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Bioaccumulation and Biotransformations of Organic Material-Borne Selenium in Mosquitofish (Determination of Dimethylselenoxide in Solution Using HPLC-HG-AAS)

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PROJECT SUMMARY

Formation of superoxides and oxides such as dimethylselenoxide (DMSeO) from oxidative reactions of methanselenol (CH_3SeH) and dimethylselenide [(CH_3)$_2$Se] is one of the important mechanisms causing Se toxicity in fish and waterfowl. Because of a lack of analytical methods for directly measuring the superoxides and oxides, the mechanisms responsible for causing deformity of fish and waterfowl have not been studied in detail. In this study, an HPLC-HG-AAS system has been used to directly measure dimethylselenoxide (DMSeO) in solution. An HPLC was used to separate DMSeO with other HG-active Se compounds such as selenomethionine (Semet) and selenite [Se(IV)], and an optimum concentration of $NaHB_4$ and HCl was used in the HG system to maximally increase DMSeO signal and decrease interferences from other HG-active Se species, and AAS was used to determine it. A detection limit of DMSeO was <5 µg/L. Recovery of spiked DMSeO in drainage water was close to 100%. This new method will be helpful to study the mechanisms of Se toxicity in fish and waterfowl.
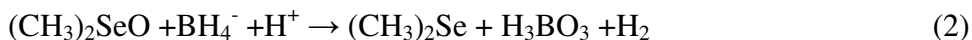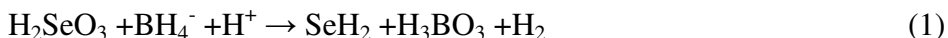
Introduction

Selenium (Se) contamination of wetlands receiving Se-containing water occurs throughout the United States (Engberg and Cappellucci, 1993; Lemly, 1994; Lemly et al., 1993; Presser et al., 1994). Bioaccumulation and transformations of Se in organisms living in these wetlands, through the food chain, creates serious hazards to fish and waterfowl (Hamilton, 2004; Lemly, 1992; Ohlendorf, 1989; Presser and Ohlendorf, 1987; Saiki and Lowe, 1987). Although toxicity of different Se compounds to fish and waterfowl has been studied, the mechanisms of causing deformity of fish and waterfowl have not been illustrated in detail partially because of a lack of analytical methods for some important Se compounds in fish and waterfowl.

One important proposed mechanism of Se toxicity to fish and waterfowl involves the transformation of different Se forms to CH_3Se^- and dimethylselenide [DMSe, (CH_3)$_2$Se], which either enter a redox cycle and generates superoxide, oxides, and oxidative stress, or forms free radicals that bind to and inhibit important enzymes and proteins (Goeger and Ganther, 1994; Spallholz and Hoffman, 2002). Goeger and Ganther (1994) reported that DMSe can be oxidized to dimethylselenoxide (DMSeO) by microsomes from rat liver and lung and by flavin-containing monooxygenase from pig liver. DMSe is considered a detoxification metabolite of different organic and inorganic forms of Se in organisms. Oxidation of DMSe to DMSeO by these enzymes would increase toxicity to organisms due to its higher toxicity than DMSe. DMSeO can also undergo redox cycling and indirectly affect toxicity of Se in organisms. Therefore, developing an accurate method to directly determine these Se oxides would help us to understand Se toxicity in fish and waterfowl.

The hydride generation (HG) technique has been widely used in Se determination due to its low interference problem and high sensitivity. Commonly used agents in a HG system are sodium tetryhydroborate ($NaBH_4$) and HCl. $NaBH_4$ is a reducing agent and can be used for conversion of different Se species present in aqueous solution into volatile hydrides. The reaction between $NaBH_4$ and Se in solution is sensitive to pH. pK_1 of selenous acid [Se(IV)] is approximately 2.5 and it can be fully protonated below pH 2. Therefore, under an acidic condition, Se(IV) can react with $NaNH_4$ to produce selenide [Se(-II)]. Selenic acid [Se(VI)] is not fully protonated (pK_1= -3). Hence, Se(VI) is inactive in the HG system. Some of the organic Se species such as selenomethionine (Semet), selenocystine (Secys), and selenoethionine (Seeth) have been reported to be capable of producing volatile Se in a HG system (Ipolyi et al., 2001;

Chatterjee at al., 2001). For example, similar to Se(IV), Semet has a pK_1 of 2.28, and it is protonated under acidic conditions. Under optimum conditions, Semet after a reaction with $NaBH_4$ can be decomposed to methylhydrogenselenide, which finally dimerizes to volatile DMSe (Chatterjee at al., 2001; Ipolyi et al., 2001). DMSeO [$(CH_3)_2SeO$] has a similar structure to Se(IV) (H_2SeO_3) and it can convert to volatile DMSe as Se(IV) converts to SeH_2 after a reaction with BH_4^- under acidic conditions.

$$H_2SeO_3 + BH_4^- + H^+ \rightarrow SeH_2 + H_3BO_3 + H_2 \qquad\qquad (1)$$

$$(CH_3)_2SeO + BH_4^- + H^+ \rightarrow (CH_3)_2Se + H_3BO_3 + H_2 \qquad\qquad (2)$$

DMSe and SeH_2 formed in a HG system can be transported to the quartz atomizer by nitrogen gas, and then decomposed to Se atoms to generate signals for AAS determination. Therefore, DMSeO can be directly determined in a system coupled with an HPLC, an interface of HG, and an AAS (Fig. 1).

The purpose of this study was to develop a method to directly determine DMSeO in solution. Interferences from other HG-active Se compounds can be removed by choosing an optimum concentration combination of BH_4^- and HCl in a HG system to increase the DMSeO signal and decrease signals of other HG-active Se species. This new developed method will provide a base for further detailed studies of Se toxicity in fish and waterfowl.

Materials and methods

Selenomethionine, methylselenomethionine (metSemet), Secys, and selenium(IV) oxide were purchased from Sigma (St. Louis, MO). DMSeO was provided by Dr. Howard E. Ganther (University of Wisconsin, Madison) and TMSeCl was provided by Dr. I.S. Palmer (University of South Dakota). $NaBH_4$ and other chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

The equipment required for the direct determination of DMSeO is shown in Fig. 1 and Table 1. The HPLC column was directly linked to the continuous HG with a 15-cm Teflon tubing. After a separation of different forms of Se by an HPLC column, the column effluent flowed to the HG system, and then reacted with $NaBH_4^-$ and HCl to produce volatile SeH_2 or DMSe, which were transported to the quartz atomizer by nitrogen gas, and then decomposed to Se atoms to generate signals for AAS determination.

Results and Discussion

The laboratory Se standard consisted of three Se species [DMSeO 250 µg/L, Semet, 1500 µg/L and Se(IV), 120 µg/L] selected in the initial method development. DMSeO is a target Se species; Semet represents organic Se species which are capable of partially producing volatile Se in a HG system, and Se(IV) is used as a reference because it can be fully protonated under acidic conditions.

After injection of 50 µL of the standard solution, three distinct peaks were detected with a retention time of 82.3, 111, and 365 s, which represented DMSeO, Semet, and Se(IV), respectively. It appeared that the peak size of three Se species was related to the concentrations

of BH_4^- and HCl used in the HG system (Figs. 2 and 3). At a given concentration of BH_4^- ranging from 0.05 to 0.6%, there were small changes of DMSeO peak area in a HCl concentration range of 0.05- 10 N. The peak area was relatively higher [6.21-6.87 (ABS × s)] when a lower concentration of 0.05% BH_4^- was used, and was relatively lower [4.44-4.67 (ABS × s)] when a higher concentration of 0.6% BH_4^- was used. In contrast, the peak area of Semet was higher [8.49-9 (ABS × s)] with a higher level (0.6%) of BH_4^-. There was no detectable signal of Semet when a lower level (0.05%) of BH_4^- and higher HCl concentrations of 3, 6, and 10 N were used. The perk area of Se(IV) was relatively higher when 0.3% of BH_4^- was used. There was no detection of Se(IV) at a low level (0.05 N) of HCl. Because the peaks of DMSeO and Semet passing out from the column were very close, higher concentrations of Semet could affect accurate measurement of DMSeO. Therefore, in order to remove possible interferences from Semet, an optimum condition for maximal detection of DMSeO without the detection of Semet is needed for direct measurement of DMSeO. Our results revealed that this optimum condition can be obtained by using 0.05% BH_4^- and 10 N HCl in the HG system. By using this optimum condition, there were also no detectable signals of the other three HG-active organic Se species (Secys, metSemet, and $TMSe^+$) at a level of 1 mg/L (data not shown).

By using 0.05% BH_4^- and 10 N HCl in the HG system and a mobile DI water/10 mM NaOH solution of 40/60% (Table 1), the concentration of DMSeO with a range of 0 to 500 µg/L was well correlated to the peak areas detected by HPLC-HG-AAS (Fig. 4). Correlation coefficient (r^2) was 0.998. Peak areas from five replicate injections of 250 µg/L DMSeO were very close (Fig. 5), with a low standard deviation (7.01±0.067 ABS × s). In order to test whether this optimum condition can be used in real samples to detect DMSeO, a drainage water sample with an EC of 10.2 dS/m was spiked DMSeO (250 µg/L). After six replicate injections of 50 µL, the recovery of DMSeO was close 100% (7.00±0.056 ABS × s).

In order to increase the detection limit of DMSeO with an optimum condition described above, we increased sample injection volume from 50 to 200 µL and tested a concentration range of 0, 5, 10, 25, 50, 100, 250 µg/L. Results showed that the concentration of DMSeO with a range of 0 to 250 µg/L was also well correlated to the peak areas detected by AAS (Fig. 6). Correlation coefficient (r^2) was 0.999. Peak areas from five replicate injections of 250 µg/L DMSeO were also very close (Fig. 5). Detection limit of DMSeO was < 5 µg/L.

Conclusion

This study showed that a coupled HPLC-HG-AAS with an optimum concentration of BH_4^- and HCl in the HG system is capable of directly detecting DMSeO at a low level of 5 µg/L in solution. This new method will help us to study the possible oxidation of volatile DMSe transformed from other Se species, and thus understand the mechanisms of Se toxicity in fish and waterfowl.

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PUBLICATIONS

Zhang, Y. Q., B.C. Okeke, and W. T. Frankenberger, Jr. 2007. Bacterial reduction of selenate to elemental selenium utilizing molasses as a carbon source. Biores. Technol. (in press).

Zhang, Y. Q. and W. T. Frankenberger, Jr. 2007. Supplementing *Bacillus* sp. RS1 with *Dechloromonas* sp. HZ for enhancing selenate reduction in agricultural drainage water. Sci. Total Environ. 372: 397-405.

Table 1. HPLC-HG-AAS system and operating parameters

HPLC	Waters 1525
Columns	Dionex IonPac AS11 analytical column (4 x 50 mm) and Dionex IonPac AS11 analytical column (4 x 250 mm)
Mobile phase	Pump A: DI water. Pump B: 10 mM NaOH
	Flow rate: 1.2 mL/min
	0-1.3 min: A/B, 97/3%
	1.3-3.9 min: A/B, from 97/3% to 40/60%
	3.9-8 min: A/B, 40/60%
Injection volume	50 µL
HG	Varian VGA-76
NaBH4	1 mL/min, concentration range: 0-0.6% in 0.1% NaOH
HCl	1 mL/min, concentration range: 0-10 N
AAS	Varian SpectrAA-10
Wavelength	196 nm
Bandwidth	1.0 nm
Flame	Air-acetylene
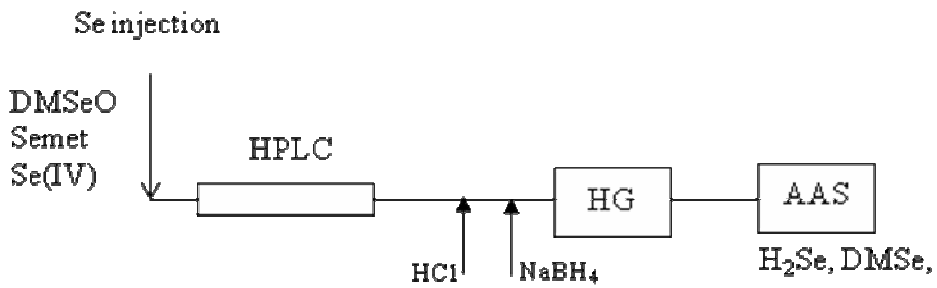
Fig. 1. An HPLC-HG-AAS schematic diagram for determining DMSeO in solution
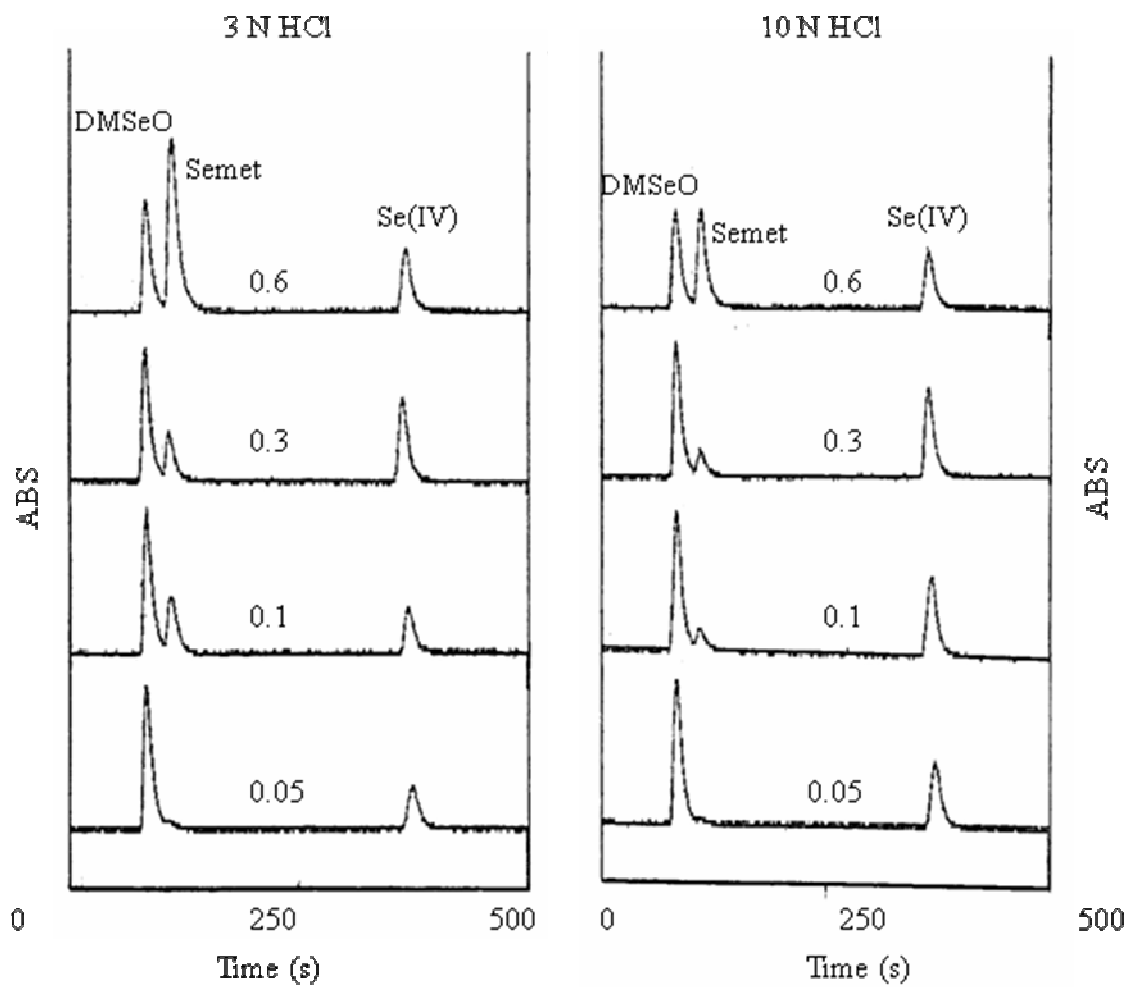
Fig. 2. HPLC-HG-AAS chromatograms of DMSeO, Semet, and Se(IV) with various concentrations of NaBH$_4$ (0.05, 0.1, 0.3, and 0.6%) and HCl (left figure, 3 N HCl and right figure, 10 N). Numbers in the figures showing percentage of NaBH$_4$.
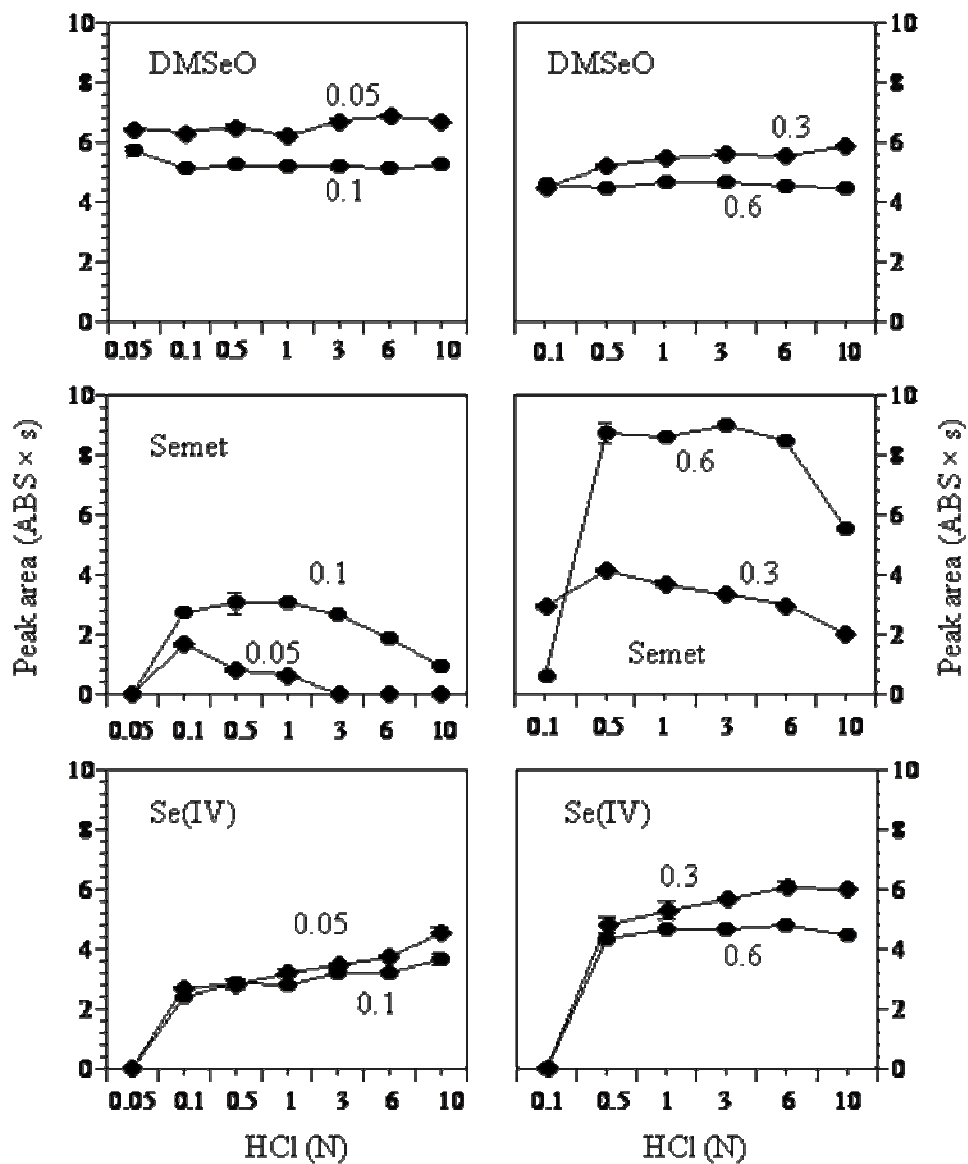
Fig. 3. Changes of peak area of DMSeO, Semet, and Se(IV) with various concentrations of NaBH$_4$ (0.05, 0.1, 0.3, and 0.6%) and HCl (0.05, 0.1, 0.5, 1, 3, 6, and 10 N). Numbers in the figures showing percentage of NaBH$_4$.
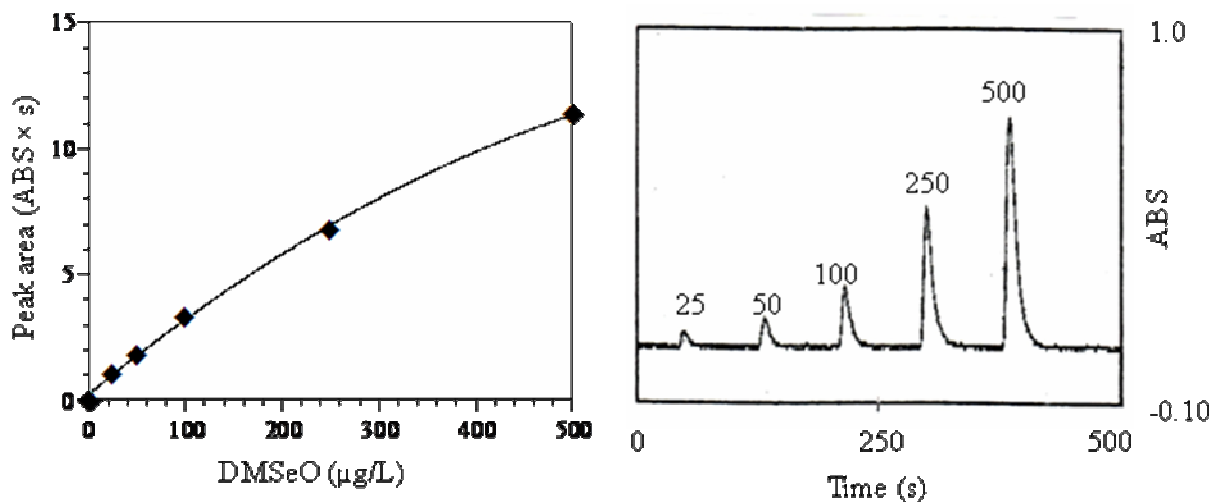
Fig. 4. A calibration curve of DMSeO using an injection (50 µL) of 25, 50, 100, 250, and 500 µg/L (left figure). A HPLC-HG-AAS chromatogram of DMSeO. Numbers in the figures showing concentrations of DMSeO (right figure).
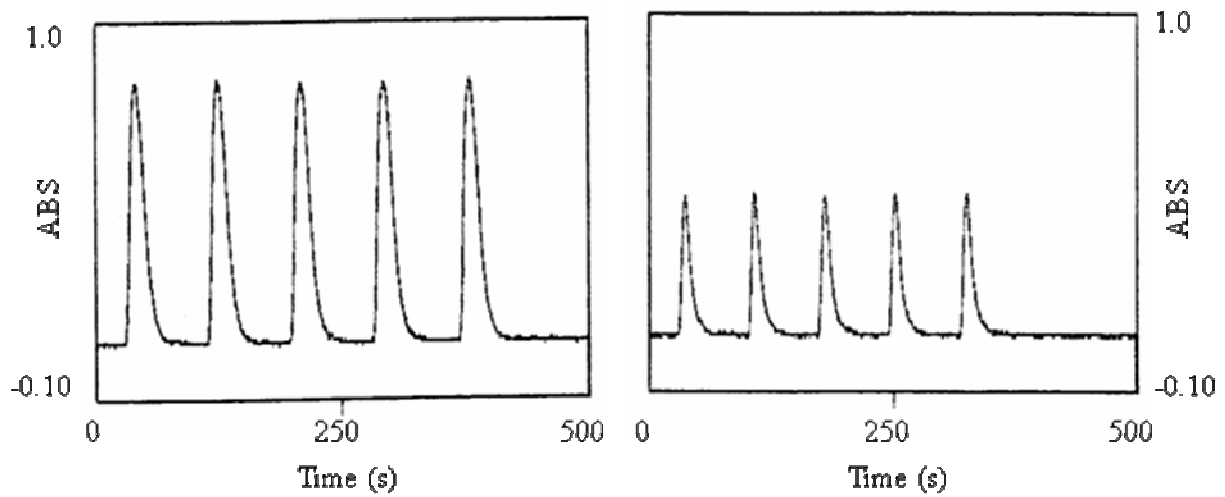
Fig. 5. HPLC-HG-AAS chromatograms of DMSeO with multiple injections of 250 µg/L. Left figure: 200 µL injection; Right figure: 50 µL injection.
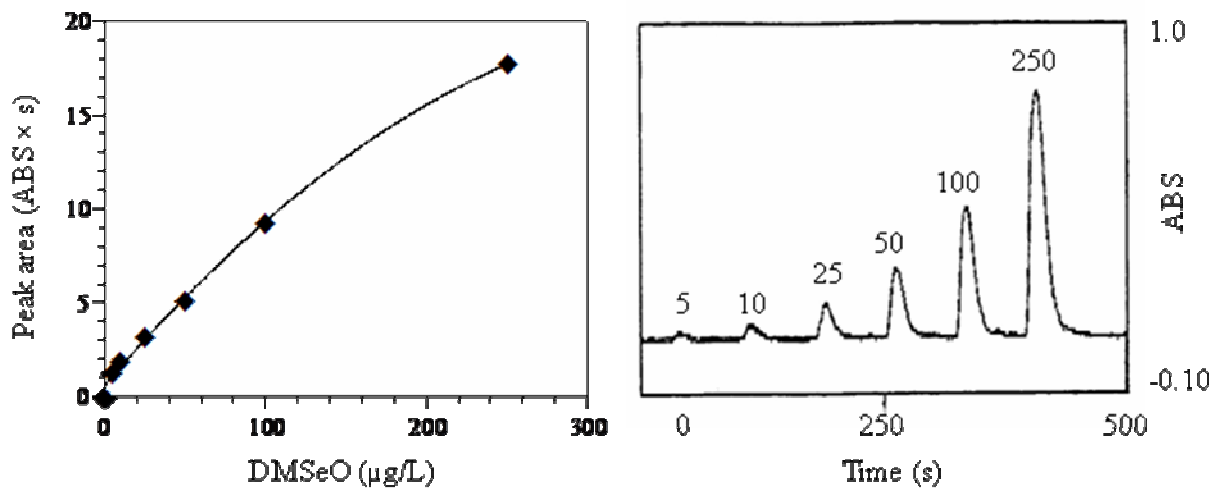
Fig. 6. A calibration curve of DMSeO using an injection (200 µL) of 5, 10, 25, 50, 100, and 250 µg/L (left figure). A HPLC-HG-AAS chromatogram of DMSeO. Numbers in the figures showing concentrations of DMSeO (right figure).