile Selenium Species in Plants Using Gas Chromatography/Inductively Coupled Plasma Mass Spectrometry

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Abstract: Gas chromatography/inductively coupled plasma mass spectrometry (GC/ICP-MS) coupled with solid phase micro-extraction can provide a simple, extremely selective and sensitive technique for the analysis of volatile sulfur and selenium compounds in the headspace of growing plants. In this work, the technique was used to evaluate the volatilization of selenium in wild-type and genetically-modified *Brassica juncea* seedlings. By converting toxic inorganic selenium in the soil to less toxic, volatile organic selenium, *B. juncea* might be useful in bioremediation of selenium contaminated soil.

Key words: gas chromatography/inductively coupled plasma mass spectrometry (GC/ICP-MS); solid phase micro-extraction (SPME); selenium speciation; bioremediation; *Brassica juncea*; soil

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Several instrumental methods can be used for the detection and determination of volatile selenium (Se) and sulfur (S) compounds. These rely primarily on gas chromatography (GC) for separation and different detection techniques, including atomic emission detection (AED), mass spectrometric detection (MS), and flame photometric detection (FPD). The present work addresses the use of gas chromatography/inductively coupled plasma mass spectrometry (GC/ICP-MS) in conjunction with an attractive sample introduction method for volatile species, solid-phase micro-extraction (SPME). Sampling and preconcentration can be combined into a single step using the SPME technique, which allows simultaneous adsorption and up to 1 000-fold preconcentration of low molecular weight analytes. To achieve the elemental speciation, an element specific detection with high sensitivity and selectivity is necessary. The ICP-MS meets these requirements.

GC combined with selective SPME minimizes the possibility of co-eluting matrix-based interferences, eliminating the need for conventional sample preparation steps. Therefore, headspace SPME with GC separation and ICP-MS is the hyphenated system of choice for volatile Se and S volatiles from plants. This system allows performing speciation analysis without significant changes in analyte composition. This will be useful for the extraction and further characterization of volatile Se and S species formed in several plants (for example, garlic, onion, etc.). In this application note, the applicability of the developed approach is demonstrated with *Indian mustard*, *Brassica juncea*, seedlings.

1 Phytoremediation and phytovolatilization

Phytoremediation is defined as the use of living plants to remediate contaminated soil or groundwater through removal, degradation, or containment of the pollutants. It is an in situ technique that is gaining public acceptance.

It is known that Se is mainly volatilized as dimethyl selenide, which is 500 – 700 times less toxic than its inorganic forms. This is beneficial in comparison to the biovolatilization of volatile organic compounds (VOCs), which are released to the atmosphere without any changes. Volatilization studies of Se from plant tissues may provide a mechanism of Se detoxification[1].

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2 Experimental conditions

This study was carried out with two different *Brassica juncea* lines：a transgenic line transformed with tDNA including the Se-cysteine methyl transferase（SMT）gene and wild-type plants. Homozygous SMT and wild-type seeds were sterilized by rinsing them on a rocking platform in 96% ethanol for 30 seconds，then in 0.65% sodium chlorate（$NaClO_3$）solution for 30 minutes，and finally in sterile deionized water for 5 – 10 minutes.

Sterilized seeds were sown in a grid pattern in each magenta box on half-strength MS-medium（Sigma）with 10 g/L sucrose and 5 g/L phytagar（Sigma）. After 2 days at 4 ℃，the seeds were gently placed in 20 mL vials（approximately 5 cm high and 2 cm in diameter）with half-strength hydroponic solution and left to stand for 1 day.

The next day，Se-containing solutions were added as Na_2SeO_3，Na_2SeO_4，Se-methionine，and KSeCN to a final Se concentration of 200 μmol/L，and a rubber septum was placed on the top and capped to seal. Control plants received the same treatment，but without the Se-spike. The vials were placed under constant light in a controlled environment room maintained at 25 ℃ for 1 week.

Afterwards，the SPME fiber was exposed for 10 minutes to the vial headspace（HS），then immediately inserted into the GC injection port and left to stand for 3 minutes 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 [2]，the SPME fiber was conditioned between runs for approximately 5 minutes in a hot injector port.

3 Results and discussion

Separation and identification of the Se compounds and stability of the different species GC conditions for GC/ICP-MS were optimized by direct injection of a daily-prepared solution of dimethyl selenide，dimethyl diselenide and diethyl diselenide（DMeSe，DMeDSe，and DEtDSe）standards in methanol and pentane. The final optimum separation conditions are summarized in Table 1.

Table 1 Operating conditions for GC/ICP-MS

GC	
Model	Agilent 6890A
Column	Capillary column DB-5 （5% dimethylpolysiloxane）
Split ratio	5：1
Column size	30 m × 0.320 mm i. d. × 0.25 μm
Inlet liner	SPME injection sleeve，0.75 mm i. d.
Carrier gas	He，2.4 mL/min
Injection port temperature	200 ℃
Column temperature	35 ℃，4 min isothermal；then 15 ℃/min to 125 ℃；then 5 min isothermal.
ICP-MS	
Model	Agilent 7500s
Optional gas	5% N_2 or O_2
RF power	1 100 W
Ar plasma gas flow rate	15 L/min
Ar carrier gas flow rate	0.50 L/min
Ar auxiliary gas flow rate	1.00 L/min
Isotope monitored	^{77}Se，^{78}Se and ^{33}S，^{34}S
Interface	Uncoated，deactivated，approx. 1 m，0.320 mm i. d.，in a stainless-steel tube surrounded by an Ar/ N_2 gas flow. Heating via two auxiliary ports at 200 ℃.

Fresh standard solutions must be used since several-day-old solutions stored in the refrigerator showed the presence of a few unknown species. Fig. 1 shows the GC/ICP-MS chromatogram obtained from a 1-week-old solution containing 100 μg/L（ppb）each（as individual compounds）of dimethyl sulfide，dimethyl disulfide and diethyl disulfide（DMeS，DMeDS，and DEtDS），and the corresponding Se analogs（DMeSe，DMeDSe，and DEtDSe）. Samples were introduced using the HP-SPME technique. However，seven different species can be observed（in the Se trace），one of them with the intensity comparable to the standards. The compound eluting at about 8.5 minutes，peak 8，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. Further characterization of the unknown compounds was performed using GC/MS.

The mass spectra of Et-Se-S-Et can be easily misinterpreted as that of diethyl selenone，Et-Se（O_2）-Et（as observed in the literature with Me-Se-S-Me and dimethyl selenone）. However，simultaneous capabilities of ICP-MS to selectively monitor S and Se signals allow the confirmation of the compound (see Fig. 2 where both elements, S and Se, can be observed). 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 on either DB-1 or DB-5 capillary columns.

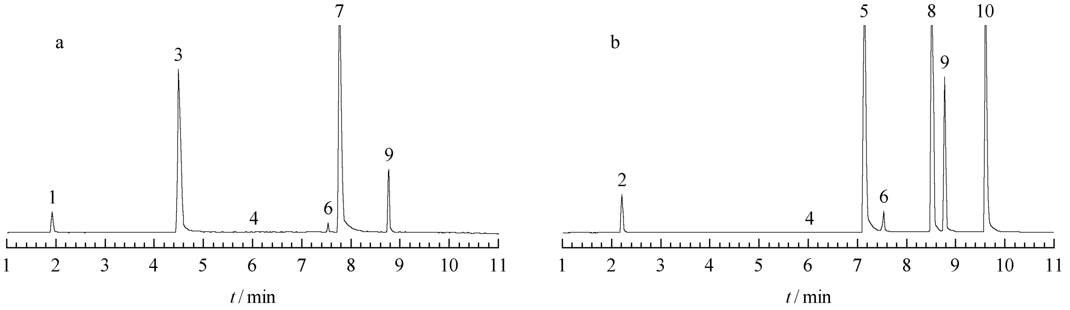

Fig. 1 GC/ICP-MS chromatograms of mixed volatiles containing sulfur (a) and selenium (b)

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.

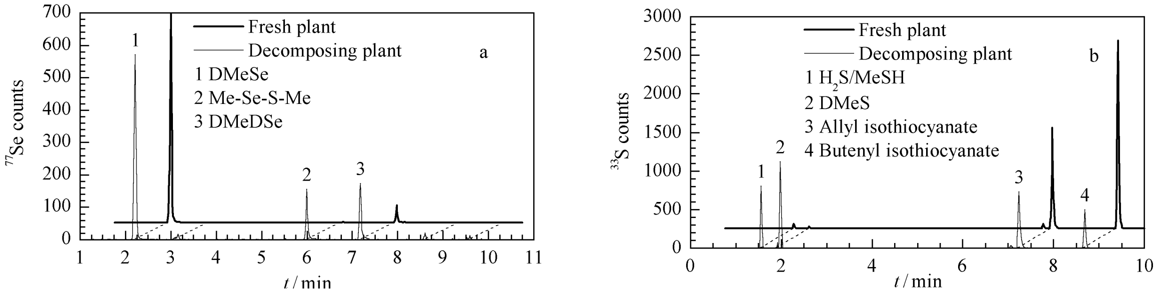

Fig. 2 S-(a) and Se-(b) containing volatile species in wild-type *Brassica juncea* treated with SeCN—

4 Analytical figures of merit

Detection limits (DLs), precision for five manual injections, and linearity up to 0.5 mg/L (ppm) were evaluated for DMeS, DMeDS, DEtDS and the corresponding Se analogs; these parameters were evaluated using both direct injection and HS-SPME sampling.

Observe the results in Table 2 that were obtained for optimal conditions of RF power and carrier gas flow using 5% N_2 as optional gas. This table also illustrates retention time (RT), peak width at half-height, and precision of the isotope ratio (78/77 for Se and 34/33 for S). As seen in the table, because of the preconcentration on the SPME fiber, the increase in detection capability for all the species when using SPME as sample introduction is dramatic (about three orders of magnitude). This shows high promise for detection of volatile Se species at sub-picogram and S species at picogram levels, a significant improvement over literature values for S[3]. 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 4 000 μg/L (ppb) in the case of SPME. Note 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).

Table 2 Analytical performance characteristics for test compounds by SPME/GC/ICP-MS

Compound	DL	RT/min	Peak width at half-height/s	Method precision$^{2)}$ (RSD/%)	Precision of the isotope ratio$^{3)}$ (RSD/%)
DMeS	300 ng/L(ppt)	1.94 ± 0.02	1.8	7	2.1
DMeDS	80 ng/L(ppt)	4.56 ± 0.02	2.3	12	1.7
DEtDS	25 ng/L(ppt)	7.78 ± 0.02	1.7	10	2.2
DMeSe	7 ng/L(ppt) (65 μg/L(ppb))$^{1)}$	2.21 ± 0.01	2.4	7	1.1
DMeDSe	1 ng/L(ppt) (7 μg/L(ppb))$^{1)}$	7.13 ± 0.01	2.0	8	2.3
DEtDSe	1 ng/L(ppt) (7 μg/L(ppb))$^{1)}$	9.58 ± 0.01	1.6	13	2.2

1) DL for direct injection (1 μL solutions in pentane); 2) Evaluated at the level of 100 DL (n = 4); 3) 34/33 for S and 78/77 for Se.

5 Detection of volatile species in *Brassica juncea*

Brassica juncea plants were grown hydroponically in nutrient solutions inside a glass vial with a rubber septum cover through which the SPME needle was pierced. Two main Se compounds are present from *Brassica juncea*：DMeSe and DMeDSe. Due to the ultratrace DLs available with this instrumentation (GC/ICP-MS), it was possible to observe minor additional Se containing species that were not identifiable at that level by GC/MS. Enrichment of samples with different Se sources：Na_2SeO_3, Na_2SeO_4, Se-methionine, and KSeCN 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 accumulate Se mainly as inorganic forms in root and shoot tissues, whereas selenite and Se-methionine treated plants accumulate Se in the form of Se-methyl selenocysteine. The Se-methionine is volatilized much more readily than selenate or selenite to form mainly DMeSe through an intermediate selenonium compound (Se-methyl-selenomethionine). On the other hand, DMeDSe could be produced through Se-methyl-selenocysteine, but to a much lesser extent than DMeSe in *Brassica tissues*. Very little literature exists 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.

According to the results previously published, the production of DMeSe is dramatically increased when the Se source is Se-methionine, described above. The enrichment with KSeCN did not produce any significantly different species to those obtained with any of the other Se enrichments (mainly DMeSe and minimum amount of DMeDSe). Further identification studies by GC/MS indicated that the main volatile species present in the *Brassica juncea* headspace are allyl isothiocyanate and 3-butenyl isothiocyanate. The ratio of these two isothiocyanates is found to be 2∶1, and the ratio is not affected by the different Se treatment procedures. Allyl isothiocyanate is the volatile S compound released during the decomposition of leaf tissues of *Brassica juncea*. These isothiocyanates have been observed on each one of the plants analyzed, and their levels have proved to be constant despite the treatment or modification used. Therefore, these species could be used as an in vivo internal standard for normalization of the Se signal for day-to-day variations.

When the plants are left unrefrigerated and natural decomposition starts to occur, the production of H_2S and DMeS seems to increase. Fig. 2 shows the comparison of the Se and S traces in the fresh plant and in the old one.

6 Conclusions

The coupled technique of HS-SPME/GC/ICP-MS has proven suitable for the speciation of volatile Se species in plants. The ultratrace DLs achieved permits the speciation of these compounds at very low levels in biological samples, such as plants, and requires minimal sample treatment.

Use of auxiliary gases, such as oxygen and nitrogen, increases the sensitivity of GC/ICP-MS for Se. Maximum sensitivity for volatile Se species can be achieved by mixing 5% nitrogen with the argon carrier 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 ICP-MS 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.

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