2007

Simultaneous speciation of arsenic and selenium in human urine by high-performance liquid chromatography inductively coupled plasma mass spectrometry

FM Pan

JF Tyson

PC Uden

Simultaneous speciation of arsenic and selenium in human urine by high-performance liquid chromatography inductively coupled plasma mass spectrometry†

Fumin Pan, Julian F. Tyson and Peter C. Uden

A procedure for the simultaneous determination of six selenium species and six arsenic species in human urine by ion-pair, reversed-phase liquid chromatography coupled to ICP-MS has been developed. Selenium species, trimethylselenonium ion (TMSe), selenocystine (SeCys), selenite (Se^{IV}), selenourea (SeUr), selenomethionine (SeMet), selenoethionine (SeEt), and arsenic species, arsenocholine (AsC), arsenobetaine (AsB), dimethylarsinic acid (DMA), methylarsonic acid (MMA), arsenite (As^{III}), arsenate (As^{V}) were separated on a C_8 reversed phase column by a mobile phase of 13.0 mmol l^{-1} tetrabutylammonium hydroxide (ion-pair reagent) and 1.3% methanol at pH 5.7–5.8 (adjusted with malonic acid). The total analysis took less than 10 min with isocratic elution at a flow rate of 1.0 ml min^{-1}, and was free from chloride interference due to the complete separation of the analyte species from chloride. The detection limits, in synthetic urine for an injection volume of 50 μL, ranged between 0.1 and 0.4 μg l^{-1} for the six arsenic species (except AsC) and between 0.7 and 2 μg l^{-1} for the six selenium species (at m/z 78). The repeatability was less than 10% (RSD) for 1 μg l^{-1} arsenic species and 5 μg l^{-1} selenium species, except for AsC. Quantification was based on response factors in a synthetic urine matrix relative that of As^{V} in the mobile phase. The response factors for selenium species showed considerable compound dependence. Recoveries from spiked urine samples ranged from 81% to 103%, except for SeUr and SeEt, which were not recovered. The method was applied to the determination of arsenic and selenium species in urine, which only required filtering through a 0.45 μm membrane filter and dilution with mobile phase in order to measure arsenic and selenium urinary metabolites.

Introduction

Arsenic is widespread in nature and is drawing increasing attention due to its high toxicity and water source contamination in several parts of the world.[1] Chronic arsenic exposure may increase the risk of skin, lung, and bladder cancers or damage the immune and cardiovascular systems.[2] It is well known that the toxicity of arsenic depends on its chemical form, and inorganic arsenic species, arsenite and arsenate, are believed to be more toxic than organic species. However, recent studies show that the methylated trivalent arsenic species found in human urine[3] may be more toxic than inorganic arsenic.[4,5] Human exposure normally occurs through consumption of arsenic-containing food and drink, whose arsenic content varies from <0.1 to 25 μg g^{-1}.[6] After metabolism, inorganic arsenic species, which have been methylated, are excreted in urine as DMA and MMA, while organic arsenic species such as AsB and AsC are excreted unchanged.[7]

Within a small intake window, selenium plays an important biological role in human health. It is an essential element that also has cancer prevention properties.[8] Selenium is present at the active site of glutathione peroxidase and acts as a redox center to protect cells from oxidation damage.[9] Selenium deficiency has been linked to some diseases in countries such as China;[10] DNA damage by the generation of reactive oxygen species could arise from excess selenium.[11] The multiple roles of selenium depend on its chemical form and concentration. Foods provide mainly organoselenium compounds such as selenomethionine, selenocysteine and *Se*-methylselenocysteine, while most supplements contain selenomethionine bound in selenized yeast and sometimes selenate or selenite. Urine is a major excretion route for consumed selenium.[12] Francesconi and Pannier[13] reviewed the previous studies of urinary metabolites and concluded that selenosugars should be considered to be the major urinary selenium species (rather than the trimethylselenonium ion), while for other compounds, such as selenomethionine and selenocystine, more evidence is needed if they are to be accepted as typical urine metabolites.

There is evidence that arsenic and selenium species can interact in biological systems and cause mutual detoxification.[14] Moxon first reported the antagonism between arsenic and selenium when he observed that arsenite in drinking water could protect animals from selenium toxicity.[15] One solid proof of interaction is the identification of the As–Se

Department of Chemistry, University of Massachusetts, Amherst, MA 01002, USA. E-mail: Tyson@chem.umass.edu; Fax: +1-413-545-4490; Tel: +1-413-545-0195

† Electronic supplementary information (ESI) available: results of optimization studies. See DOI: 10.1039/b703713a

compound seleno-bis(*S*-glutathionyl)arsonium ion, $[(GS)_2AsSe]^-$, in bile excretion.[16] The proposed mechanisms of the antagonistic interactions between As and Se, including direct as well as metabolic interactions, have been reviewed by Zeng *et al*.[14]

Urinary arsenic and selenium are known to be highly correlated with recent arsenic and selenium intake.[17,18] The chemical forms and quantities of urinary arsenic and selenium are useful indicators to probe the status, the interactions and the possible metabolic pathways of arsenic and selenium in the body. Techniques for the simultaneous speciation of arsenic and selenium are needed to support such studies.

Commonly used element-specific detection techniques, such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) can be coupled to HPLC. As a robust and sensitive combination, HPLC-ICP-MS provides a powerful technique for the determination arsenic and selenium.[19]

Ion-exchange and ion-pair, reversed-phase chromatographic methods have been widely used for separating arsenic and selenium compounds in biological materials including urine.[20,21] Arsenic species have been mostly separated by ion-exchange, while selenium species have been mostly separated by ion-pairing, reversed-phase procedures.[22] Eight arsenic compounds, As^{III}, As^{V}, DMA^{III}, DMA^{V}, MMA^{III}, MMA^{V}, AsB and AsC were separated on both anion- and cation-exchange columns.[23] Separation of anionic, cationic and neutral selenium species, namely Se^{VI}, SeUr, SeMet, SeEt and $TMSe^+$, was achieved on a reversed-phase column with sodium 1-butanesulfonate and tetrabutylammonium hydroxide as ion-pair reagents.[24] Only a limited number of the separation systems have been reported for the simultaneous speciation of arsenic and selenium. Orero Iserte *et al*. devised an anion-exchange separation of As^{III}, As^{V}, DMA, MMA, Se^{IV} and Se^{VI} on a Hamilton PRP-X100 column with an $NH_4H_2PO_4$ mobile phase,[25] and applied the method to the analysis of sediment extracts. Do *et al*. separated ten arsenic and selenium species, mainly found in the environment and in mammals, on a reversed-phase column, with tetrabutylammonium phosphate as the counter-ion.[26] The separated species were collected in 75 µl fractions and quantified by ETAAS. The retention time of chloride, a key interferent if ICP-MS detection were to be used, was not investigated. Le *et al*. achieved baseline separation of thirteen inorganic and organic arsenic and selenium species on a reversed-phase column with hexanesulfonate as the ion-pair reagent;[27] however, for urine analysis they used a different column and a different mobile phase, though elevated temperatures were still needed (70 °C) to decrease the separation time from 37 to 18 min.

We have developed an ion-pair, reversed-phase, C_8 HPLC procedure with ICP-MS detection for the simultaneous determination of six arsenic species, AsC, AsB, DMA, MMA, As^{III}, As^{V}, and six selenium species, TMSe, SeCys, Se^{IV}, SeUr, SeMet, and SeEt, in human urine within 10 min. The isocratic separation is at room temperature, with detection of selenium at m/z 78 and arsenic at m/z 75, for which the chloride interference was removed by chromatographic separation. The method was developed to study possible interactions between arsenic and selenium metabolism as evidenced by urinary temporal profiles of compounds containing these elements following ingestion of seafood and/or a selenium supplement. Urine samples were analyzed following filtering and dilution with mobile phase.

Experimental

Instrumentation

The chromatographic system consisted of a Spectra Physics Model 8810 precision isocratic pump (San Jose, CA, USA), a Rheodyne Model 7010 sample injector (Rohnert Park, CA, USA) equipped with a 50 µL sample loop and a Waters Symmetry C_8 reversed-phase column 250 mm × 4.6 mm id, containing 5 µm particles (Milford, MA, USA). The chromatographic eluent (1.0 ml min^{-1}) was coupled directly to the cross-flow nebulizer and the Scott-type, double-pass spray chamber of the spectrometer. The ICP-MS instrument was a PerkinElmer Model Elan 6100 DRC-e (Norwalk, CT, USA). The operating parameters are summarized in Table 1. To achieve the best sensitivity, the ICP-MS conditions indicated in Table 1 were tuned daily by aspirating a standard solution of 10 µg l^{-1} of In, Mg, Pb, Ce, and Ba. Ion intensities at m/z 75, 78, 82 were recorded with the PE Elan time-resolved analysis software. Quantification was performed in the peak area mode using OriginPro7.5 (OriginLab, USA). The pH was measured with an Accumet 915 pH meter Fisher (Fairlawn, NJ, USA) which was calibrated daily using pH 4.0 and pH 7.0 standard solutions.

Reagents

All chemicals were of analytical grade and were used without further purification. High-purity water was produced by an E-pure Deionized Water System (Barnstead, Dubuque, IA, USA). Selenomethionine, selenocystine, selenoethionine, selenourea, sodium selenate, sodium selenite and arsenobetaine

Table 1 Operating conditions for HPLC and ICP-MS instruments

HPLC	
Column	Waters Symmetry C_8 (250 × 4.6 mm id, 5 µm particle size)
Mobile phase	13.0 mmol l^{-1} tetrabutylammonium hydroxide 1.3% methanol pH 5.7–5.8 adjusted with malonic acid
Flow rate	1.0 ml min^{-1}
Injection volume	50 µl
Column temperature	Ambient
ICP-MS	
Rf power	1125 W
Plasma Ar flow	15.00 l min^{-1}
Auxiliary Ar flow	1.18 l min^{-1}
Nebulizer Ar flow	0.90 l min^{-1}
Lens voltage	8.60 V
Data acquisition mode	Time resolved analysis
Dwell time	200 ms
Sweeps/reading	1
Reading/replicate	3000
Replicates	1
Isotopes monitored	^{75}As, ^{78}Se, ^{82}Se

were purchased from Sigma (St. Louis, MO, USA), trimethylselenonium iodide from Organometallics Inc. (East Hampstead, NH, USA), sodium arsenite and sodium arsenate from Fisher, monomethylarsonic acid (MMA) from ChemService Inc. (West Chester, PA, USA), and dimethylarsinic acid (DMA) from Pfaltz & Bauer (Waterbury, CT, USA). Arsenocholine was provided by Dr X.-C. Le of the University of Alberta. Stock 1000 mg l^{-1} (as Se or As) solutions were prepared in 10.0 ml of deionized water and stored in a freezer at −20 °C. Working standard solutions in the μg l^{-1} range were prepared daily by appropriate dilution.

Tetrabutylammonium hydroxide (TBAH) obtained, as a 1.0 mol l^{-1} solution in methanol, from Aldrich (St Louis, MO, USA), was diluted as needed to prepare various mobile phase compositions, the pH of which was adjusted by the addition of 1.0 mol l^{-1} malonic acid (Eastman, Rochester, NY, USA) or 20% NH_4OH (EMD, Gibbstown, NJ, USA). Synthetic urine, prepared according to the procedure of Gammelgaard *et al*.,[28] contained 55 mmol l^{-1} of sodium chloride, 67 mmol l^{-1} of potassium chloride, 2.6 mmol l^{-1} of calcium sulfate, 3.2 mmol l^{-1} of magnesium sulfate, 19.8 mmol l^{-1} of sodium dihydrogen sulfate, 29.6 mmol l^{-1} of sodium sulfate, 310 mmol l^{-1} of urea and 9.8 mmol l^{-1} of creatinine in deionized water. These reagents were all purchased from Fisher, except magnesium sulfate (Mallinckrodt), creatinine (Sigma) and urea (EMD). The selenium supplement (Schiff Products, Salt Lake City, UT, USA) was purchased from a local supermarket. This material consists of tablets, each of which contains 200 μg of selenium (as Se), a value that has been verified.[29] It is also known that in this material about 80% of the selenium is present as selenomethionine (or the corresponding selenoxide).[30] The pollock seafood (Matlaws, National Fish & Seafood Inc., Gloucester, MA, USA) was bought from a local supermarket and stored at −20 °C. This material contained 0.29 mg kg^{-1} of arsenic (as As).[31]

Urine samples

Urine contains high concentrations of urea, proteins, sodium and potassium,[32] which often cause matrix interference effects. Several procedures have been proposed to minimize these interferences, such as C_{18} solid-phase extraction to remove the organic matrix,[33] crown ether extraction to remove potassium and sodium,[34] methanol extraction for de-salting,[35] and ethanolic precipitation for de-salting and de-proteinization.[36] Another difficulty is the low analyte concentrations, which has led to attempts to preconcentrate prior to speciation.[37] These various clean-up and concentration procedures have brought other problems, such as the oxidation of selenosugars,[37] the loss of inorganic selenium,[38] the oxidation of monomethylated selenium[39] and the degradation of selenosugars,[35] all of which affect the accuracy and precision of the quantification. These results indicate that the sample treatment should be as simple as possible, involving only filtration and dilution and that speciation analysis should be carried out with fresh samples. Therefore, it was decided only to filter the urine samples through a 0.45 μm filter and dilute them (1 + 4) with mobile phase on the day of analysis.

Urine samples were collected in the morning, afternoon and evening from a male volunteer and mixed for the recovery tests in which 5 μg l^{-1} of arsenic species and 10 μg l^{-1} of selenium species were added. Urine samples were collected 11 h after consumption of a selenium dietary supplement and/or seafood, stored in polyethylene sample containers at 4 °C and analyzed within one week. Prior to analysis, urine samples were filtered through a 0.45 μm filter (Whatman, Florham Park, NJ) and diluted (1 + 4) with mobile phase.

Method development and validation

Optimization. The retention characteristics of the arsenic and selenium species were considered to be mainly determined by their hydrophobic nature and their charge. Therefore, both the concentration of the ion-pairing reagent, tetrabutylammonium hydroxide (TBAH), and the pH could influence the separation. The pH was adjusted with malonic acid, as reported by Zheng *et al*.[24] The concentration of TBAH, mobile phase pH value and injection volume were optimized in this study to get baseline separations with good peak shapes for both the arsenic and the selenium species. The concentration of methanol was kept at the minimum value that arose from the dilution of the stock TBAH solution, which was supplied dissolved in methanol. The cyclic alternating variable search strategy was adopted in line with our previous approaches to complex optimizations.[40] In this procedure, each variable is changed in turn, while the others are held constant, until a maximum in the response factor (or figure of merit) space has been found. The process is then repeated for a second and, possibly, a third time. This has the advantage over a procedure in which more than one variable is changed simultaneously that at the conclusion of the process, the shape of the factor space is known as a function of each of the parameters. The concentration of ion-pairing reagent was varied from 5 mmol l^{-1} to 13 mmol l^{-1}, initially at pH 6.0, and the pH was varied from 5.0 to 7.0. In order to achieve higher sensitivity and lower detection limits, a large injection volume is preferred. However, increasing the sample volume will decrease separation efficiencies, by increasing peak broadening and/or producing peak overlap. Sample volumes of 20, 50, or 100 μl were injected.

It is well known that the argon dimers $^{40}Ar_2^+$ and $^{40}Ar^{38}Ar$ interfere with the major isotope of selenium at m/z 80 (49.6%) and 78 (23.8%). The dynamic reaction cell (DRC) has usually been used to minimize the background caused by the argon dimers. However, it was observed that the sensitivities for both the ^{75}As and the ^{78}Se signals were decreased (up to 50-fold in some cases) by reaction with methane in the DRC. Without a DRC, the background signal at m/z 78 was constant throughout the chromatographic run at 15 000 cps; with the DRC, the background was about 300 cps. As the standard deviation of the signal equals its square root, it may be estimated that a lower signal to noise ratio (and hence limit of detection) is obtained under the higher sensitivity conditions.

In the study of human urine, the molecular ions $^{40}Ar^{37}Cl^+$, $^{12}C^{35}Cl_2^+$ and $^{40}Ar^{35}Cl^+$ strongly interfere with the ^{77}Se, ^{82}Se and ^{75}As isotopes, respectively. The average Cl^- concentration in human urine may be as high as 5 mg ml^{-1},[41] which after a

5-times dilution would be decreased to about 1 mg ml^{-1}. To evaluate the effect of Cl^- on the chromatographic separation, arsenic and selenium standards prepared with the presence of 1 mg ml^{-1} Cl^- (as NaCl) were injected into the HPLC-ICP-MS system. A solution containing only 1 mg ml^{-1} Cl^- (as NaCl) was also injected.

Analytical performance. To characterize the performance of the method, the following figures of merit were evaluated: (a) calibration range (solutions covering the concentration range of 0–100 μg l^{-1} in both mobile phase and synthetic urine matrices were injected), (b) limit of detection (defined as the concentration giving a signal equal to 3 times the standard deviation of the peak area, for $n = 6$, of 1 μg l^{-1} As and 5 μg l^{-1} Se in both mobile phase and synthetic urine matrices), and (c) precision (expressed as the RSD of 6 injections of 1 μg l^{-1} arsenic species and 5 μg l^{-1} selenium species in either mobile phase or synthetic urine).

For quantification, peak area response factors for each species relative to the response for As^V in the mobile phase were calculated for both mobile phase and synthetic urine matrices. The factors were calculated for the injection of the same concentration of the element for each of the 12 species. The concentrations included 1, 5, 10, 50, and 100 μg l^{-1} for the arsenic species and 5, 10, 50, and 100 μg l^{-1} for the selenium species. The response factors for each species were calculated as the average of the response factors for all concentrations. Matrix effects were investigated by spiking arsenic (5.0 μg l^{-1}) and selenium (10.0 μg l^{-1}) standards individually into a real urine matrix and measuring recoveries based on the calculated response factors.

Results and discussion

Optimization

Chromatograms showing the influence of the variation of TBAH concentration on the ion-pairing HPLC-ICP-MS chromatogram of arsenic and selenium standards are provided in the Electronic Supplementary Information (ESI)†. As the TBAH concentration was increased from 5 to 13 mmol l^{-1}, the resolution between As^{III} and AsB improved, and stabilized for TBAH concentrations greater than 8 mmol l^{-1}, although the peaks were still partially overlapping. All other arsenic peaks were baseline separated over the entire range of TBAH concentrations, though the peaks for AsC and AsB, As^{III} and DMA, DMA and MMA moved closer with increasing TBAH concentration. Meanwhile, the total elution time for all arsenic peaks was decreased from about 10 to 7 min. Higher TBAH concentrations (greater then 13 mmol l^{-1}) are not recommended as the resolution between some arsenic peaks caused by the tailing of peaks for AsC, As^{III} and DMA becomes worse. The peaks for SeUr and SeMet were separated at low TBAH concentration (less than 8 mmol l^{-1}), but with increasing concentration they co-eluted and then switched elution sequence, becoming once again baseline separated when the TBAH was 13 mmol l^{-1}. Again, the total elution time for all selenium species was decreased from about 11 to 9 min. Considering both arsenic and selenium chromatograms under the influence of TBAH concentrations, 13 mmol l^{-1} of TBAH was selected as the optimum concentration. The retention times of all arsenic and selenium species were sensitive to pH values between 5.0 and 6.0 (details shown in the ESI†). At pH 5.0, peaks for all selenium species were baseline separated, but the peaks for AsB, As^{III} and DMA were not resolved. As the pH was increased from 5.5 to 6.0, the resolution between peaks for As^{III} and DMA was improved while the resolution between AsC and AsB, and DMA and MMA was poorer. At pH 6.0, TMSe and SeCys co-eluted. The effect of pH values between 5.5 and 6.0 on the resolutions of those peaks was further investigated. It was found that at pH 5.7–5.8, the overlap between TMSe and SeCys, and between As^{III} and DMA, were minimized, and this value was chosen. The effect of changing sample injection volume on the retention times and peak shapes of arsenic and selenium compounds for the optimum TBAH concentration and pH value are also shown in the ESI†. Resolutions were not affected for injection volumes of 20 and 50 μl, and so the larger volume was selected.

The optimized chromatographic conditions are shown in Table 1. As can be seen from Fig. 1, baseline separations of all

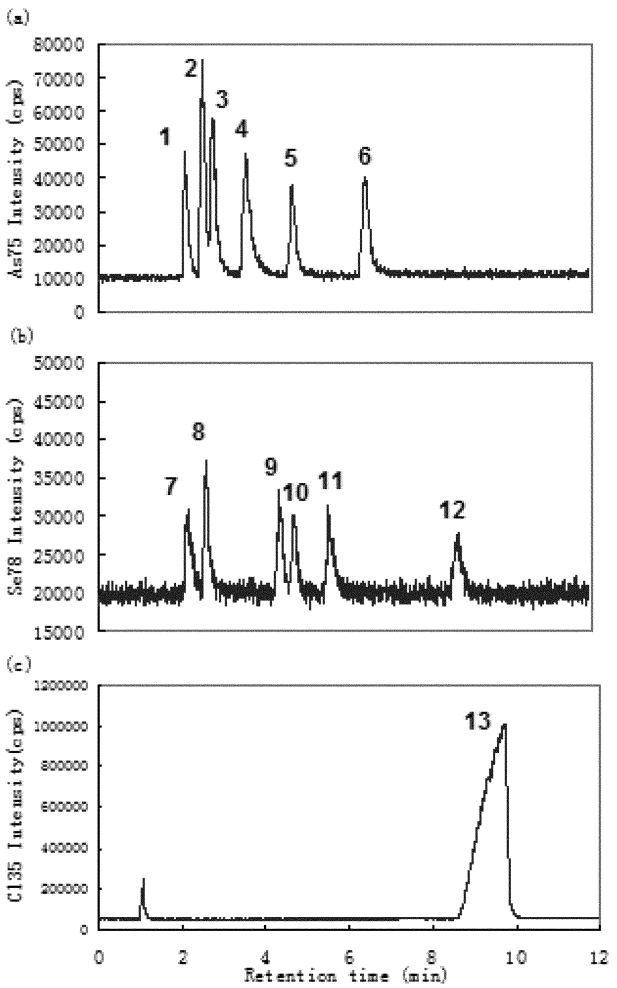

Fig. 1 Chromatograms of standard solution in the mobile phase containing six arsenic compounds, and six selenium compounds (10 μg l^{-1}) and 1000 μg ml^{-1} Cl^- (as NaCl). Peak identification: 1, AsC; 2, AsB; 3, As^{III}; 4, DMA; 5,MMA; 6, As^V; 7, TMSe; 8, SeCys; 9, SeMet; 10, SeUr; 11, Se^{IV}; 12, SeEt; and 13 Cl^-.

peaks, except for those of AsB and As^{III}, within 10 min were obtained. Although selenate was originally included in the compounds studied, it was not included in the compounds determined in the urine samples as there are no definitive data demonstrating that selenate is a typical constituent of urine.[13] The retention time for selenate is about 40 min. The addition of chloride had no effect on the selenium chromatograms, but was visible as a late eluting broad peak in the arsenic chromatograms that was well separated from the final As^{V} peak. The chromatogram shown in Fig. 1(c) shows the signal at m/z 35 which includes a significant contribution from chlorine. A better signal to noise ratio was obtained for selenium at m/z 78 and measurements were made at this rather than at m/z 82.

Analytical performance

The calibration curves for the six arsenic and six selenium standards were linear over the range of 0–100 μg l^{-1} in both mobile phase and synthetic urine matrices with correlation coefficients all greater than 0.995. The relative response factors are shown in Table 2. These values show some interesting variations, suggesting that the ICP-MS response is not compound-independent. As the purity of the AsC material is not known (though it is not accompanied by any other arsenic-containing compounds), it is possible that the low value for this species is due to inaccuracy in the calculated concentration. The effect would appear to be more pronounced for the selenium species. The values normalized to the value for selenite are also shown in Table 2, from which it can be seen that the response for selenium compounds in which the selenium is bound to carbon are significantly lower than the response for the oxoanion SeO_3^{2-}. To our knowledge, the issue of compound dependency in quantitative speciation with ICP-MS detection has not been reported previously, although differences for the ICP-OES responses of arsenite and arsenate have been observed,[42] but not definitively explained. This phenomenon, which we have also observed with ICP-OES and selenium compounds, is the subject of further study.[29] Our current hypothesis is that the differences in sensitivity arise from differences in atomization efficiencies, and hence ionization efficiencies, within the central channel of the plasma, *i.e.*, the different compounds give rise to a variety of molecular species (such as hydrides, alkyls, carbonyls and oxides) due to incomplete atomization during the residence time in the plasma.

The spike recoveries from a real urine sample for arsenic (5.0 μg l^{-1}) and selenium (10.0 μg l^{-1}) are also shown in Table 2. The values ranged from 81% to 103% except for those for SeUr and SeEt, whose signals were completely suppressed by the urine matrix. Generally, the recovery values for the arsenic species were higher than those of the selenium species.

The detection limits, summarized in Table 3, ranged from 0.09 to 0.2 μg l^{-1} (for arsenic species) and 0.5 to 1 μg l^{-1} (for selenium species) for the mobile phase matrix, and from 0.1 to 0.4 μg l^{-1} (for arsenic species) and 0.7 to 2 μg l^{-1} (for selenium species) for a synthetic urine matrix. These values are suitable for the direct speciation of arsenic and selenium in human urine samples, according to the reported concentrations of arsenic and selenium in urine, and are comparable to previously reported values.[24,43,44] The repeatabilities, also shown in Table 3, were less than 10% RSD for 1 μg l^{-1} arsenic species and 5 μg l^{-1} selenium species spiked in both the mobile phase and a synthetic urine matrix, except for AsC, the RSD of which was 15%.

Peak identification

The chromatograms of a urine sample collected 11 h after seafood consumption and the same urine sample spiked with individual arsenic standards are shown in Fig. 2. A total of 7 arsenic-containing peaks can be seen. Of these, six could be identified by retention time matching, with one minor unknown peak just before 6 min. In agreement with previously reported distributions,[41] the major urinary arsenic species was DMA. Hulle *et al.*[20] investigated the unknown species present in ion-exchange chromatograms of urine samples after ingestion of food containing arsenosugars, and concluded that the arsenosugars were completely transformed into DMA, MMA and unknown metabolites. Therefore, the unknown peak in Fig. 2 is assigned as an arsenosugar metabolite. As can be seen from Fig. 3(a), the only selenium species that could be identified in urine collected 11 h after 1.0 mg selenium supplementation was $TMSe^{+}$, though this was not the major species. Two minor unknown species, unknown 1 (with a retention time of 3.8 min) and unknown 2 (with a retention time of 5.1 min) were also found. The major species, with a

Table 2 Response factors and recovery

	Response factors relative to As^{V}					
Species	Mobile phase	Synthetic urine	Average	RSD (%)	Relative to Se^{IV}	Recovery[a] (%)
AsC	0.699	0.783	0.741	5.8		85 ± 11
AsB	1.25	1.32	1.28	3.2		97 ± 5
As^{III}	1.02	1.04	1.03	4.7		96 ± 4
DMA	1.12	1.20	1.16	6.4		98 ± 3
MMA	0.955	1.02	0.989	2.6		94 ± 6
As^{V}	1.00	1.02	1.01	4.6		101 ± 4
TMSe	0.279	0.272	0.276	5.3	0.701	81 ± 2
SeCys	0.252	0.303	0.278	4.9	0.706	100 ± 9
SeMet	0.297	0.322	0.310	5.4	0.786	84 ± 6
SeUr	0.321	0.243	0.282	10	0.716	0
Se^{IV}	0.374	0.413	0.394	12	1.00	103 ± 4
SeEt	0.253	0.214	0.233	7.0	0.591	0

[a] The ± terms are 95% confidence intervals, $n = 3$.

Table 3 Detection limit and repeatability for 1 μg l^{-1} (As) and 5 μg l^{-1} (Se) spiked mobile phase or synthetic urine. (n = 6)

	Repeatability, RSD (%)		Detection limit[a]/μg l^{-1}	
Species	Mobile phase	Synthetic urine	Mobile phase	Synthetic urine
AsC	5.1	14	0.15	0.43
AsB	5.3	6.4	0.16	0.19
As^{III}	3.1	5.0	0.09	0.15
DMA	5.6	3.3	0.17	0.10
MMA	5.1	5.3	0.15	0.16
As^{V}	3.2	4.0	0.10	0.12
TMSe	9.3	8.4	1.4	1.3
SeCys	4.0	4.7	0.60	0.71
SeMet	3.4	6.7	0.51	1.0
SeUr	7.5	10	1.1	1.5
Se^{IV}	4.7	8.3	0.71	1.3
SeEt	5.5	6.7	0.83	1.0

[a] Second digit shown for information.

retention time of 6.1 min, is seen in Fig. 3(b), which is a continuation of the chromatogram with an expanded time scale and a compressed response scale. The peak for this major species partially overlapped that for the spiked Se^{IV} to form a broad peak. Franscesconi and Pannier have presented[13] a thorough evaluation of the literature relating to selenium speciation in urine (both rat and human) and concluded that methyl 2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside is ''firmly established'' as the ''major selenium metabolite after supplementation with ... selenized yeast'' in urine. Recent results[45] for separation on a 150 mm × 4.6 mm C_{18} reversed-phase HPLC column with ICP-MS detection showed this major metabolite eluting between 7 and 8 min, with a mobile phase flow rate of 1.0 ml min^{-1}. Therefore, the largest peak (at 6.1 min) shown in Fig. 3(b) is assigned to this selenosugar. This major species was detected in all urine samples collected 2, 4, 6, 9, 11, 13, 21 and 25 h after ingestion of a 1.0 mg selenium supplement.[31] In agreement with the results of recently published studies, TMSe was not the major selenium metabolite, though it was detectable. This is in contrast to the findings of Gammelgaard and Bendahl,[46] who did not detect

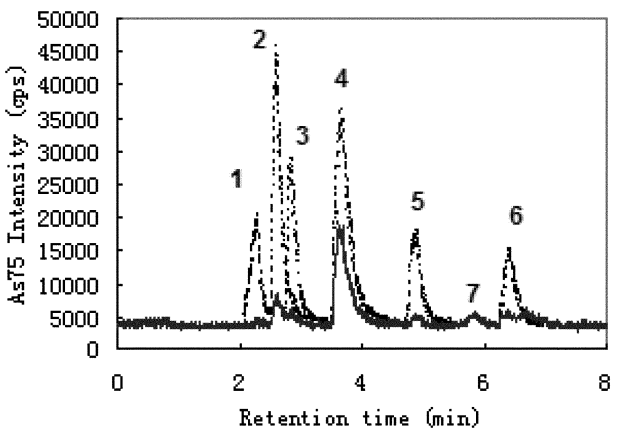

Fig. 2 Chromatograms of a urine sample (collected 11 h after seafood consumption, continuous line) and the same urine sample spiked with 5 μg l^{-1} (as As) of six arsenic species (broken line). Peak identification: 1, AsC; 2, AsB; 3, As^{III}; 4, DMA; 5,MMA; 6, As^{V}; 7, unknown.

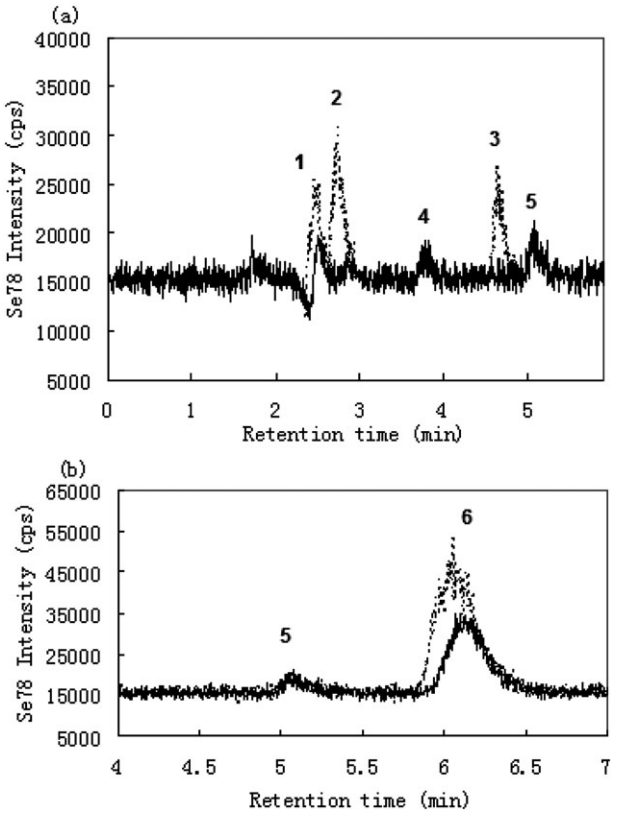

Fig. 3 Chromatograms of a urine sample (collected 11 h after 1.0 mg selenium supplementation, continuous line) and the same urine sample spiked with 10 μg l^{-1} (as Se) of (a) four selenium species, respectively (broken line), (b) Se^{IV} (broken line). Peak identification: 1, TMSe; 2, SeCys; 3, SeMet; 4, unknown 1; 5, unknown 2; 6, Se^{IV}/methyl 2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside.

TMSe (< 0.5 μg l^{-1}) in urine even after supplementation with 2 mg of selenium as selenized yeast. It should be noted that in ref. 13, Franscesconi and Pannier refer to methyl 2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside as ''selenosugar **2**'', whereas in the later paper,[45] this compound is labelled ''selenosugar **1**''. Gammelgaard and co-workers,[46,47] who refer to this compound as *Se*-methyl-*N*-acetylgalactosamine, also consider that it is the major metabolite in urine after supplementation with selenized yeast. The small peak just before three minutes is possibly also a selenosugar, as this would be consistent with previous results[45] in which methyl 2-amino-2-deoxy-1-seleno-β-D-galactopyranoside was shown to elute at around 3 min under the conditions for which methyl 2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside eluted at about 7 min.

Conclusions

It is possible to separate six arsenic species (AsC, AsB, MMA, DMA, As^{III}, As^{V}) and six selenium species (Se^{IV}, SeCys, TMSe, SeEt, SeMet, SeUr) in urine in less than 10 min by a method based on a reversed-phase ion-pair chromatography, at room temperature, with isocratic elution, coupled with ICP-MS detection down to concentrations of between 0.1 and 1 μg l^{-1}. The procedure is free from Cl^{-} interference, as chloride

elutes after all of the arsenic (and selenium) species of interest have eluted, and requires only minimal sample pre-treatment. Quantification in a real urine matrix, based on response factors relative to the response for As^V, would be possible for all species except SeUr and SeEt. Significant differences in response factors for selenium compounds were observed, which is the subject of on-going further study. The method is suitable for application to a study of the urinary excretion and metabolite distribution of arsenic and selenium when ingested simultaneously and separately. Such a study would provide further information about the interactions between arsenic and selenium in human metabolism.[31]

Acknowledgements

We thank Professor David Reckhow, Civil and Environmental Engineering, University of Massachusetts Amherst, for access to the plasma source mass spectrometer and Professor X.-C. Le, University of British Columbia, for the gift of the arsenocholine.

References

1 C. K. Jain and I. Ali, *Water Res*., 2000, **34**, 4304.
2 A. A. Duker, E. J. M. Carranza and M. Hale, *Environ. Int*., 2005, **31**, 631.
3 H. V. Aposhian, E. S. Gurzau, X. C. Le, A. Gurzau, S. M. Healy, X. Lu, M. Ma, L. Yip, R. A. Zakharyan, R. M. Maiorino, R. C. Dart, M. G. Torcus, D. Gonzalez-Ramirez, D. L. Morgan, D. Avram and M. M. Aposhian, *Chem. Res. Toxicol*., 2000, **13**, 693.
4 E. Dopp, L. M. Hartman, A. M. Florea, U. van Recklinghausen, R. Pieper, B. Shokouhi, A. W. Rettenmeier, A. V. Hirner and G. Obe, *Toxicol. Appl. Pharmacol*., 2004, **201**, 156.
5 J. C. Petrick, F. Ayala-Fierro, W. R. Cullen, D. E. Carter and H. V. Aposhian, *Toxicol. Appl. Pharmacol*., 2000, **163**, 203.
6 E. H. Larsen, G. Pritzl and S. H. Hansen, *J. Anal. At. Spectrom*., 1993, **8**, 557.
7 X. C. Le, W. R. Cullen and K. J. Reimer, *Clin. Chem*., 1994, **40**, 617.
8 L. C. Clark, G. F. Combs, Jr, B. W. Turnbull, E. H. Slate, D. K. Chalker, J. Chow, L. S. Davis, R. A. Glover, G. F. Graham, E. G. Gross, A. Krongrad, J. L. Lesher, Jr, H. K. Park, B. B. Sanders, Jr, C. L. Smith and J. R. Taylor, *J. Am. Med. Assoc*., 1996, **276**, 1957.
9 T. Nakane, K. Asayama, K. Kodera, H. Hayahibe, N. Uchida and S. Nakazawa, *Free Radical Biol. Med*., 1998, **25**, 504.
10 M. P. Rayman, *Lancet*, 2000, **356**, 233.
11 L. Letavayova, V. Vickova and J. Brozmanova, *Toxicology*, 2006, **227**, 1.
12 K. T. Suzuki, *J. Health Sci*., 2005, **51**, 107.
13 K. A. Francesconi and F. Pannier, *Clin. Chem*., 2004, **50**, 2240.
14 H. Zeng, E. O. Uthus and G. F. Combs, Jr, *J. Inorg. Biochem*., 2005, **99**, 1269.
15 A. L. Moxon, *Science*, 1938, **88**, 81.
16 J. Gailer, G. N. George, I. J. Pickering, R. C. Prince, S. C. Ringwald, J. E. Pemberton, R. S. Glass, H. S. Younis, D. W. DeYoung and H. V. Aposhian, *J. Am. Chem. Soc*., 2000, **122**, 4637.
17 Y. Shiobara, Y. Tamami and K. T. Suzuki, *Toxicol. Appl. Pharmacol*., 1998, **152**, 309.
18 X. C. Le, X. Lu and X. F. Li, *Anal. Chem*., 2004, **76**, 26A.
19 K. A. Francesconi and D. Kuehnelt, *Analyst*, 2004, **129**, 373.
20 M. V. Hulle, C. Zhang, B. Schotte, L. Mees, F. Vanhaecke, R. Vanholder, X. R. Zhang and R. Cornelis, *J. Anal. At. Spectrom*., 2004, **19**, 58.
21 S. Wangkarn and S. A. Pergantis, *J. Anal. At. Spectrom*., 2000, **15**, 627.
22 J. A. Caruso and M. Montes-Bayon, *Ecotoxicol. Environ. Saf*., 2003, **56**, 148.
23 K. T. Suzuki, B. K. Mandal and Y. Ogra, *Talanta*, 2002, **58**, 111.
24 J. Zheng, M. Ohata and N. Furuta, *J. Anal. At. Spectrom*., 2002, **17**, 730.
25 L. O. Iserte, A. F. Roig-Navarro and F. Hernández, *Anal. Chim. Acta*, 2004, **527**, 97.
26 B. Do, S. Robinet, D. Pradeau and F. Guyon, *J. Chromatogr., A*, 2001, **918**, 87.
27 X. C. Le, X. F. Li, V. Lai, M. Ma, S. Yalcin and J. Feldmann, *Spectrochim. Acta, Part B*, 1998, **53**, 899.
28 B. Gammelgaard and O. Jons, *J. Anal. At. Spectrom*., 1999, **14**, 867.
29 E. N. Dodova, J. F. Tyson and P. C. Uden, in preparation.
30 P. O. Amoako, C. L. Kahakachchi, E. N. Dodova, J. F. Tyson and P. C. Uden, *J. Anal. At. Spectrom*., 2007, DOI: 10.1039/b703381h.
31 F. Pan, PhD Dissertation, University of Massachusetts, Amherst, 2007.
32 K. Wrobel, K. Wrobel and J. A. Caruso, *Anal. Bioanal. Chem*., 2005, **381**, 317.
33 M. A. Quijano, A. M. Gutierrez, M. C. Perez-Conde and C. Camara, *Talanta*, 1999, **50**, 165.
34 B. Gammelgaard, O. Jons and L. Bendahl, *J. Anal. At. Spectrom*., 2001, **16**, 339.
35 Y. Ogra, K. Ishiwata, H. Takayama, N. Aimi and K. T. Suzuki, *J. Chromatogr., B: Biomed. Appl*., 2002, **767**, 301.
36 R. J. Kraus, S. J. Foster and H. E. Ganther, *Anal. Biochem*., 1985, **147**, 432.
37 B. Gammelgaard, L. Bendahl, N. W. Jacobsen and S. Sturup, *J. Anal. At. Spectrom*., 2005, **20**, 889.
38 V. Diaz-Huerta, J. Szpunar, R. Lobinski, M. L. Fernandez-Sanchez and A. Sanz-Medel, *J. Anal. At. Spectrom*., 2003, **18**, 1471.
39 Y. Ogra, T. Hatano, M. Ohmichi and K. T. Suzuki, *J. Anal. At. Spectrom*., 2003, **18**, 1252.
40 S. Greenfield, M. S. Salman, M. Thomsen and J. F. Tyson, *J. Anal. At. Spectrom*., 1989, **4**, 55.
41 E. H. Larsen, G. Pritzl and S. H. Hansen, *J. Anal. At. Spectrom*., 1993, **8**, 557.
42 L. L. Yu, T. A. Butler and G. C. Turk, *Anal. Chem*., 2006, **78**, 1651.
43 W. J. Christian, C. Hopenhayn, J. A. Centeno and T. Todorov, *Environ. Res*., 2006, **100**, 115.
44 R. Ritsema, L. Dukan, T. R. Navarro, W. V. Leeuwen, N. Oliveira, P. Wolfs and E. Lebret, *Appl. Organomet. Chem*., 1998, **12**, 591.
45 D. Kuehnelt, N. Kienzl, P. Traar, N. H. Le, K. A. Francesconi and O. Takafumi, *Anal. Bioanal. Chem*., 2005, **383**, 235.
46 B. Gamelgaard and L. Bendahl, *J. Anal. At. Spectrom*., 2004, **19**, 135.
47 L. Bendahl and B. Gamelgaard, *J. Anal. At. Spectrom*., 2004, **19**, 950.