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REVIEW

Simultaneous speciation of arsenic (As(III), MMA, DMA, and As(V)) and selenium (Se(IV), Se(VI), and $SeCN^-$) in petroleum refinery aqueous streams

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Abstract High-performance liquid chromatography (HPLC) coupled to an ICP-MS with an octapole reaction system (ORS) has been used to carry out quantitative speciation of selenium (Se) and arsenic (As) in the stream waters of a refining process. The argon dimers interfering with the ^{78}Se and ^{80}Se isotopes were suppressed by pressurizing the octapole chamber with 3.1 mL min^{-1} H_2 and 0.5 mL min^{-1} He. Four arsenic species arsenite—As(III), arsenate (As(V)), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA)—and three inorganic Se species—selenite Se(IV), selenate Se(VI), and selenocyanate ($SeCN^-$)—were separated in a single run by ion chromatography (IC) using gradient elution with 100 mmol L^{-1} NH_4NO_3, pH 8.5, adjusted by addition of NH_3, as eluent. Repeatabilities of peak position and of peak area evaluation were better than 1% and about 3%, respectively. Detection limits (as 3σ of the baseline noise) were 81, 56, and 75 ng L^{-1} for Se(IV), Se(VI), and $SeCN^-$, respectively, and 22, 19, 25, and 16 ng L^{-1} for As(III), As(V), MMA, and DMA, respectively. Calibration curve R^2 values ranged between 0.996 and 0.999 for the arsenic and selenium species. Column recovery for ion chromatography was calculated to be 97±6% for combined arsenic species and 98±3% for combined selenium species. Because certified reference materials for As and Se speciation studies are still not commercially available, in order to check accuracy and precision the method was applied to certified reference materials, BCR 714, BCR 1714, and BCR 715 and to two different refinery samples—inlet and outlet wastewater. The method was successfully used to study the quantitative speciation of selenium and arsenic in petroleum refinery wastewaters.

Keywords Speciation · Selenium · Arsenic · Wastewaters · Stream waters · HPLC-ICP-MS

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Introduction

Trace arsenic and selenium speciation analysis is normally performed by HPLC-ICP-MS coupling [1–3]. In natural waters, arsenic exists predominantly in inorganic forms (arsenite and arsenate), and methylated less toxic metabolites dimethylarsinic (DMA) and monomethylarsonic (MMA) are generated by microorganisms under oxidizing conditions [4]. Selenium usually exists in four oxidation states in the environment: selenate (Se(VI)), selenite ((Se(IV)), elemental selenium (Se(0)), and selenide (Se(II)).

Some oil refinery and mining wastewater contains significant concentrations of selenocyanate ($SeCN^-$) [5–7]. Sour crude oils produced from geological formations containing seleniferous marine shales often contain high levels of selenium. $SeCN^-$, and other reduced chemical species, for example hydrogen sulfide, are formed in the reducing environment. As crude oil is processed in refinery operations, selenium is concentrated in the wastewater. The treatment of selenium in waters is a difficult challenge because the mobility of the selenium species is quite different. Conventional wastewater-treatment processes, for example coagulation with ferric salts, are not effective for removal of $SeCN^-$, because of its low affinity for iron

hydroxide at neutral pH. Aeration of the refinery wastewater does not result in obvious oxidation of $SeCN^-$ at neutral pH. $SeCN^-$ can be oxidized to Se(IV) and Se(VI) in aerobic biological water-treatment processes, analogous to thiocyanate oxidation. However, the biological processes are not effective for the removal of the selenium species [8]. Because all three inorganic Se species of interest occur as anions under ambient conditions, anion-exchange chromatography (AEC) has been the most widely used mode of LC separation for waters [9], whereas ion pairing [10], reversed-phase HPLC [11], cation-exchange chromatography [12], and vesicle-mediated HPLC [13, 14] have been successfully used for separation of organic Se species in biological matrices.

A reason for simultaneous speciation of both elements can be found in countering their toxic effects [15, 18–20]. Several methods have been reported to this end, and have profited from the multielement capability of ICP-MS [16, 17].

Because of the ionizable character of arsenic and selenium species, ion-exchange and ion-pair chromatography have mainly been used to separate non-volatile compounds. Arsenic species separation was successfully performed by ion chromatography, in contrast with selenium, of which only the inorganic forms have been separated by this technique.

The objectives of the current study were to develop simultaneous HPLC separation followed by a sensitive and specific detection of the arsenic (As(III), MMA, DMA, and As(V)) and selenium (selenite, or Se(IV), selenate, or Se(VI), and $SeCN^-$) species mainly found in natural surroundings.

Experimental

Instrumentation

An HPLC-ICP-MS system was used for the speciation analysis. A Metrosep A Supp10 (250×4.0 mm) (Metrohm, Herisau, Switzerland) separation column based on a high-capacity styrene–divinylbenzene copolymer having a particle size of only 4.6 μm, protected by a guard column, was used. Separation of the seven species on the Metrohm column was complete in 20–22 min. Gradient elution using 100 mmol L^{-1} NH_4NO_3 (Scharlau, Barcelona, Spain), pH 8.5, adjusted by the addition of NH_3 (Trace Select, Fluka)) was used. Details of the gradient are given in Table 1. The eluent was pumped by a Agilent HPLC 1100 into an Agilent 7500c inductively coupled plasma mass spectrometer (ICP-MS) with an octapole ion guide operated in RF-only mode (Agilent Technologies, Tokyo, Japan). Hydrogen, 4 mL min^{-1}, was introduced into the octapole cell as reaction gas. The column effluent was connected using PEEK tubing to a Meinhard nebulizer with a Scott double-pass quartz spray chamber cooled to 2 °C. The torch position and ion-lens voltage settings were optimized daily for optimum sensitivity with a 10 ng g^{-1} Li, Co, Y, Tl, and Ce mixture in 1% (*w*/*w*) HNO_3 solution. A solution of 1 % (*w*/*w*) HNO_3 was also used to check the background level caused by polyatomic argon interferences. The integration time chosen for each mass was 0.1 s and the masses selected were 75, 76, 77, 78, 79, 80, 81, 82, and 83. Integration of the ICP-MS chromatographic peaks was performed using Origin 5.0 software (Microcal Software, Northampton, MA, USA). Instrument operating conditions are summarized in Table 2.

Table 1 Gradient elution program

Event step	Time (min)	Ammonium nitrate (A)[a]+ water (B), (%)
1	0–4	3+97
2	4–5	30+70
3	5–7	70+30
4	7–20	70+30
5	21–25	3+97

[a] 100 mmol L^{-1} NH_4NO_3 (Scharlau, Barcelona, Spain) pH 8.5 adjusted by addition of NH_3 (Trace Select, Fluka)

Reagents and materials

Ultra-pure water with a resistivity of 18.2 MΩ cm^{-1}, obtained from Milli-Q water-purification system (Millipore, Bedford, MA, USA), and analytical-grade reagents were used to prepare all eluents, standard stock solutions, and dilutions.

Sodium arsenite ($NaAsO_2$), disodium acid arsenate heptahydrate ($Na_2HAsO_4.7H_2O$), and dimethylarsinic acid sodium salt ($Na(CH_3)_2AsO_2.3H_2O$) were from Sigma (St Louis, MO, USA). Monomethylarsonic acid disodium salt ($Na_2CH_3AsO_3.6H_2O$) was from Carlo Erba (Milano, Italy). Sodium selenite (Na_2SeO_3) was from Sigma, sodium selenate (Na_2SeO_4) from Merck (Darmstadt, Germany), and potassium selenocyanate (KSeCN) from Aldrich (Milwaukee, WI, USA). Stock solutions of arsenic and selenium containing approximately 1000 mg L^{-1} As or Se were prepared in water and maintained at 4 °C in the dark. Appropriate dilutions of the stock solution were prepared daily, by weight, using double-deionized water to obtain the required concentration. All solutions were prepared in high-density polyethylene bottles on a balance. Individual species standard solutions were analyzed by IC-ICP-MS to assess any change in elemental speciation. No change in the elemental species of the standard solutions was observed over the duration of the experiments. The mobile

Table 2 Instrumental operating conditions and data acquisition conditions

Chromatographic conditions	
Separation column	Metrosep A Supp10 (250×4.0 mm)
Eluent	Gradient elution program—Table 1
Flow rate	1 mL min^{-1}
Sample loop	100 μL
ICP-MS conditions	
Plasma conditions	
Rf power	1480 W
Nebulizer gas flow rate	1.15 L min^{-1}
Reaction conditions	
H_2 flow rate	3.1 mL min^{-1}
He flow rate	0.5 mL min^{-1}
Cell entrance	−30 V
Cell exit	−21 V
Octapole bias	−13 V
QP bias	−14 V
Data-acquisition conditions (for semiquantitative analysis)	
Points per peak	6
Acquisition time per point	0.1 s
Replicates	1
Data-acquisition conditions (for total As and Se determination)	
Monitored isotopes	75–83
Points per peak	3
Acquisition time per point	4 s
Replicates	5
Data-acquisition conditions (for chromatographic analysis)	
Monitored isotopes	75–83
Integration time	0.1 s
Points per peak	1

phases used ammonium nitrate buffers prepared from ammonium nitrate (NH_4NO_3; Scharlau, Barcelona, Spain). The pH was adjusted by addition of NH_3 (Trace Select, Fluka). These solutions were filtered through a 0.22-μm membrane before use.

Sample preparation

Sample collection and pre-treatment

Industrial wastewater samples (internal stream waters (wastewater inlet) and treated effluents (wastewater outlet)) were collected from a Brazilian petroleum refinery. The pH of the samples were 8.2 and 7.1 for wastewater inlet and outlet, respectively. No acid was added to prevent species transformation. After collection, the samples were stored at 4 °C in dark borosilicate glass flasks. Before injection, water samples were filtered through Nylon 0.45 μm syringe filters (Chromafil; Macherey-Nagel) into 50-mL polypropylene tubes (Sarstedt, Germany) and diluted, whenever possible, for total Se determination and speciation analysis.

Because certified reference materials for As and Se speciation studies are still not commercially available, in order to check accuracy and precision, the developed methodology was applied to certified reference materials, BCR 714, BCR 1714, and BCR 715 and to two different samples—inlet and outlet wastewater.

Procedures

Semiquantitative and quantitative analysis

The samples to be analyzed were diluted 20-fold with 1% (*w*/*w*) HNO_3 and spiked with the internal standard elements: Be, Sc, Ga, Y, Rh, In, Tb, Re, and Th at ca. 10 ng mL^{-1} in order to compare the measured intensities for these elements with those in a response table previously established for the instrument. The estimated concentrations of the analytes were obtained using the Agilent software. Data acquisition conditions for selected isotopes of every element are shown in Table 2.

ICP-MS detection of arsenic and selenium was optimized for the highest signal-to-noise ratio. Although the most abundant selenium isotope is ^{80}Se (49.6% abundance), the presence of Ar_2 species in the argon plasma results in a high background when *m*/*z* 80 is measured. Ar_2 species of *m*/*z* 80 and two other minor isotopes of argon, ^{38}Ar (0.063% abundance) and ^{36}Ar (0.337% abundance), are also responsible for the high background when two other selenium isotopes, ^{78}Se (23.8% abundance) and ^{76}Se (9.4% abundance), are measured. Therefore, ^{77}Se (7.6% abundance) was chosen for monitoring. Although the presence of $ArCl^+$ can lead to a potentially high background at *m*/*z* 77, the HPLC mobile phase excluded Cl species and the system was optimized so that the background and potential interference from $ArCl^+$ species in the determination of ^{77}Se were minimal.

Results and discussion

Ion chromatography operating conditions and performance

A typical chromatogram obtained from a solution containing 5 ng mL^{-1} (as element) of As(III), As(V), MMA, DMA, Se(IV), Se(VI), and $SeCN^-$ in the LC mobile phase is shown in Fig. 1. It is apparent all seven species studied could be well separated in less than 22 min.

In order to enhance the ionization and improve sensitivity for the As and Se species, 0.5% methanol was added to the mobile phase.

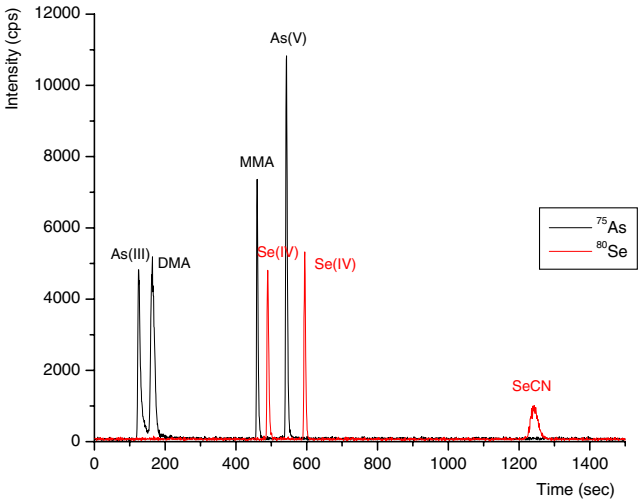

Fig. 1 Ion chromatogram showing separation of the arsenic species As(III), DMA, MMA, and As(V), and the selenium species Se(IV), Se (VI), and $SeCN^-$ (5 ng of each species)

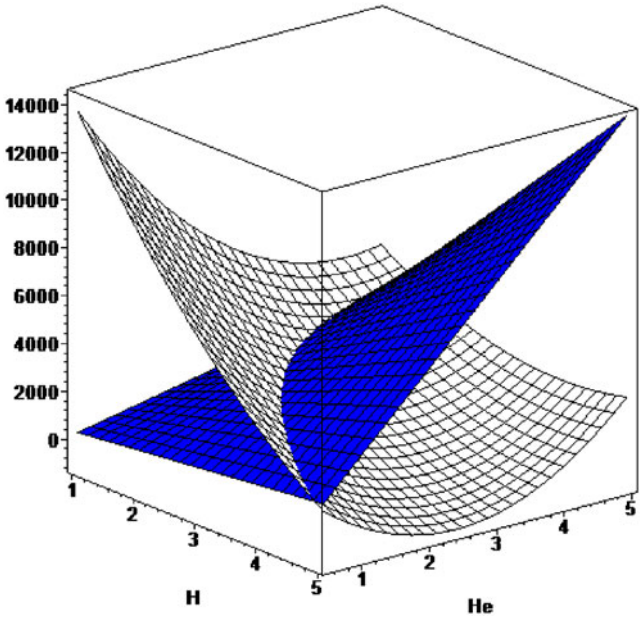

Fig. 2 Response surfaces for ^{75}As (*white*) and ^{80}Se (*blue*)

The reproducibility of chromatographic retention times and peak areas was calculated by taking the standard deviation of six replicates. For the arsenic and selenium species retention time reproducibility ranged from 0.1 to 0.9% RSD and peak area reproducibility ranged from 0.4 to 1.1% RSD. The chromatographic detection limits were calculated by taking three times the standard deviation of seven replicates for the blank peak areas (3δ) divided by the slope of the calibration curves (IUPAC). The detection limits ranged from 16 to 25 ng L^{-1} for arsenic species and 81 to 56 ng L^{-1} for selenium species. The calibration curve R^2 value ranged between 0.996 and 0.999 for arsenic and selenium species. Column recovery for the ion chromatographic run was calculated to be 97±6% for combined arsenic species and 98±3% for combined selenium species. The chromatographic figures of merit are summarized in Table 3.

Octapole collision/reaction cell (ORS) operating conditions

Key conditions of the ICP-MS octapole collision/reaction cell (ORS), including gas flow rates and ion lens voltages, were adjusted for simultaneous removal of As and Se interferences. In collision/reaction cell techniques, different gases can be efficiently used to eliminate argon-based polyatomic ions without affecting the analyte of interest. In this work hydrogen and/or helium was introduced to the ORS in order to efficiently remove interferences ($^{40}Ar^{38}Ar^+$, $^{40}Ar^{40}Ar^+$, and $^{40}Ar^{35}Cl^+$) while keeping maximum sensitivity for the most abundant selenium isotopes ^{78}Se (23.8% abundance) and ^{80}Se (49.6% abundance), and for ^{75}As. Gas flow rates for hydrogen and helium were optimized using D-optimal design methodology. D-optimal designs are one form of design provided by a computer algorithm. Traditional experimental designs (full factorial designs, fractional factorial designs, and response surface designs) are appropriate for calibrating linear models in experimental settings where factors are relatively unconstrained in the region of interest. In some cases, however, models are necessarily nonlinear. In other cases, certain treatments (combinations of factor levels) may be expensive or infeasible to measure. *D-optimal designs* are model-specific designs that address these limitations of traditional designs; they can also be

Table 3 Chromatographic figures of merit

	Retention time (% RSD)	Peak area (% RSD)	Se/As detection limits (ngL^{-1})	Calibration curve R^2 value
As(III)	0.8	0.6	22	0.999
MMA	0.2	1.1	19	0.999
DMA	0.1	0.5	25	0.997
As(V)	0.1	0.4	16	0.999
Se(IV)	0.2	0.7	81	0.999
Se(VI)	0.1	0.8	56	0.998
$SeCN^-$	0.9	0.9	75	0.996

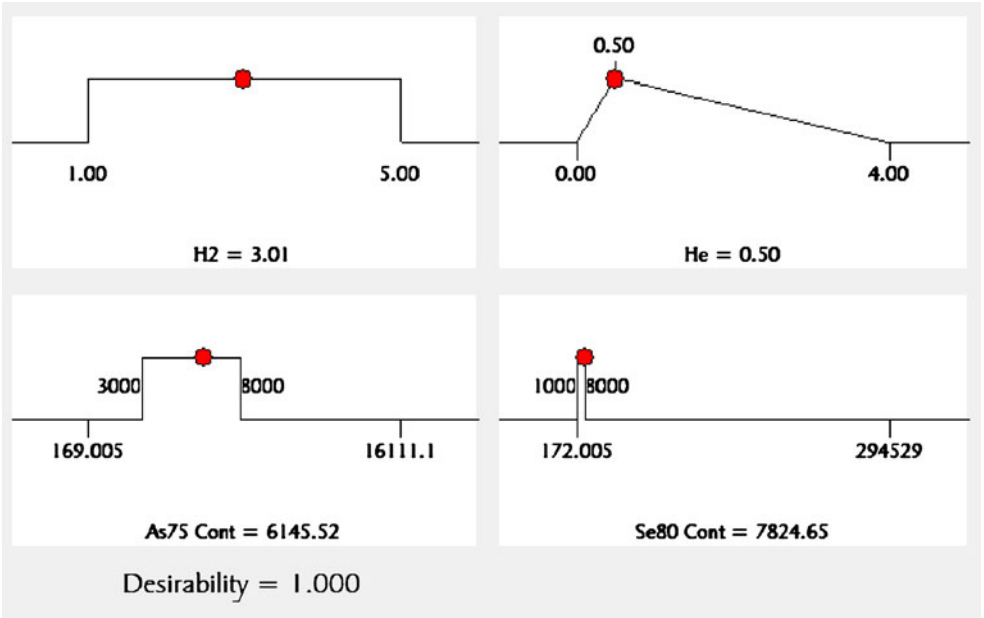

Fig. 3 Desirability 1 functions

optimized when the design-space is constrained, for example, when the mathematical process-space contains factor-settings that are practically infeasible, e.g. because of safety concerns. These types of computer-aided designs are particularly useful when classical designs do not apply [21].

A response surface method (RSM) is designed to estimate interaction, quadratic effects, and the local shape of the response surface is investigated. RSM design is used to find optimal process settings. Figure 2 was plotted using the Eqs. (1) and (2) obtained from the experimental design. The surface of ^{80}Se was rescaled because of the great difference in the intensity magnitude. To optimize both elements simultaneously the desirability 1 approach was used. This is a popular method that assigns a "score" to a set of responses and chooses factor settings that maximize that score. Figure 3 shows the point chosen gives desirability 1.

$$^{75}\mathrm{cps} = 21153.369 - 5325.939{*}H_2 - 5692.225{*}\mathrm{He} + 314.272{H_2}^2 + 570.340{*}\mathrm{He}^2 + 575.013{*}H_2{*}\mathrm{He} \quad (1)$$

$$1/\sqrt{^{80}\mathrm{cps}} = -6,769{*}10^{-4} - 3,267{*}10^{-3}{*}H_2 - 4,981{*}10^{-3}{*}\mathrm{He} + 1,379{*}10^{-3}{*}\mathrm{He}^2 + 2,866{*}H_2{*}\mathrm{He} \quad (2)$$

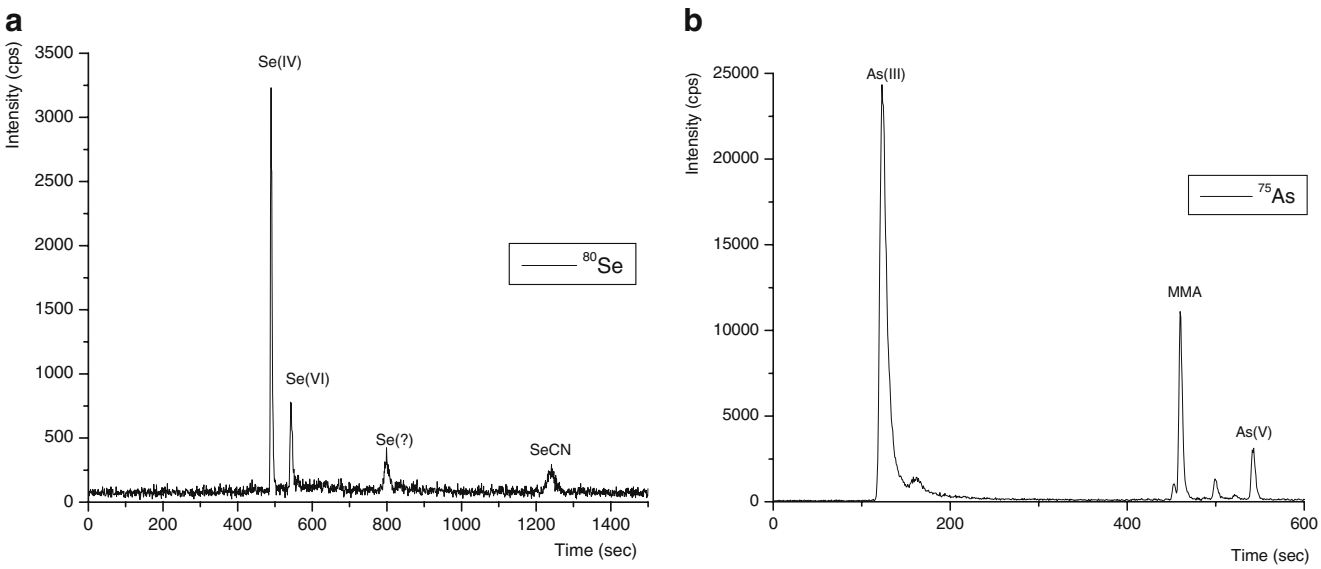

Fig. 4 Ion chromatograms obtained from characteristic process wastewater inlet selenium species (**a**) and arsenic species (**b**) in sample I

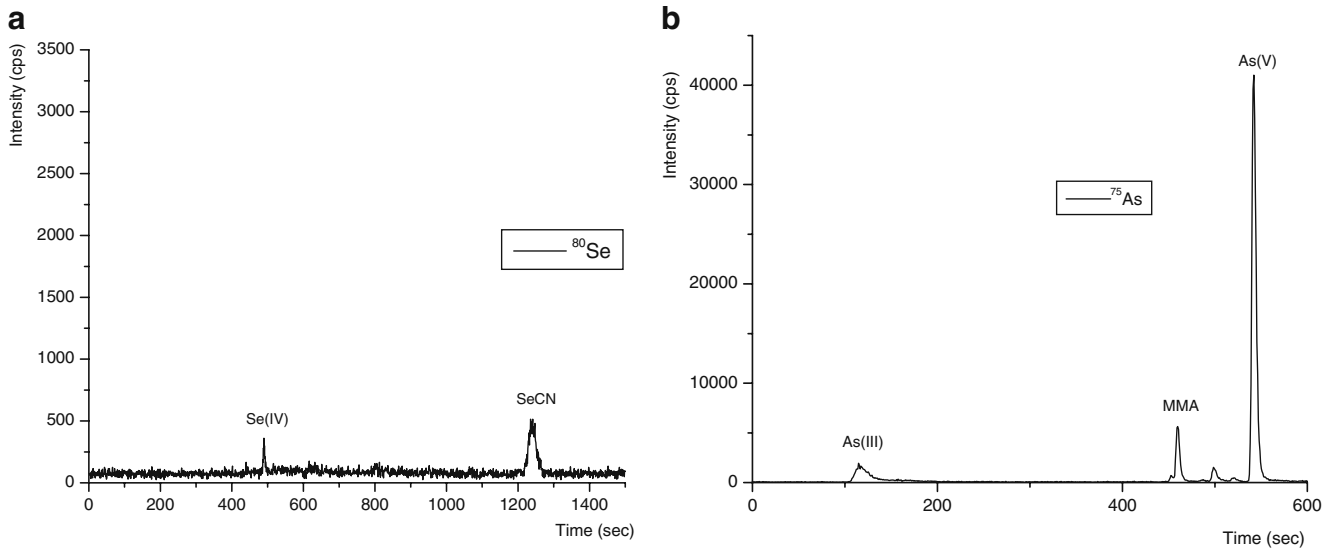

Fig. 5 Ion chromatograms obtained from characteristic process wastewater outlet selenium species (**a**) and arsenic species (**b**) in sample II

The optimization was performed in total analysis, in the range 0–5 mL min^{-1} H_2 and 0–7 mL min^{-1} He, chosen after preliminary experiment for the ORS. A blank solution, 5 g L^{-1} NaCl in ultrapure water, and solutions containing 0.5–20 μg L^{-1} As and Se in the same matrix were measured. For all the experiments, regression and bias factors, and regression coefficients were checked and shown to be significant. Ion lens settings were also optimized in a chlorine matrix spiked with 10 μg L^{-1} As and Se.

The potential of the different ion lenses disposed around the C/RC was also investigated, to enable better focusing of the ion beam and kinetic energy discrimination; the values selected were −14 V for quadrupole bias, −13 V for octapole bias, −20 V for cell entrance, −20 V for cell exit, and −45 V for plate bias.

A mixture of 3.1 mL min^{-1} H_2 and 0.5 mL min^{-1} He was found to be suitable for the removal of both $ArAr^+$ and $ArCl^+$ interferences.

Application to petroleum wastewater samples

Removal of $SeCN^-$ from petroleum refinery wastewater is a two-step process involving, first, its reduction to Se(0) or Se^{2-} by Fe(0) and, further, precipitation as FeS or Se(0) co-precipitation with $Fe(OH)_3$. According to Meng et al [22], the process efficiency is higher under aerobic conditions. It was supposed that the corrosion process helps to remove the oxide layer on the surface activating the Fe(0). Similar effects should be observable for Se(VI). According to Murphy [23], Se(VI) is also removed by Fe(0) by formation of Se(0) and the efficiency is, again, higher under aerobic conditions.

However, under the conditions applied in the studied refinery only $SeCN^-$ and Se(IV) are removed from the wastewater; Se(VI) is not affect by the process (Figs. 4a and 5a). Furthermore, despite the apparent removal of all As(III) and MMA, partial oxidation of arsenic to As(V) is observed (Figs. 4b and 5b).

Therefore, as a consequence of the applied wastewater treatment approximately 80% of both Se and As are removed and the fraction remaining is observed as the oxidized form (As(V) and Se(VI)) (Table 4).

Conclusion

Key conditions of the ICP-MS octapole collision/reaction cell including gas flow rates and ion lens voltages, were adjusted for the simultaneous removal of As and Se interferences. The use of C/RC has a greater effect on the detection limits for selenium than for arsenic.

Table 4 Determination of As and Se species—evaluation of total and species concentrations

		Inlet	Outlet
As (μg L^{-1})	As(III)	123.2	2.5
	MMA	44.3	5.3
	DMA		
	As(V)	4.8	21.6
	Σ species	172.3	29.4
	Total	174.6	31.2
Se (μg L^{-1})	Se(IV)	52.1	3.2
	Se(VI)	19.4	
	$SeCN^-$	18.7	15.4
	Σ species	90.2	18.6
	Total	103	20.3

The merits of coupling HPLC and ICP-MS for arsenic and selenium speciation analysis in stream waters from a refining process have been demonstrated. The concentrations of various arsenic and selenium compounds have been determined. Based on the developed procedure, it is possible, with a single chromatographic run to obtain speciation information for both arsenic and selenium present in a water sample.

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