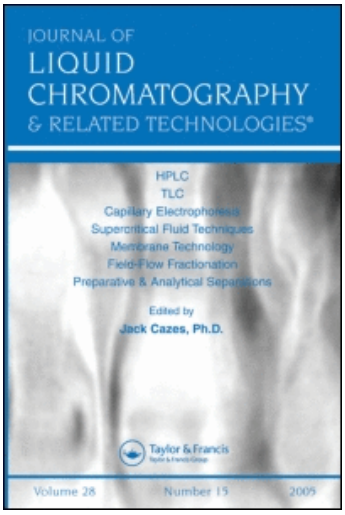


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# Simultaneous Speciation of Arsenic and Selenium Compounds by ION-Chromatography with Inductively Coupled Plasma Mass Spectrometry as Elemental Specific Detector

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# SIMULTANEOUS SPECIATION OF ARSENIC AND SELENIUM COMPOUNDS BY ION-CHROMATOGRAPHY WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY AS ELEMENTAL SPECIFIC DETECTOR

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## ABSTRACT

The feasibility of simultaneous speciation of arsenic and selenium compounds after ion chromatographic separation was investigated using inductively coupled plasma mass spectrometry (ICP-MS) as multielement specific detector. Separation conditions were optimized. Under the optimized conditions, six arsenic species: arsenous acid [As (III)], methylarsonic acid (MA), dimethylarsinic acid (DMA), arsenic acid [As(V)], arsenobetaine (AsB), arsenocholine (AsC), and four selenium species: selenous acid [Se(IV)], trimethylselenonium cation ($TMSe^+$), selenocystine (SeCys), selenomethionine (SeMet), can be separated in the same injection within 8 minutes and determined at environmental concentration levels. The detection limits are estimated in the range of 0.04-0.6 μg/L for arsenic compounds and 0.18-0.35 μg/L for selenium compounds.

## INTRODUCTION

Arsenic and selenium compounds are widely distributed in the biosphere, the different species of arsenic and selenium play an important role in biological and environmental samples. There is considerable evidence to indicate that toxicity and physiological behavior of arsenic depend on its chemical form, arsenous acid [As(III)] and arsenic acid [As(V)] are very toxic, whereas dimethylarsinic acid (DMA), methylarsonic acid (MA), and arsenocholine (AC) are generally less toxic and arsenobetaine (AB) seems to be non-toxic.[1] Selenium shows dual toxic and essential characteristics within a very narrow range.[2] The toxic dose of selenium is very much dependent on its chemical form, with different toxicity for organic and inorganic compounds. Arsenic and selenium speciation became, therefore, the subject of increasing interest in recent years.



Of the many methods used for metal speciation, liquid chromatography coupled to various element-specific detectors is increasingly used in speciation studies. In particular, inductively coupled plasma mass spectrometry (ICP-MS)[3,4] has been frequently chosen for chromatographic detection due to its ease in interfacing as an on-line detector and its ability to both separate interferences from significant peaks and simultaneously determine several elements with very high sensitivity. The combination of HPLC and ICP-MS provides the potentiality for multiement-speciation with a considerable decrease in analysis time, that is, several elements can be monitored simultaneously after the chromatographic separation. Since selenium functions as an antagonist to counteract to the toxicity of arsenic[5], it is essential to get the information on selenium species and arsenic species present in a biological and/or environmental system at same time.

There is little literature reporting the simultaneous speciation of arsenic and selenium compounds.[6-9] Schlegel[6] et al. and Colon and Barry[7] separated As(III), As(V), DMA, and selenous acid [Se(IV)] with ICP-atomic emission spectroscopy (ICP-AES) and alternating current plasma (ACP) detection, respectively. Thompson and Houk[4], Thomas[8] et al. and Guerin[9] et al. separated As(III), As(V), DMA, MA, Se(IV), and selenic acid [Se(VI)] by using HPLC-ICP-MS technique. Generally, arsenic compounds studied were limited to As(III), As(V), DMA, and MA, selenium compounds were limited to only inorganic forms. In the environment, arsenobetaine and arsenocholine are commonly existed, especially in a marine environment. Organic selenium compounds, such as selenomethionine (SeMet), selenocystine (SeCys), have been identified in selenium yeast and several selenium-enriched vegetables;[10,11] trimethylselenonium ion ($TMSe^+$), as a major metabolite of selenium, has been determined in urine and lake water samples.[12,13]

The aim of this study was to develop a fast, sensitive, and accurate simultaneous separation of six arsenic and four selenium compounds considered to be possibly present in environmental and biological samples using isocratic chromatographic conditions. Ion-exchange chromatography coupling ICP-MS as on-line element-specific detector was used. The detection limits, the linear dynamic range, and the precision of the proposed method were determined.

## EXPERIMENTAL

### Chemical and Reagents



All the chemical and reagents used in this study were of analytical grade. The water used for analytical work was purified to 18.2 MΩ cm resistivity. Stock solutions of the arsenic compounds (1000 mg/L As) were prepared from $NaAsO_2$ (Merck), $Na_2HAsO_4.7H_2O$ (Merck), methylarsonic acid (gift from Vineland Chemical Co. USA), dimethylarsinic acid (gift from Vineland Chemical Co. USA), and arsenobetaine bromide and arsenocholine bromide (synthesized in our Lab.). Selenium stock standard solutions (1000 mg/L as Se) were prepared from sodium selenate (Fluka), sodium selenite pentahydrate (Merck), DL-selenocystine (Sigma), DL-Selenomethionine (Sigma), and trimethylselenonium iodide (synthesized in our Lab.). Stock solutions were stored in the dark at 4°C. Working standards were obtained daily by dilution of the stock solutions with NANOpure water.

The mobile phase for chromatography was prepared by dissolving an appropriate amount of tartaric acid to 1 L NANOpure water to get the required concentration. The pH of the mobile phase was adjusted by dropwise addition of 15% aqueous ammonium hydroxide solution. The resulting solutions were filtered through a 0.2 μm membrane filter and degassed before use.

### Instrumentation

Ion chromatography: the chromatographic system consisted of a Milton Roy CM 4000 multi-solvent delivery system, a syringe-loading injector (Model 7125, Rheodyne six-port injection valve) with a 100 μL loop and a PRP-X100 anion-exchange column (Hamilton, Reno, NV, USA; 250 mm x 4.1 mm i.d). A guard cartridge (Hamilton) filled with the same stationary phase protected the analytical column. The chromatographic system was interfaced with the ICP-MS instrument using a 300 mm PEEK (polyether ether ketone) capillary tubing (0.25 mm i.d.) to connect the column outlet to the inlet hole of the nebulizer.

**Table 1**

**The Operating Conditions of HPLC-HHPN-ICP-MS**

| | |
|---|---|
| **HPLC** | |
| Column | PRP-X 100 anion-exchange column (250x4.1 mm. I.D.) |
| Mobile Phase | 15 m mol/L tartaric acid, pH 2.91 |
| Flow rate | 1.5 mL/min |
| Injected volume | 100 μL |
| **HHPN** | |
| Desolvation | heating 150°C, cooling 7°C |
| Nebulizer gas (argon) | 1.0 L/min |
| Back pressure | ~200 bar |
| **ICP-MS** | |
| Plasma power | 1400 W |
| Auxiliary gas flow | 1.1 L/min |
| Cooling gas flow | 13.5 L/min |
| Sampler and skimmer Cones | Ni |
| Monitored signal | $^{75}As$, $^{40}Ar$, $^{32}Cl$ or $^{77}Se$, $^{78}Se$ |
| Time/slice | 0.51 s |
| Mode of monitoring | continuous |



The chromatographic flow rate of 1.5 mL/min was found to be compatible with the nebulizer uptake rate of the ICP-MS instrument. The chromatographic system was also connected to FAAS for the optimization of chromatographic separation conditions.[14]

Inductively coupled plasma mass spectrometry: A VG plasma-Quad 2 Turbo plus (VG Elemental, Winsford, UK) ICP-MS equipped with a hydraulic high-pressure nebulizer (HHPN; Knauer, Berlin, Germany), and a Fassel-type quartz torch were used as an element-specific detector for arsenic and selenium. The efficiency of producing an aerosol with common Meinhard concentric glass nebulizer is only about one percent.[15,16] The HHPN nebulizer increases the aerosol yield up to 30%.[17] The ion intensities at m/z 75 ($^{75}As$) and m/z 78 ($^{78}Se$) were recorded with the time-resolved analysis software© Version 1a (Fisons Scientific Equipment Division, Middlesex, UK). This software is designed specifically for the acquisition of multi-element time resolved signals.

**Table 2**

**Influence of the Tartaric Acid Concentration (pH 2.91) on the Capacity Factor**

| | mmol/L | | | |
|---|---|---|---|---|
| | **5.0** | **10.0** | **15.0** | **20.0** |
| AC | 0.06 | 0.06 | 0.04 | 0.04 |
| AB | 0.34 | 0.32 | 0.33 | 0.34 |
| DMA | 0.54 | 0.54 | 0.53 | 0.53 |
| MA | 1.06 | 0.92 | 0.84 | 0.80 |
| As(III) | 0.92 | 1.16 | 1.25 | 1.44 |
| As(V) | 7.23 | 4.42 | 3.54 | 3.07 |
| $TMSe^+$ | nd | 0.00 | 0.00 | 0.00 |
| SeCys | nd | 0.35 | 0.32 | 0.30 |
| SeMet | nd | 2.52 | 2.33 | 2.34 |
| Se(IV) | nd | 4.32 | 3.57 | 3.03 |



Data from arsenic and selenium were collected and stored in definable time slices and displayed as mass/intensity/time plots. Prior to each HPLC-ICP-MS run, the ion intensity at m/z 87 ($^{87}Rb$) was checked on the rate meter to prevent the instrumental sensitivity from decreasing, aspirating the mobile phase with rubidium at a concentration of 50 ng/mL. The lens settings were adjusted as needed for optimal response of the instrument. For quantification the chromatograms were exported, peak area determined, and the concentrations calculated with external calibration curves using software written in-house.[17] The operating conditions for the HPLC-HHPN-ICP-MS are summarized in Table 1.

## RESULTS AND DISCUSSION

The optimization of the separation with regard to pH values, different tartaric acid concentrations, and ICP-MS nebulization flow rate for arsenic compounds were described in an earlier paper.[18] Since the aim of this work was to perform the separation of As and Se compounds at the same time, with the same chromatographic system, good results for arsenic with the previous system were obtained; we decided to keep the pH of the mobile phase constant and just tried to observe the effect of the tartaric acid concentration. Furthermore, it was preferable to employ isocratic elution in the chromatographic separation because of the relatively simple HPLC instrumentation required in addition to a larger sample throughput (no equilibration time between each run) that could be achieved in this manner. Table 2 summarizes the influence of tartaric acid

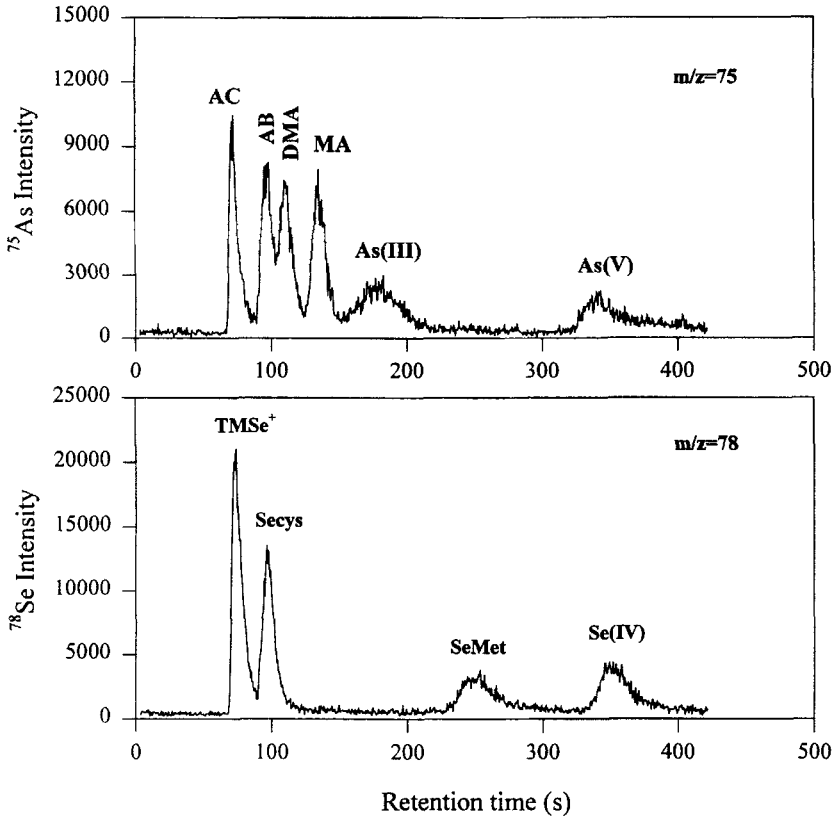


**Figure 1.** HPLC-ICP-MS chromatograms of a mixture of arsenic (0.5 ng) and selenium (1.0 ng) compounds on PRP-X 100 anion-exchange column with 15 m mol/L tartaric acid (pH 2.91) as mobile phase. Flow rate: 1.5 mL/min. Injected volume: 100 μL.

concentration on the capacity factor of investigated As and Se compounds. It was found that increasing the concentration of tartaric acid mainly affected four compounds: MA, As(III), As(V), and Se(IV). The capacity factors of MA, As(V), and Se(IV) decreased, while the capacity factor of As(III) increased. AC and $TMSe^+$ are cations, therefore, they eluted with the void volume in our chromatographic system. At pH 2.91, DMA is present as a neutral molecule ($pK_a$ 6.3) and AB is zwitterionic, thus, the increasing of tartaric acid concentration did not affect their capacity factors. In both seleno-amino-acids, SeCys and SeMet were retained on the column due to their carboxylic groups, however, no distinct variation of capacity factor was observed. Considering the resolution between arsenic compounds, 15.0 mmol/L tartaric acid, pH 2.91 was

**Table 3**

**Detection Limits and Retention Times Using Proposed HPLC-ICP-MS Method**

| Compounds | Retention Time (s) | LOD (μg/L) |
|---|---|---|
| AC | 74 ± 1 | 0.04 |
| AB | 98 ± 1 | 0.09 |
| DMA | 111 ± 2 | 0.12 |
| MA | 136 ± 2 | 0.06 |
| As(III) | 182 ± 2 | 0.20 |
| As(V) | 343 ± 2 | 0.60 |
| $TMSe^+$ | 74 ± 1 | 0.18 |
| SeCys | 98 ± 1 | 0.23 |
| SeMet | 249 ± 2 | 0.35 |
| Se(IV) | 352 ± 3 | 0.30 |



selected as optimized mobile phase. Figure 1 shows the HPLC-ICP-MS chromatograms of separation of six arsenic compounds (5 ng/mL as As) and four selenium compounds (10 ng/mL as Se) at the same injection. The separation can be realized within 8 min.

The detection limits, defined as three times the standard deviation of the blank, are summarized in Table 3. It was found that the detection limits for arsenic compounds are in the range of 0.04-0.6 μg/L As, and for selenium compounds 0.18-0.35 μg/L Se. For the determination of arsenic and selenium by ICP-MS, a potential interference is the formation of a polyatomic species ArCl ($^{38}Ar^{37}Cl$, and $^{40}Ar^{35}Cl$, m/z=75; $^{40}Ar^{37}Cl$, m/z=77 ) resulting from the combination of Ar (from plasma) and Cl (from the sample) in the plasma when high chloride content is present in a sample solution. This should result in the appearance of an additional peak in the chromatogram corresponding to the elution of chloride, and consequently, affect the quantification of arsenic and selenium. In order to investigate whether the argon chloride interferes with the determination of arsenic and selenium compounds in this study, a 1000 mg/L sodium chloride solution was introduced into the HPLC-HHPN-ICP-MS. Surprisingly, ArCl ion was not detected and no peak at m/z 77 ($^{40}Ar^{37}Cl$) was observed within 10 min under our experimental conditions. Then, a conductivity detector was connected with the used chromatographic system, and 10 mg/L $Cl^-$ was injected into the chromatographic system. It was found that $Cl^-$ eluted with a retention time of 14 min. This experimental result demonstrates that the proposed HPLC-ICP-MS method is freedom from the ArCl interference

for the determination of As and Se compounds. The linearity of the calibration curves of investigated arsenic and selenium compounds were determined for concentrations and found to be linear for all compounds in the range of 0-100 ng/mL. Reproducibility was measured by five replicate injections of a test mixture of 10 ng/mL as a relative standard deviation less than 5%.

In conclusion, this work demonstrated that the coupling of the anion-exchange chromatographic separation (PRP-X100) with ICP-MS using a hydraulic high-pressure nebulizer for sample introduction offered the resolution and sensitivity necessary to achieve simultaneous separation of six arsenic and four selenium compounds at ultratrace detection levels, which are required for the simultaneous speciation of arsenic and selenium in environmental and biological samples. The developed method meant freedom from ArCl interference.



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