Wu, Debo ; Pichler, Thomas

Simultaneous speciation analysis of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC

Journal Article	as:	peer-reviewed accepted version (Postprint)
DOI of this document*(secondary publication):		https://doi.org/10.26092/elib/3272
Publication date of this document:		02/09/2024

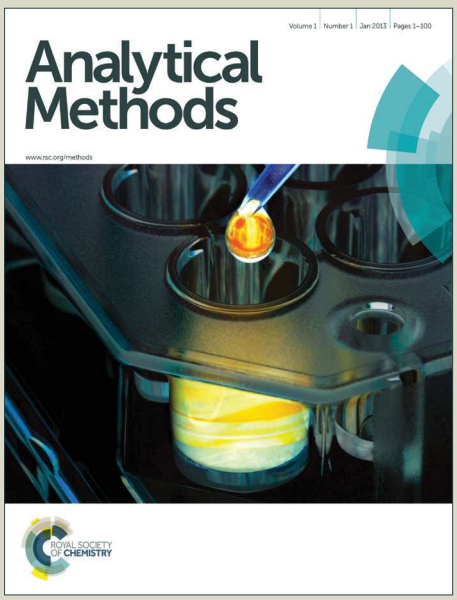
* for better findability or for reliable citation

Recommended Citation (primary publication/Version of Record) incl. DOI:

Wu, Debo / Pichler, Thomas. 2014. Simultaneous speciation analysis of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC. In: Analytical Methods, Vol. 6(14): 5112-5119, Royal Society of Chemistry. http://dx.doi.org/10.1039/C4AY01013B

Analytical Methods

Accepted Manuscript



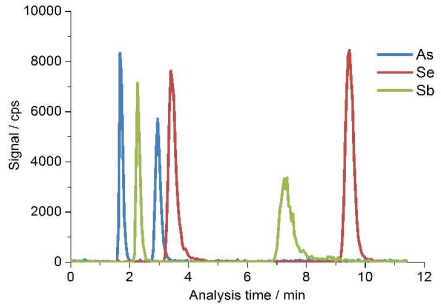
ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Simultaneous speciation analysis of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC

Debo Wu* and Thomas Pichler

A new method for the simultaneous speciation analysis of inorganic arsenic (III, V), antimony (III, V) and selenium (IV, VI) using anion exchange column.



Simultaneous speciation analysis of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC

Debo Wu*[a], Thomas Pichler[a]

[a] Geochemistry & Hydrogeology, Department of Geosciences GEO, Bremen University, 28334 Bremen, PO Box 330440, Germany

Corresponding author: Debo Wu

Tel: +49(0)421-218-65119

Fax: +49(0)421-218-65105

E-mail: debo.wu@uni-bremen.de

Abstract

A new method was developed for the simultaneous speciation analysis of inorganic arsenic (III, V), antimony (III, V) and selenium (IV, VI) in fluid samples by double-focusing sector field-inductively coupled plasma-mass spectrometry (SF-ICP-MS) coupled with high performance liquid chromatography (HPLC). A Hamilton PRX-X100 anion exchange column with EDTA (pH of 4.7) and 3% methanol as mobile phase was used for separation of the six species. The flow rate was set at 1.5 mL min^{-1}. The overall analysis time was shortened down to within 11minutes for all six desired species after a solvent gradient (linear ramp from 5 mM to 30 mM) was introduced in. The detection limits for As(III), As(V), Sb(III), Sb(V), Se(VI) and Se(IV) were 0.02 µg L^{-1}, 0.06 µg L^{-1}, 0.2 µg L^{-1}, 0.02 µg L^{-1}, 0.2 µg L^{-1} and 0.4 µg L^{-1} respectively, which were obtained from 11 replicate measurements of blank. The stability of retention time and linearity of calibration curve were also evaluated. Relative standard deviations (RSD) of ≤ 9% for retention times (at least 20 replicate measurements) and correlation coefficients (R^2) of ≥ 0.9998 for calibration curves (at least 6 replicate experiments) were obtained. Finally, the proposed method was applied to the analysis of one synthetic sample, two hot spring samples and two certified reference materials. The results showed a good spike recovery, indicating that basically no mass loss occurred during chromatographic separation. For two certified reference materials, the detected results were in good agreement with the certified values.

1. Introduction

Despite our effort to better understand the geochemistry of redox sensitive, multi species elements, such as, arsenic (As), antimony (Sb), chromium (Cr), cobalt (Co), copper (Cu) and selenium (Se), there are still large gaps in our knowledge, particularly with respect to their redox behavior in different environments. It has been realized that a full understanding of the redox behavior of these species can help us to: (a) better understand the redox behavior of these elements in different matrices;[1,2,3] (b) develop further studies in the area of toxicity and bioavailability, for example, different toxicity and bioavailability as a function of redox state;[4,5,6,7] (c) evaluate competitive adsorption of, for example, arsenic and antimony, onto hydrous ferric oxide (HFO) surfaces, which in turn would let us better predict their mobility.[8,9,10] However, conventional total element concentration determination did not provide adequate information to completely understand the effect, behavior and fate of these redox species in the environment. In view of this hyphenated analytical techniques such as HPLC-HG-AFS, ETV-ICP-MS[11] and HPLC-ICP-MS[12] were developed to obtain additional information about the distribution of individual redox species. Since the distribution of redox species of a given element in an aqueous solution greatly depends on the species distribution in other redox couples an accurate and rapid simultaneous speciation analysis method for multiple redox couples would be the logical next step towards an improved understanding of redox chemistry in natural systems.

Redox speciation analysis requires analytical technology, which basically includes species separation followed by detection, where due to often low concentrations and minute sample amounts detector can be the weak link. If it is desired to speciate more than one redox couple chromatographic separation becomes critical and difficult, because the chromatography conditions for different elements can vary substantially. For example, inorganic As species are usually separated with anion exchange chromatography with phosphate buffers at neutral pH as mobile phase[13,14] and anion exchange column such as Hamilton PRP-X100 column is most commonly used.[15,16] Se species are commonly separated with anion exchange chromatography as well, with phosphate, ammonium or citrate buffers as mobile phase.[17] However, Sb behaves quite differently, as it has complexing properties and needs the presence of a chelating agent in the mobile phase. EDTA[18,19,20] and phthalic acid[21,22,23,24] have been widely used as mobile phase. In the wide pH range from 2 to 10 in aqueous solutions, Sb(III) exists in the form of non-charged $Sb(OH)_3°$ in liquid, while Sb(V) exists as negatively charged $Sb(OH)_6^-$.[25] Theoretically, $Sb(OH)_3°$ and $Sb(OH)_6^-$ can be separated using an anion exchange column, then $Sb(OH)_3°$ would be eluted in solvent-front, and $Sb(OH)_6^-$ would be retained in the column and eluted out subsequently. However, the fact is that $Sb(OH)_6^-$ elutes close to the solvent front and $Sb(OH)_3°$ is strongly retained in the column and can not be eluted with common mobile phases similar to those used for As and Se speciation. This provides a challenging task in finding the optimum mobile phase, which would allow the separation of As, Se and Sb redox couples. A few methods for the simultaneous speciation analysis of As, Sb and Se are listed in Table 1. Only one of the methods, however, allowed complete speciation analysis of the three redox couples, and was only optimized with synthetic standards.[26] Guerin *et al*.[17] developed a speciation analysis method for As(III, V), Se(IV, VI) and Sb(V), but Sb(III) was not included. Iserte *et al*.[27] and Morita *et al*.[28] only considered the speciation analysis of two of the three elements. Regarding the stationary

phases, anion exchange was the typical choice for the simultaneous separation of those species. However, Morita *et al*.[28] used a reversed phase chromatographic column, basically because they were interested in the separation of other organic As species. For the separation of As and Se species, phosphate and hydrogen carbonate buffers are adequate, when anion exchange chromatography is used. If Sb should be separated together with Se and As species, a chelating agent, such as tartaric acid, malonic acid or EDTA is usually added.

Following a successful chromatographic separation of the three redox couples, simultaneous detection and thus speciation analysis still requires sensitive detection. ICP-AES, ICP-AAS and AFS were extensively investigated but not practically applied, because of low sensitivity and spectral interferences specially for Se.[29,30,31,32] Thus an extra analytical step, such as hydride generation (HG), was used to improve its sensitivity.[33,34,35] However, HG-ICP-AES, HG-ICP-AAS, HG-AFS are not suitable for direct Se(VI) determination, due to its inability to form a Se-hydride.[30,36,37,38]

In this paper we presented a new method for the simultaneous speciation analysis of As(III, V), Sb(III, V), and Se(IV, VI) using double-focusing sector field-inductively coupled plasma-mass spectrometry (SF-ICP-MS) coupled to high performance liquid chromatography (HPLC). In addition to low detection limits and good separation one of the goals was to keep the chromatographic conditions as simple as possible, with the idea that this should facilitate implementation of this method by others interested in the redox behavior of natural aqueous solutions.

2. Experimental

2.1 Instrumentation

2.1.1 Detection

A Thermo Scientific ELEMENT 2 sector field ICP-MS (SF-ICP-MS) was used for the detection of As(III, V), Sb(III, V), and Se(IV, VI). The instrumental conditions and tuning information are given in Table 2. The inlet system consisted of a Scott type double pass spray chamber (G.E.) and a Conikal nebulizer (G.E.), whose uptake rate was compatible with the flow rate of the HPLC (1.5 mL min^{-1}). For As and Sb, isotopes of ^{75}As and ^{121}Sb were monitored. For Se, the less abundant isotope of ^{78}Se or ^{82}Se had to be used due to the $^{40}Ar^{40}Ar$ interference on ^{80}Se. The medium and high resolution modes of the mass spectrometer were checked. However the medium resolution mode was favored because it provided a higher sensitivity than in high-resolution mode and would not cause interference for As analysis. Optimum sensitivity and signal stability after coupling to the HPLC were achieved by adjusting the nebulizer gas slightly (typical setting of 1.0 L min^{-1}). The signal intensity (based on 10 μg L^{-1} standard) for the monitored isotopes are listed in Table 2.

2.1.2 Separation

The high-pressure liquid chromatographic separations were carried out using a Thermo Scientific Accela 1250 Pump and an Hamilton PRP-X100 (Hamilton, Reno, USA) anion exchange column (250 mm × 4.1 mm, 10 μm) at a constant flow rate of 1.5 mL min^{-1}. The instrumentation further consisted of a six-port injection valve and a 50 μL sample loop. The HPLC column was connected via a capillary tube (EzyFit Nebulizer sample Tubing) to a Conikal Nebulizer (G.E.). The chromatography conditions are listed in Table 3. The pHs of all solutions were determined using a pH-meter (pH 340, WTW).

2.2 Reagents and solutions

All solutions were prepared with double deionized water obtained from a Millipore water purification system (MilliQ Advantage A10, 18 MΩ cm).

Stock solutions (1000 mg L^{-1} for each species) were prepared as follows: As(III) from As(III) oxide (As_2O_3, p.a., ACS, Reag. ≥ 99.0% Sigma-Aldrich) dissolved in 4 g L^{-1} NaOH (ACS, Reag. Merck) and preserved in 2% HCl. As(V) from sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, ACS reagent, Sigma-Aldrich) dissolved in water. Sb(III) from potassium antimonyl tartrate trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, ACS, Reag. ≥ 99%, Sigma-Aldrich) dissolved in water. Sb(V) from potassium hexahydroxoantimonate (H_6KO_6Sb, for the precipitation of sodium, ≥ 99.0%, Fluka) dissolved in water. Se(IV) from sodium selenite (Na_2O_3Se, 99%, Sigma) dissolved in water. Se(VI) from sodium selenate (Na_2O_4Se, p.a., ≥ 98.0%, Sigma-Aldrich) dissolved in water. All the stock solutions were kept at 4 °C in the dark and analytical standards were prepared daily by appropriate dilution.

The mobile phase was prepared using EDTA (p.a. AppliChem), the pH of which was adjusted with ammonium (Suprapur, Merck) and formic acid (ACS, Reag. 98-100%, Merck). Other acids such as acetic acid (for synthesis, 99-100%, Merck), phosphoric acid (Suprapur, 85%, Merck) and sulfuric acid (GR for analysis, 95-97%, Merck) were also checked. The mobile phase was filtered through a 0.45 μM membrane (Whatman) before use. To enhance plasma performance methanol (for HPLC, ≥ 99.9%, Sigma-Aldrich) was added to the mobile phase.

SRM 1643e (NIST, National Institute of Standards and Technology) and CRM-SW (High-purity Standards) were used as certified reference materials.

3. Procedure

As a first step As (As(III) and As(V)) and Sb (Sb(III) and Sb(V)) were studied separately under different chromatographic conditions (*e.g.* pH and concentration of mobile phase), with the purpose of exploring information regarding the influence of pH and concentration of the different mobile phases on the retention times. With the knowledge that EDTA works well for the separation of Se,[39] EDTA was the eluent of choice. By changing the concentration, flow rate and pH of the EDTA-based mobile phase retention times for As(III), As(V), Sb(III) and Sb(V) were obtained first individually and then later in combination for As(III, V) and Sb(III, V), and eventually As(III, V), Sb(III, V) and Se(IV, VI). The chromatographic conditions were optimized by adjusting the pH slightly with different acids. Phosphoric acid, sulfuric acid, acetic acid and

formic acid were tested for the adjustment of pH. To further optimize separation and detection various solvent gradients and different methanol concentrations (*e.g.* 1%, 2% and 3% methanol) were investigated. Those separation and detection conditions deemed most efficient were then validated and tested for stability of retention time, linearity, detection limit and recovery. Finally, the method was applied to the analyses of two hot spring samples from Indonesia labeled as J52 and J54, and two CRMs: SRM 1643e and CRM-SW.

4. Results and discussion

4.1 Development of the speciation analysis method

4.1.1 Speciation of As and Sb

Theoretically, the anions and non-charged molecules of a given elemental species, such as $H_3AsO_3°$ and $H_2AsO_4^-$, can be separated in an ion exchange column. This is caused by differences in their charges, charge densities and distribution of charge on their surfaces, which results in different degrees of binding with the ion exchanger. These binding abilities can be controlled by varying the chromatographic conditions, particularly ionic strength (*e.g.* concentration of mobile phase) and pH. Sepciation of Sb, however, adds additional complexity, because $Sb(OH)_3°$ normally precipitates in the colum. Thus a particular mobile phase is needed in order to stabilize $Sb(OH)_3°$ in solution. Since Sb has a more complexing chromatographic behavior than As and Se, we chose EDTA as a starting point for the optimization of As and Sb species separation, with the idea that EDTA could chelate Sb(III) and form a Sb(III)-EDTA anion. The first step was to investigate retention time as a function of mobile phase concentration. Concentrations of 2 mM, 5 mM, 8 mM, 10 mM and 20 mM were tested on an anion exchange PRP-X100 (250 mm × 4.1 mm, 10 μm) column. The pH of mobile phase was adjusted to 4.5. The result showed that, the retention times of As(III) and Sb(V) more or less remained constant in the concentration range from 2 mM to 20 mM, while on the other hand, the retention times of As(V) and Sb(III) decreased, particularly for Sb(III), whose retention time decreased sharply from 2 mM to 10 mM. Considering the interaction of Sb(III) and EDTA, it makes sense that the retention time of Sb(III) was more dependent on the concentration of EDTA. Taking both, resolution and retention time, into consideration, 5 mM seemed to be the optimum concentration of EDTA in the mobile phase, which allowed a relatively short analysis time and good separation of the chromatographic peaks of As(V) and Sb(III). For the flow rate of mobile phase, 1.5 mL min^{-1} was favored, as previous work[40] had shown that 1.5 mL min^{-1} worked well for the As(III) and As(V) speciation analysis on an Hamilton PRP-X 100 anion exchange column. A solution containing 10 μg L^{-1} of each As(III), As(V), Sb(III) and Sb(V) was analyzed with 5 mM EDTA as mobile phase (pH = 4.5). The chromatogram showed that each analyte had a good separation, while the separation as a whole was finished in 16 min.

Our chromatogram revealed an additional peak at around 15 min, which following Hansen *et al*.[41] was interpreted to be that of Sb(V)-polymer. Since EDTA can not chelate Sb(V), and thus Sb(V)-polymer and $Sb(OH)_6^-$ should elute at different times. Hansen *et al*.[41] assumed that the inability to convert Sb(V) complexes into one common complex may partly explain the emergence of unidentified peaks in Sb speciation analysis.[18,22,42] They suggested acidic hydrolysis of samples

with 1M HCl in the presence of chelating ligands such as EDTA and citrate. The same phenomenon was also verified when EDTA and phthalic acid combined were used as mobile phase.[43] In this study whenever the unidentified Sb(V) peak appeared in a chromatogram, it was integrated together with the Sb(V) peak for the calculations of Sb(V) linearity and recovery.

4.1.2 Speciation of As, Sb and Se

The chromatographic conditions obtained in the first step were subsequently applied for the separation of Se(IV) and Se(VI). However, the result revealed that Se speciation has a strong dependency on the acid used for pH control. Thus different acids, such as phosphoric acid, sulfuric acid, acetic acid and formic acid were tested for the adjustment of pH of the mobile phase. Eventually phosphoric acid, sulfuric acid and acetic acid were discarded, because they (specially phosphoric acid) led to a surprisingly high baseline and poor peak shape for Se(IV) and Se(VI). While we did not investigate this further one could assume that the purity of those acids may have played a role. Once formic acid was chosen as the appropriate acid separation and retention were optimized by adjusting the pH. Because pH can affect the species retention time by changing the existing forms of eluent and solute ions, a solution containing 10 μg L^{-1} of As(III), As(V), Sb(III), Sb(V) and 100 μg L^{-1} of Se(IV) and Se(VI) was analyzed using 5 mM EDTA as a mobile phase in the pH range from 4 to 5. The result revealed that the retention times of Se(VI) and the unidentified peaks for a Sb(V)-polymer were shortened by increasing the pH from 4 to 5, while other species remained more or less the same position, indicating less dependence on pH. The behavior of Se(VI) could be explained solely based on the physicochemical properties of the eluent. EDTA is known for its various protonated forms depending on pH; from H_6EDTA^{2+} at very acidic condition to $EDTA^{4-}$ at very basic conditions. It could function as a powerful competing anion and thus has a strong influence on polyvalent anions such as SeO_4^{2-}. The Sb(V)-polymer was affected greatly by pH, probably due to hydrolysis and change of charged situation of Sb(V)-polymer in response to the change in pH. Increasing pH shortened of the total analysis time, but at the same time causing deterioration of the Sb(III) peak shape. Noteworthy, the "unwanted" peak of Sb(V)-polymer became larger with increasing pH, an observation in accordance with Hausen *et al*.[41] who postulated that the Sb(V)-polymer peak would become smaller at a lower pH because of acidic hydrolysis. Compromisingly, a pH value of 4.5 was favored as the optimum acidic condition for the mobile phase.

4.2 Optimization of chromatographic conditions

4.2.1 Addition of methanol

Methanol is one of the most commonly used organic compounds for modification of chromatographic conditions, because it can be used to improve signal intensity during ICP-MS detection and changes retention time during speciation analysis.[17,44] Thus, the logic next step in method development was to investigate the potential to improve separation and detection by adding methanol to the mobile phase. The result was that signal intensity was enhanced greatly for As (As(III) and As(V)) and Se (Se(IV) and Se(VI)) when methanol was added to the mobile phase. The intensity for Sb species, however, was only slightly improved. A suggested explanation for this phenomenon is that the loading of carbon-containing polyatomic ions into the

plasma leads to a strongly increased population of C^+ and/or carbon-containing polyatomic ions and the degree of ionization of a given analyte is improved by transfer of electrons to the carbon ions (or other carbon-containing ions) from that analyte.[45] This would indicate that As and Se species were not fully ionized in the plasma. A combination of 3% methanol and 5 mM EDTA provided the optimum chromatographic conditions although it slightly increased analysis time. To offset this increase the mobile phase pH was changed from 4.5 to 4.7. This shortened the overall analysis time, without remarkable effect on the quality of the peak shapes. The chromatogram obtained under what was considered optimum chromatographic conditions (5 mM EDTA in combination with 3% methanol at pH of 4.7 adjusted with formic acid) showed that all species had a good signal sensitivity and detection was finished in just under 22 min. These species eluted in the order of As(III), Sb(V), As(V), Se(IV), Sb(III) and Se(VI).

4.2.2 Application of a solvent gradient

The first four peaks (As(III), Sb(V), As(V) and (Se(IV)) were sufficiently separated and could be eluted in the first 4 min after injection. However, the remaining peaks for Sb(III) and Se(VI) came much later; at 8.3 min for Sb(III) and 19.1 min for Se(VI), indicating that they were strongly retained in the column, which can also be seen from their broad peak shape and long retention time, thus leading to a pretty long overall analysis time of more than 22 minutes. In order to shorten the separation time and to improve the detection limit of Sb(III) and Se(VI), a further modification was made by applying 30 mM EDTA combined with 3% methanol as a solvent gradient which is a common strategy in chromatography.[46] The gradient program was: 0 - 4.5 min, 97% 5 mM EDTA and 3% methanol; 4.5 - 5.5 min, linear ramp to 97% 30 mM EDTA and 3% methanol; 5.5 - 11min, 97% 30 mM EDTA and 3% methanol. With this gradient setting the first four peaks were kept in their original position and shape, while the two remaining peaks had improved elution time and peak shape. Thus, the sensitivity of Sb(III) and Se(VI) was improved which ensured a lower detection limit and shortened the overall analysis time to 11 min. Despite the variation in composition of the mobile phase, no shift of the base line was observed, which could be a byproduct of applying a gradient during HPLC separation. A chromatogram of a standard solution containing 0.2 μg L^{-1} As(III,V) and Sb(III,V), and 0.5 μg L^{-1} Se(IV,VI) is presented in Fig. 1.

4.3 Validation

Following development a complete evaluation was conducted to evaluate stability of retention time, linearity, detection limit and spike recovery of the method.

4.3.1 Stability of retention time

To determine the retention times for the six species replicate measurements were made. The relative standard deviation (RSD) was calculated in order to check its stability (Table 4. Mean ± RSD, $n \geq 20$). Table 4 shows that the retention times for As(III), As(V), Sb(III), Sb(V), Se(IV) and Se(VI) were 1.70, 2.94, 7.14, 2.28, 3.38 and 9.36 min, respectively. Low RSD of 2% for As(III) and Sb(V) and 4% for As(V) and Se(IV) were obtained from more than 20 replicate measurements. Even after the eluent gradient was introduced, Sb(III) and Se(VI) still had RSDs of less than 10% (8% for Sb(III) and 9% for Se(VI)).

4.3.2 Detection limit

The limit of detection (LOD) was calculated according to the recommendation of the IUPAC (International Union of Pure and Applied Chemistry), as the corresponding concentration of 3 times the standard deviation for the signal/noise (S/N) ratio for each species. Blank solution (Milli-Q water) spiked with concentrations close to the detection limit of all six desired species (0.2 μg L^{-1} for As(III,V) and Sb(III,V), and 0.5 μg L^{-1} for Se(IV,VI)) were measured 11 times to calculate the corresponding detection limits. Fig. 1 showed the chromatography close to the detection limit. The detection limits for As(III), As(V), Sb(III), Sb(V), Se(VI) and Se(IV) were 0.02 μg L^{-1}, 0.06 μg L^{-1}, 0.2 μg L^{-1}, 0.02 μg L^{-1}, 0.2 μg L^{-1} and 0.4 μg L^{-1} respectively (Table 4), which in general were better than the LODs for those methods listed in Table 1. In addition to S/N, peak height and peak area for each species were also calculated to confirm their detection limits and similar results were obtained.

4.3.3 Linearity

The linearity of each calibration curve was examined for different concentration ranges with at least 5 standard points. The linearity for As(III, V) and Sb(III, V) was investigated for the concentration range from 0.5 to 75 μg L^{-1}, and the range from 5.0 to 200 μg L^{-1} was used for Se(IV, VI), which are the regulatory concentration ranges for many different water types, including ground water.[47,48] The results showed that detection in these concentrations ranges was linear, as demonstrated by excellent correlation coefficients. The linear correlation coefficients (R^2), obtained from at least 6 replicate experiments, were 0.9999 for As(III) and Se(IV), and 0.9998 for As(V) (Table 4).

4.3.4 Recovery

To our knowledge, only a few methods focused on the simultaneous analysis of As, Se and Sb species in fluid samples have been reported. In the absence of a certified reference material (CRM), which contains As(III, V), Sb(III, V) and Se(IV, VI) the strategy was to carry out spike recovery experiments for each species, which enabled us to perform a quantitative evaluation of the method. The chromatographic recovery for every individual species was estimated by calculating the quantity of the species eluted from the column as a percentage of the amount injected into the column.

Firstly, a synthetic sample prepared with deionized water with low concentrations approximately from 1.0 to 2.5 μg L^{-1} for all species were analyzed. A duplicate sample was spiked with 1.0 μg L^{-1} As(III, V), Sb(III, V) and 2.0 μg L^{-1} Se(IV, VI). Both the synthetic sample and the spiked one were measured 3 times and the spike recovery was calculated (Table 5). Subsequently, spike recoveries were tested using two "real" samples labeled as J52 and J54. The two samples were from two hot springs in Cisolok and Patuha on Java Island, Indonesia. The initial temperature and pH for sample J52 in the field were 102.0 ℃ and 8.1 and the initial temperature and pH for sample J54 were 32.9 ℃ and 1.0. Both J52 and J54 were chloride rich samples; 305.6 mg L^{-1} for J52 and 35.2 mg L^{-1} for J54. The samples were acidified with HCl and preserved in the dark at room temperature after sampling. These two samples were diluted 5 fold with deionized water prior to spiking to bring the concentrations into the calibration and linearity range of the method.

For sample J52, 20 μg L^{-1} As(III, V), 1.0 μg L^{-1} Sb(III, V) and 5.0 μg L^{-1} Se(IV, VI) were added as spikes. For sample J54, 5 μg L^{-1} As (III), Se(IV, VI) and 0.5 μg L^{-1} Sb(III, V), As(V) were added. Each sample (with and without spikes) was measured at least 3 times and the results are listed in Table 5. It can be seen that: (i) Except the recovery of As(III) for synthetic sample (89%) and Sb(III) for sample J52 (123%), all the other species had a spike recovery of 90 - 110%, indicating that almost no mass loss happen in chromatography. (ii) In the two analyzed hot spring samples, the concentration of As is much higher than Sb and Se. In sample J52 only As(V) and Sb(V) were detectable probably because of oxidation caused by inappropriate preservation. (iii) Although, in the two hot spring samples no Se species were determined. However, the good recovery (98.8% and 107.6% for Se(IV), and 97.8% and 109.4% for Se(VI)) indicated that the proposed method could be successfully applied to Se(IV, VI) speciation analysis in real samples.

4.4 Application and matrix interference

The proposed method was finally applied to two CRMs: SRM 1643e (NIST, National Institute of Standards and Technology) and CRM-SW (High-purity Standards). It is worth noting that in the CRM-SW no Sb species were present. The two CRMs were measured without dilution, and 3 replicate measurements were carried out. In SRM 1643e As(V) was predominant and only trace amounts of As(III) were detected. In the case of Sb and Se, only Sb(V) and Se(IV) were detected. In CRM-SW, only As(V) and Se(IV) were detected. The results were in good agreement with the certified values (Table 6).

Matrix interferences were evaluated, including the potential $^{40}Ar^{35}Cl^{+}$ interference on As analysis, and the matrix effect on species retention time and results. In order to check if Cl^{-} can cause interference for As species, a standard solution containing 10 μg L^{-1} of the six desired species and 500 μg L^{-1} Cl^{-} was injected into the column. The result showed that Cl^{-} was eluted at 6.11 min, indicating that it would not cause any interference for As analysis because As(III) and As(V) were eluted at 1.70 and 2.94 min respectively. The retention times of the six species were evaluated again when analyzing the two hot spring samples and the two CRMs. The results showed no shift for those species detected in the hot spring samples. In SRM 1643e and CRM-SW, the detected species generally showed a slightly earlier elution than the standards. However, no obvious matrix influence could be inferred.

5. Conclusion

In this paper, a new simultaneous speciation analysis method focused on inorganic redox species of As(III, V), Sb(III, V), and Se(IV, VI) based on HPLC-SF-ICP-MS was developed using an anion exchange column. EDTA combined with 3% methanol was used as mobile phase. The total analysis time was less than 11 minutes by introducing in a solvent gradient. The inlet system of detection consisted of a scott type double pass spray chamber and a conikal nebulizer. All species were measured free of interference. Linear correlation coefficients of ≥ 0.9998 for all calibration curves were obtained. The method showed a low detection limit, generally lower than related report, for each desired species. Most species in the analyzed samples had a spike recovery of 90 - 110%. No inter-conversion between species or mass loss during chromatography was observed. The study confirmed the complexing property of Sb that Sb(III) rather than Sb(V) had a strong

affinity in the column, and Sb(V) existed in the form of $Sb(OH)_6^-$ and Sb(V)-polymer. The method is characterized with simple eluent composition, short overall analysis time, low detection limit, good linearity and reliable repeatability of retention time, and thus could be safely applied to a variety of fluid samples.

Acknowledgements

We thank Maria Jose Ruiz-Chancho for her constructive help at the early age of the study. Also thank Budi Joko Purnomo for his donation of the two hot spring samples collected from Java, Indonesia. TP acknowledges support by the German Research Foundation (DFG).

Reference

1. C. Breuer and T. Pichler, *Chem. Geol.*, 2013, **348**, 2–14.

2. N. Vodyanitskii, Yu, *Eurasian Soil Sci.*, 2010, **43**, 37–46.

3. A.-K. Leuz, 2006.

4. R. E. Price, J. London, D. Wallschläger, M. J. Ruiz-Chancho, and T. Pichler, *Chem. Geol.*, 2013, **348**, 48–55.

5. B. J. Mailloux, E. Alexandrova, A. R. Keimowitz, K. Wovkulich, G. A. Freyer, M. Herron, J. F. Stolz, T. C. Kenna, T. Pichler, M. L. Polizzotto, H. Dong, M. Bishop, and P. S. K. Knappett, *Appl. Environ. Microbiol.*, 2009, **75**, 2558–2565.

6. R. E. Price, J. P. Amend, and T. Pichler, *Appl. Geochemistry*, 2007, **22**, 2595–2605.

7. V. Jamier, L. A. Ba, and C. Jacob, *Chem. Eur. J.*, 2010, **16**, 10920–10928.

8. Y. Nakamaru and K. Sekine, *Soil Sci. Plant Nutr.*, 2008, **54**, 332–341.

9. K. M. Campbell, D. Malasarn, C. W. Saltikov, D. K. Newman, and J. G. Hering, *Environ. Sci. Technol.*, 2006, **40**, 5950–5955.

10. S. C. Wilson, P. V Lockwood, P. M. Ashley, and M. Tighe, *Environ. Pollut.*, 2010, **158**, 1169–1181.

11. Y. Li, B. Hu, M. He, and G. Xiang, *Water Res.*, 2008, **42**, 1195–1203.

12. M. Moldovan, E. M. Krupp, A. E. Holliday, and O. F. X. Donard, *J. Anal. At. Spectrom.*, 2004, **19**, 815–822.

13. J. Zheng, H. Hintelmann, B. Dimock, and M. S. Dzurko, *Anal. Bioanal. Chem.*, 2003, **377**, 14–24.

14. J. A. Day, M. Montes-Bayón, A. P. Vonderheide, and J. A. Caruso, *Anal. Bioanal. Chem.*, 2002, **373**, 664–668.

15. L. S. Milstein, A. Essader, E. D. Pellizzari, R. a Fernando, and O. Akinbo, *Environ. Int.*, 2002, **28**, 277–83.

16. A. J. Bednar, J. R. Garbarino, M. R. Burkhardt, J. F. Ranville, and T. R. Wildeman, *Water Res.*, 2004, **38**, 355–364.

17. T. Guerin, M. Astruc, A. Batel, and M. Borsier, *Talanta*, 1997, **44**, 2201–2208.

18. N. Miekeley, S. R. Mortari, and A. O. Schubach, *Anal. Bioanal. Chem.*, 2002, **372**, 495–502.

19. S. Canepari, E. Marconi, M. L. Astolfi, and C. Perrino, *Anal. Bioanal. Chem.*, 2010, **397**, 2533–2542.

20. M. Krachler, H. Emons, and J. Zheng, *Trends Anal. Chem.*, 2001, **20**, 79–90.

21. P. Smichowski, Y. Madrid, and C. Cámara, *Fresenius. J. Anal. Chem.*, 1998, **360**, 623–629.

22. H. R. Hansen and S. A. Pergantis, *Anal. Chem.*, 2007, **79**, 5304–5311.

23. J. Zheng, A. Iijima, and N. Furuta, *J. Anal. At. Spectrom.*, 2001, **16**, 812–818.

24. G. Ceriotti and D. Amarasiriwardena, *Microchem. J.*, 2009, **91**, 85–93.

25. K. Takayanagi and D. Cossa, *Water Res.*, 1997, **31**, 671–674.

26. T. Lindemann, a. Prange, W. Dannecker, and B. Neidhart, *Fresenius. J. Anal. Chem.*, 1999, **364**, 462–466.

27. L. Orero Iserte, a. F. Roig-Navarro, and F. Hernández, *Anal. Chim. Acta*, 2004, **527**, 97–104.

28. Y. Morita, T. Kobayashi, T. Kuroiwa, and T. Narukawa, *Talanta*, 2007, **73**, 81–6.

29. P. C. Uden, *Anal. Bioanal. Chem.*, 2002, **373**, 422–431.

30. X. Dauchy, M. Potin-Gautier, A. Astruc, and M. Astruc, *Fresenius. J. Anal. Chem.*, 1994, **348**, 792–805.

31. C. B'Hymer and J. A. Caruso, *J. Chromatogr. A*, 2006, **1114**, 1–20.

32. J. Bowman, B. Fairman, and T. Catterick, *J. Anal. At. Spectrom.*, 1997, **12**, 313–316.

33. J. Y. Cabon and C. Louis Madec, *Anal. Chim. Acta*, 2004, **504**, 209–215.

34. H. Wu, X. Wang, B. Liu, Y. Liu, S. Li, J. Lu, J. Tian, W. Zhao, and Z. Yang, *Spectrochim. Acta Part B At. Spectrosc.*, 2011, **66**, 74–80.

35. B. A. Fernandez, C. V Temprano, M. R. F. Campa, A. Sanz-Medel, and P. Neil, *Talanta*, 1992, **39**, 1517–1523.

36. I. Ipolyi and P. Fodor, *Anal. Chim. Acta*, 2000, **413**, 13–23.

37. L. Kozak and P. Niedzielski, *Anal. Lett.*, 2011, **44**, 2312–2320.

38. R. C. Richter, K. Swami, S. Chace, and L. Husain, *Fresenius. J. Anal. Chem.*, 1998, **361**, 168–173.

39. B. R. E. Wolf, S. A. Morman, J. M. Morrison, and P. J. Lamothe, *Simultaneous Speciation of Arsenic , Selenium , and Chromium by HPLC-ICP-MS*, Denver, 2008.

40. M. J. Ruiz-Chancho, T. Pichler, and R. E. Price, *Chem. Geol.*, 2013, **348**, 56–64.

41. C. Hansen, B. Schmidt, E. H. Larsen, B. Gammelgaard, S. Stürup, and H. R. Hansen, *Analyst*, 2011, **136**, 996–1002.

42. S. Foster, W. Maher, F. Krikowa, K. Telford, and M. Ellwood, *J. Environ. Monit.*, 2005, **7**, 1214–1219.

43. S. Amereih, T. Meisel, E. Kahr, and W. Wegscheider, *Anal. Bioanal. Chem.*, 2005, **383**, 1052–1059.

44. N. Ulrich, *Anal. Chim. Acta*, 1998, **359**, 245–253.

45. E. H. Larsen and S. Stürup, *J. Anal. At. Spectrom.*, 1994, **9**, 1099–1105.

46. K. Müller, B. Daus, J. Mattusch, H.-J. Stärk, and R. Wennrich, *Talanta*, 2009, **78**, 820–826.

47. J. A. Plant, D. G. Kinniburgh, P. L. Smedley, F. M. Fordyce, B. A. Klinck, and B. G. Survey, *Treatise on Geochemistry*, 2006, 9, 17–66.

48. M. Filella, N. Belzile, and Y. Chen, *Earth-Science Rev.*, 2002, **59**, 265–285.

Table 1. Review of various chromatography methods of simultaneous separation of inorganic species of As, Sb and Se.

	Matrix	Column	Eluent	Species	Method Information	Reference
As **Se**	Sediment extracts	Anion exchange Hamilton PRX-X100 (250 mm × 4.1 mm, 10 μm)	Gradient elution: A: 10 mM $NH_4H_2PO_4$ pH = 6 (with ammonia) B: 200 mM $NH_4H_2PO_4$ pH = 6 (with ammonia)	As(III) As(V) MA(V) DMA(V) Se(IV) Se(VI)	Flow = 1.0 mL min^{-1} Injection volume = 100 μL Time: < 10 min LOD*: 2 - 40 ng g^{-1} Spike recovery: 80-120%, (except As)	27
As **Se** **Sb**	Standards	Dionex IonPac AS14, (250 mm × 4 mm) with IonPak AG 14, (50 mm × 4 mm)	Gradient elution: A: 2 mM ammonium hydrogen carbonate + 2.2 mM tartaric acid pH = 8.2 (with ammonia) B: 2 mM ammonium hydrogen carbonate + 45 mM tartaric acid pH = 8.2 (with ammonia)	As(III) As(V) MA(V) DMA(V) Se(IV) Se(VI) Sb(III) Sb(V)	Flow = 1.0 mL min^{-1} Injection volume = 50 μL Time: ≈ 15 min LOD*: 4.5 μg L^{-1} (Sb(III) and Se species) 0.5 μg L^{-1} (others) RSD#(retention time): < 2%	26
As **Sb**	Hot spring water, fish sample	Develosil C30-UG-5 (250 mm × 4.6 mm, 5 μm)	Isocratic elution: 10 mM sodium butanesulfonate + 4 mM malonic acid + 4 mM tetramethylammonium hydroxide + 0.1% (v/v) methanol 20mM ammonium tartrate pH = 2.0	As(III) As(V) MA(V) DMA(V) AsB AsC TMAO TeMA Sb(III) Sb(V)	Flow = 0.75 mL min^{-1} Injection volume = 10 μL Time: ≈ 12 min LOD*: 0.2 ng mL^{-1} (As) 0.5 ng mL^{-1} (Sb) RSD#: < 2% and 3% (for As and Sb)	28
As **Sb** **Se**	Water samples	Anion exchange Hamilton PRX-X100 (250 mm × 4.1 mm, 10 μm)	Isocratic elution: 12.5 mM $(NH_4)_2HPO_4$ + 3% (v/v) methanol pH = 8.5 (with NH_4OH)	As(III) As(V) MMA DMA Sb(V) Se(IV) Se(VI)	Flow = 1.5 mL min^{-1} Injection volume = 100 μL Time: ≈ 20 min	17

AsC: arsenocholine; TMAO: trimethylarsine oxide; TeMA: tetramethylarsonium ion; MA: methylarsonate; DMA: dimethylarsinate; AsB: arsenobetaine;
* Limit of detection
# Relative standard deviation

Table 2. The ICP-MS conditions used in the measurement.

Nebulizer	Conikal nebulizer (G.E.)
Nebulizer gas	Around 1.0 L min^{-1}
Spray chamber	Scott type double pass spray chamber (G.E.)
Resolution mode	High resolution (HR), Medium resolution (MR)
Monitored isotopes	^{75}As, ^{78}Se(or ^{82}Se), ^{121}Sb
Signal intensity (cps/10 μg L^{-1})	MR: ^{75}As: 6.0×10^4; ^{78}Se: 1.6×10^4; ^{121}Sb: 1.2×10^5 HR: ^{75}As: 1.0×10^4; ^{78}Se: 3.3×10^3; ^{121}Sb: 3.0×10^4

Table 3. The chromatography conditions used during analysis.

Column	PRP-X100 (250 mm × 4.1 mm, 10 μm) (Hamilton, Reno, USA)
Mobile phase	0 - 4.5 min: 5 mM EDTA (97%) + methanol (3%) 4.5 - 5.5 min: linear ramp to 30 mM EDTA (97%) + methanol (3%) 5.5 - 11 min: 30 mM EDTA (97%) + methanol (3%)
pH	4.7 (adjusted with Formic acid)
Flow rate	1.5 mL min^{-1}
Injection volume	50 μL
Species	As (III, V), Sb (III, V), and Se (IV, VI)

Table 4 The concentration range, correlation coefficient, detection limit and retention times for six species. The correlation coefficients were obtained from at least 6 replicate experiments. The detection limit was calculated from 11 replicate measurements of blank. The retention times were obtained from at least 20 replicate measurements.

	Concentration range / μg L^{-1}	Correlation coefficient / R^2	Limit of detection (LOD) / μg L^{-1}	Retention time / min
As(III)	0.5 - 75	0.9999	0.02	1.70 ± 0.02
As(V)	0.5 - 75	0.9998	0.06	2.94 ± 0.04
Sb(III)	0.5 - 75	0.9998	0.2	7.14 ± 0.08
Sb(V)	0.5 - 75	0.9998	0.02	2.28 ± 0.02
Se(IV)	5.0 - 200	0.9999	0.2	3.38 ± 0.04
Se(VI)	5.0 - 200	0.9998	0.4	9.36 ± 0.09

Table 5 Spike recovery of a synthetic sample and two hot spring samples J52 and J54. The data was obtained from at least 3 replicate measurements for each sample.

	Synthetic sample			**Sample J52**			**Sample J54**		
	Spike (μg L^{-1})	Determined (μg L^{-1})	Recovery (%)	Spike (μg L^{-1})	Determined (μg L^{-1})	Recovery (%)	Spike (μg L^{-1})	Determined (μg L^{-1})	Recovery (%)
As(III)	0.0	1.03 ± 0.02	—	0.0	0	—	0.0	3.89 ± 0.02	—
	1.0	1.92 ± 0.01	89.0	20.0	18.83 ± 0.83	94.2	5	8.59 ± 0.31	94
As(V)	0.0	1.21 ± 0.01	—	0	19.45 ± 0.56	—	0.0	0	—
	1.0	2.11 ± 0.01	90	20.0	38.83 ± 0.70	96.9	0.5	0.51 ± 0.01	102
Sb(III)	0.0	1.58 ± 0.02	—	0	0	—	0.0	0.40 ± 0.01	—
	1.0	2.53 ± 0.03	95	1.0	1.23 ± 0.09	123	0.5	0.90 ± 0.00	100
Sb(V)	0.0	1.56 ± 0.01	—	0	0.83 ± 0.06	—	0.0	0.38 ± 0.02	—
	1.0	2.62 ± 0.07	106	1.0	1.77 ± 0.11	94	0.5	0.87 ± 0.01	98
Se(IV)	0.0	2.21 ± 0.06	—	0	0	—	0.0	0	—
	2.0	4.10 ± 0.06	94.5	5.0	4.94 ± 0.59	98.8	5	5.38 ± 0.04	107.6
Se(VI)	0.0	2.08 ± 0.01	—	0	0	—	0.0	0	—
	2.0	3.95 ± 0.06	93.5	5.0	4.89 ± 0.20	97.8	5	5.47 ± 0.08	109.4

Table 6 The results of certified reference materials SRM 1643e and CRM-SW. Data obtained from 3 replicate measurements.

Sample	Species	Determined (μg L^{-1})	Certified (μg L^{-1})
SRM 1643e	As(III)	0.62 ± 0.07	60.45 ± 0.72
	As(V)	58.03 ± 0.87	
	Sb(III)	ND	58.30 ± 0.61
	Sb(V)	58.40 ± 2.63	
	Se(IV)	11.85 ± 0.51	11.97 ± 0.14
	Se(VI)	ND	
CRM-SW	As(III)	ND	20.00 ± 0.00
	As(V)	20.80 ± 1.40	
	Se(IV)	4.42 ± 0.39	4.00 ± 0.00
	Se(VI)	ND	

ND: not determined.

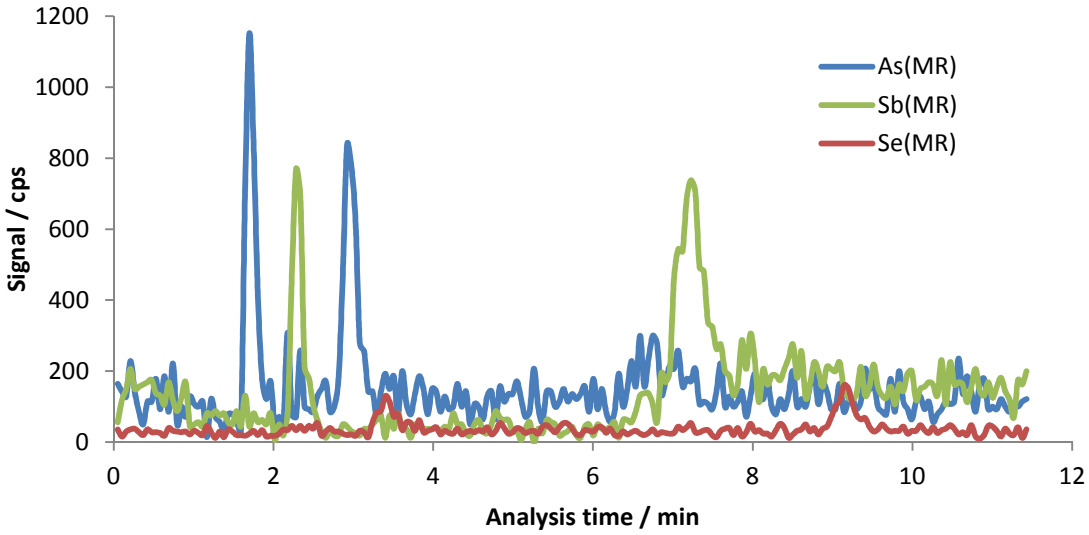


Fig. 1 Chromatogram of a standard containing 0.2 μg L^{-1} As(III,V) and Sb(III,V), and 0.5 μg L^{-1} Se(IV,VI); The peaks from left to right are As(III), Sb(V), As(V), Se(IV), Sb(III) and Se(VI) respectively.