

## Quantification of Selenium Species in Petroleum Refinery Wastewaters using Ion Chromatography Coupled to Post-Column Isotope Dilution Analysis ICP-MS

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A metodologia analítica de diluição isotópica (IDA) pós-coluna foi empregada para realizar ensaios de especiação quantitativa de selênio (Se) em amostras de efluentes industriais através do acoplamento da cromatografia de íons ao ICP-MS, contendo um sistema composto por uma célula de reação (ORS). As interferências ocasionadas pelos dímeros de argônio nos isótopos  $^{78}\text{Se}$  e  $^{80}\text{Se}$  foram suprimidas pressurizando-se a câmara do octapolo com  $4\text{ mL min}^{-1}$  de hidrogênio. Selenito [Se (IV)], selenato [Se (VI)] e selenocianato [SeCN<sup>-</sup>] foram separados por cromatografia de íons (IC) utilizando eluição isocrática com  $20\text{ mmol L}^{-1}\text{ Na}_2\text{CO}_3/2\text{ mmol L}^{-1}\text{ NaHCO}_3$ . Após a separação, os íons carbonato presentes no eluente foram convertidos a  $\text{CO}_2$  em linha por uma membrana supressora auto-regenerante, a fim de evitar a obstrução do nebulizador ou dos cones de interface do ICP-MS. Limites de detecção ( $3\sigma$  da flutuação da linha de base) de  $0,18$ ,  $0,13$  e  $1,0\text{ ng g}^{-1}$  foram atingidos para Se (IV), Se (VI) e SeCN<sup>-</sup>, respectivamente. A quantificação do selênio, separadamente em cada espécie, foi executada empregando a análise de diluição isotópica (IDA) pós-coluna, adicionando de modo contínuo uma solução enriquecida com  $^{77}\text{Se}$  ao efluente da coluna supressora. Finalmente, as razões entre os isótopos  $^{78}\text{Se}/^{77}\text{Se}$  ou  $^{80}\text{Se}/^{77}\text{Se}$  foram monitoradas e a concentração da espécie correspondente de selênio avaliada. A metodologia proposta foi aplicada com sucesso para o estudo de especiação quantitativa do selênio em efluentes de refinarias de petróleo.

A post-column Isotope Dilution Analysis (IDA) methodology has been used to carry out the quantitative speciation of selenium (Se) in industrial wastewaters by ion chromatography coupled to ICP-MS with an octapole reaction system (ORS). The interfering argon dimers on the  $^{78}\text{Se}$  and  $^{80}\text{Se}$  isotopes were suppressed by pressurizing the octapole chamber with  $4\text{ mL min}^{-1}$  of hydrogen. The inorganic Se species selenite [Se (IV)], selenate [Se (VI)] and selenocyanate [SeCN<sup>-</sup>] were separated by ion chromatography (IC) using isocratic elution with  $20\text{ mmol L}^{-1}\text{ Na}_2\text{CO}_3/2\text{ mmol L}^{-1}\text{ NaHCO}_3$  as eluent. After the separation, the eluent carbonates were converted to  $\text{CO}_2$  on-line by a membrane self-regenerating suppressor, in order to avoid clogging problems in the nebulizer or in the cones of the ICP-MS interface. Detection limits (as  $3\sigma$  of the baseline noise) of  $0,18$ ,  $0,13$  and  $1,0\text{ ng g}^{-1}$  were achieved for Se (IV), Se (VI) and SeCN<sup>-</sup> respectively. Quantification of selenium in each separated species was performed using post-column isotope dilution analysis by continuous mixing of an enriched  $^{77}\text{Se}$  spike solution with the effluent from the suppressor column. Finally, the  $^{78}\text{Se}/^{77}\text{Se}$  or  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios were monitored and the concentration of the corresponding selenium species was evaluated. The proposed methodology has been successfully applied to study the quantitative speciation of selenium in petroleum refinery wastewaters.

**Keywords:** selenium speciation, wastewaters, post-column isotope dilution analysis, inductively coupled plasma mass spectrometry, octapole reaction system

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## Introduction

Sour crude oils produced from geological formations containing seleniferous marine shale often contain high levels of selenium.<sup>1</sup> Due to its chemical similarity with sulfur, selenium follows the whole oil refining process being concentrated into the wastewater.<sup>2</sup> Inorganic selenium is most commonly found in refinery wastewaters as selenate, Se (VI), and selenite, Se (IV), both highly soluble in water. Also, a significant concentration of selenocyanate ( $\text{SeCN}^-$ ), formed in the reducing environment, may be present.<sup>3</sup> As inorganic forms of Se are potentially toxic at high concentrations in aqueous samples,<sup>4</sup> the removal of selenium in industrial wastewaters is mandatory before discharging them into the environment. Most of such treatments for selenium removal depend on the selenium species, e.g. adsorption methods are effective to remove selenite anions but not so effective for  $\text{SeCN}^-$ , in such a way that this compound has to be oxidized before the adsorption step. Of course, the determination of such Se species, owing to the different effects of the various Se species which may be essential or toxic, depending on its oxidation state and concentration,<sup>5</sup> is today of great interest. Therefore the development of analytical methods able to provide reliable, fast and accurate determinations of the different selenium species is required. Ion chromatography (IC) is probably the most commonly used separation method for selenium anionic species in waters.<sup>6-9</sup> Since selenium concentrations in some waters may be extremely low and the concentration of potential interferences may be orders of magnitude higher, a sensitive and selective detector should be used. On-line coupling of a chromatographic system with ICP-MS enables the determination of very low levels of elemental species due to the low detection limits of the detector, its high elemental specificity, multi-element capabilities and possibility of performing isotopic measurements.<sup>10-14</sup> Unfortunately, the measurement of the most abundant Se isotopes ( $^{80}\text{Se}^+$  and  $^{78}\text{Se}^+$ ) by ICP-MS with quadrupole analyzers (ICP-QMS) is seriously affected by polyatomic interferences (e.g.  $^{40}\text{Ar}_2^+$  at  $m/z = 80$  or  $^{40}\text{Ar}^{38}\text{Ar}^+$  at  $m/z = 78$ ). The use of high resolution ICP-MS instruments for this purpose is limited due to the high resolving powers necessary ( $R=10000$ ) which lead to a significant loss of sensitivity. An interesting alternative to overcome those selenium interferences is the use of ICP-MS instruments equipped with collision/reaction cell, as they are able to remove some spectral interferences by pressurizing the cell chamber with gases such as He,  $\text{H}_2$ ,  $\text{O}_2$ ,  $\text{NH}_3$  and  $\text{CH}_4$ .<sup>15</sup>

Nevertheless, ion chromatography eluents cause some problems for on-line ICP-MS detection because they usually contain high concentration of dissolved

salts, used either as buffers or as the eluent itself. This high salt content may alter the plasma properties and thereby affect its analytical performance or may cause gradual clogging of the nebulizer and the cones in the plasma interface (so affecting the long-term stability and robustness of the analytical method). Those problems could be circumvented modifying the eluent after the separation without affecting the separated analytes. This may be carried out by using an anion self-regenerating suppressor column (ASRS) that consists of a continuously operating cation-exchange membrane device where protons are generated electrolytically. Thus, the cations from the eluent (usually  $\text{Na}^+$ ) are replaced continuously by hydronium ions by diffusion from outside of the membrane. In this way eluent anions as carbonate or hydroxide are neutralized and converted to  $\text{CO}_2$  or  $\text{H}_2\text{O}$  which do not affect the nebulizer, the plasma or the extraction interface.<sup>16</sup>

In spite of the development of hyphenated techniques for elemental speciation studies in environmental samples, the reliable quantification of the separated species is still a difficult task. An attractive approach to get reliable determinations of the selenium bound to the different species is the application of isotope dilution analysis (IDA) online with HPLC-ICP-MS.<sup>17</sup> Heumann *et al.*<sup>17</sup> proposed two different modes of operation for this purpose: species-specific and species-unspecific spiking modes, the former can be used only when isotopically enriched species are available. The species-unspecific spiking mode would allow accurate quantification even with unknown composition and structure of the compounds if enriched species-specific standards are not available.<sup>19-21</sup> Here an isotope enriched solution is mixed on-line with the column eluent after the chromatographic separation and, as a consequence, isotopic equilibration between the separated element species and the Se enriched isotope spike is only obtained in the plasma of the ICP-MS. Previous works in our laboratory have shown the applicability of this IDA technique to the precise and accurate determination of selenium species in different biological materials including human serum,<sup>19</sup> yeast and wheat flour enzymatic extracts<sup>20</sup> and cod muscle.<sup>21</sup>

However, ICP-MS sensitivity has to be species independent to correctly apply this methodology. Thus, a post column isotope dilution methodology has been investigated here for the reliable quantification of selenium species in wastewaters from a petroleum refinery. The separation of Se (IV), Se (VI) and  $\text{SeCN}^-$  species was carried out by ion chromatography using a membrane suppressor for the elimination of interferences.

The quantification was carried out by  $^{78}\text{Se}/^{77}\text{Se}$  and  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios measurements using a  $\text{H}_2$  flow for the elimination of polyatomic interferences from argon

dimers on the  $^{78}\text{Se}$  and  $^{80}\text{Se}$  isotopes.<sup>22</sup> A selenium speciation report in such kind of samples by HPLC-ICP-MS is found in the literature.<sup>23</sup> However, conventional quadrupole mass analyzer and methodologies have been used. The particular combination of techniques here reported, aiming at a robust and an accurate determination of selenium species in waste water, has not been reported so far.

## Experimental

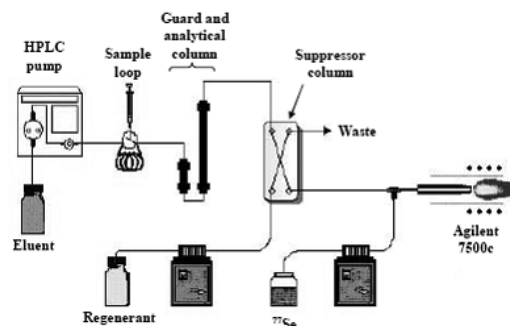
### Instrumentation

The separation was performed using a Dionex model DX-120 ion chromatography system (Sunnyvale, CA, USA), equipped with a 75  $\mu\text{L}$  sample loop. A carbonate selective anion-exchange column (Dionex AS9-HC) and guard column (Dionex AG9-HC) were used. The eluent consisted of 20  $\text{mmol L}^{-1}$   $\text{Na}_2\text{CO}_3$  plus 2  $\text{mmol L}^{-1}$   $\text{NaHCO}_3$  (1  $\text{mL min}^{-1}$ ). After separation, the column effluent is passed through the Anion Self Regenerating Suppressor (ASRS-ULTRA, 4 mm, Dionex) operated at a suppression current of 100 mA in the external water source mode.

Selenium determination was carried out on an inductively coupled plasma mass spectrometer (ICP-MS) Agilent 7500c with an octapole ion guide operated in RF-only mode (Agilent Technologies, Tokyo, Japan). Hydrogen, 4  $\text{mL min}^{-1}$ , was introduced into the octapole cell as reaction gas. The column effluent was connected using PEEK tubing (Dionex) to a Meinhard nebulizer with a Scott double-pass quartz spray chamber cooled down to 2  $^\circ\text{C}$ . The torch position and ion lens voltage settings were optimized daily for optimum sensitivity with a 10  $\text{ng g}^{-1}$  Li, Co, Y, Tl and Ce mixture in 1% (m/m)  $\text{HNO}_3$  solution. A solution of 1% (m/m)  $\text{HNO}_3$  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 76, 77, 78, 79, 80, 81, 82 and 83. Integration of the ICP-MS chromatographic peaks was performed using the Origin 5.0 software (Microcal Software Inc. Northampton, MA, USA). Instrumental operating conditions are summarized in Table 1 and a schematic diagram of the setup system is shown in Figure 1. The continuous addition of the spike (0.1  $\text{mL min}^{-1}$ ) was performed by a peristaltic pump model HP4 from Scharlau Science (Barcelona, Spain).

### Reagents and materials

Ultra-pure water with a resistivity of 18.2  $\text{M}\Omega \text{ cm}^{-1}$  obtained from Milli-Q water purification system (Millipore Co., Bedford, MA, USA) and analytical grade reagents



**Figure 1.** Experimental setup for the inorganic selenium speciation in wastewaters by online IDA with IC-ICP-MS.

**Table 1.** Instrumental operating conditions and data acquisition parameters

Chromatographic parameters	
Separation columns	Dionex Ion Pac AS 9-HC (250 $\times$ 4 mm id) and AG 9-HC (50 $\times$ 4 mm id)
Eluent	20 $\text{mmol L}^{-1}$ $\text{Na}_2\text{CO}_3$ / 2 $\text{mmol L}^{-1}$ $\text{NaHCO}_3$
Flow rate	1 $\text{mL min}^{-1}$
Sample loop	75 $\mu\text{L}$
Suppressor	ASRS-ULTRA (4 mm)
Regenerant	Distilled water (5 $\text{mL min}^{-1}$ )
ICP-MS parameters	
Plasma parameters	
Rf power	1500 W
Nebulizer gas flow rate	1.12 $\text{L min}^{-1}$
Reaction parameters	
$\text{H}_2$ flow rate	4 $\text{mL min}^{-1}$
Cell entrance	-30 V
Cell exit	-21 V
Octapole bias	-13 V
QP bias	-12 V
Data acquisition parameters (for semiquantitative analysis)	
Points per peak	6
Acquisition time per point	0.1 s
Replicates	1
Data acquisition parameters (for total Se determination)	
Monitored isotopes	76-83
Points per peak	3
Acquisition time per point	4 s
Replicates	5
Data acquisition parameters (for chromatographic analysis)	
Monitored isotopes	76-83
Integration time	0.1 s
Points per peak	1

were used to prepare all eluents, standard stock solutions and dilutions.

Enriched  $^{77}\text{Se}$  ( $91.1 \pm 0.7\%$ ) was supplied from Cambridge Isotope Laboratories (Andover, MA, USA) as elemental powder and it was dissolved in the minimum volume of sub-boiled nitric acid and diluted to volume with ultra-pure water. The concentration of this standard solution, ( $5.31 \pm 0.03$ )  $\mu\text{g g}^{-1}$ , was established by reverse isotope dilution analysis as is discussed in detail elsewhere.<sup>22</sup> A standard solution of 1000  $\text{mg L}^{-1}$  of Se as  $\text{SeO}_2$  stabilized in 2-3% (v/v) nitric acid Suprapur® was purchased from Merck (Darmstadt, Germany). Sodium selenite  $\text{Na}_2\text{SeO}_3$  from Sigma (St. Louis, MO, USA), sodium selenate  $\text{Na}_2\text{SeO}_4$  from Merck (Darmstadt, Germany), and potassium selenocyanate  $\text{KSeCN}$  from Aldrich (Milwaukee, WI, USA) were used to prepare 1000  $\text{mg L}^{-1}$  stock solutions. The purity of these Se standards was  $> 99\%$ . All solutions were prepared in high density polyethylene bottles on a weight basis and stored at 4 °C in the dark. 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. Daily working standards were prepared by dilution of the stock solutions. For the mobile phase, sodium hydrogen carbonate  $\text{NaHCO}_3$  and sodium carbonate  $\text{Na}_2\text{CO}_3$  from Merck (Darmstadt, Germany) were used. HPLC eluent was freshly prepared and degassed with helium prior to use. Nylon 0.45  $\mu\text{m} \times 13$  mm syringe filters from Waters (Millipore Co., Bedford, MA, USA) were used for filtration of samples.

### Procedures

#### Sample collection and pre-treatment

Industrial wastewater samples were collected from a Brazilian petroleum refinery. They are related to internal stream waters (CAO and CAC samples) and treated effluents (BA sample). The pHs of the samples were 8.3, 8.9 and 7.3 for CAO, CAC and BA, respectively. No acid was added to prevent species transformation. After collection, the samples were stored at 4 °C in dark borosilicate glass flasks. Prior to injection, water samples were filtered through nylon 0.45  $\mu\text{m}$  syringe filters.

#### Semi-quantitative analysis

The samples to be analyzed were diluted 20-fold with  $\text{HNO}_3$  1% (m/m) and spiked with the internal standard elements: Be, Sc, Ga, Y, Rh, In, Tb, Re and Th at *ca.* 10  $\text{ng mL}^{-1}$  levels in order to compare the measured intensities for these elements with a previously

established response table of the instrument. The estimated concentrations of the analytes were obtained using the Agilent software. Data acquisition parameters for selected isotopes of every element are shown in Table 1.

#### Total Se determination by IDA-ICP-MS

The total selenium concentration in the wastewaters was determined by IDA-ICP-MS after five-fold dilution of the samples with ultra pure water. The samples were spiked with the appropriate amount of  $^{77}\text{Se}$  enriched standard solution, calculated in previous work.<sup>22</sup> Plastic polypropylene containers were used to prepare all solutions on a weight basis. The ICP-MS and data acquisition parameters are given in Table 1.

All isotope ratio measurements were corrected using a dead time of 47 ns. For accurate isotope ratio measurements, the Se intensity signals were corrected for  $\text{SeH}^+$  and  $\text{BrH}^+$  interferences using mathematical equations previously described.<sup>22</sup> A natural standard of selenium was also measured between the samples to calculate the mass bias correction factor using an exponential model.<sup>24</sup>

Finally, the isotope dilution equation<sup>25</sup> was applied to determine the total selenium concentration by IDA-ICP-MS using the  $^{78}\text{Se}/^{77}\text{Se}$  and  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios.

#### Post-column isotope dilution analysis

The quantification of each separated selenium species was performed by post-column isotope dilution analysis. A  $^{77}\text{Se}$ -enriched standard solution of appropriate concentration to obtain the optimum spike to sample ratio<sup>25</sup> was continuously introduced (at 0.1  $\text{mL min}^{-1}$ ) using a peristaltic pump through a T piece. The data acquisition parameters are shown in Table 1.

The obtained intensity chromatograms ( $\text{counts s}^{-1}$ ) were converted to mass flow chromatograms ( $\text{ng min}^{-1}$ ) for quantification of the selenium species. For this purpose, the time resolved intensity counts were first corrected taking into account the detector dead time (47 ns). After smoothing of the data using moving average ( $n = 15$ ) in order to reduce the noise level and to guarantee optimum peak definition, mathematical corrections were applied to correct for  $\text{BrH}^+$  and  $\text{SeH}^+$  interferences<sup>22</sup> with the factors obtained using natural Se and Br standards introduced post-column instead of the enriched spike and using the chromatographic mobile phase as matrix between each triplicate sample determination. Then, the  $^{78}\text{Se}/^{77}\text{Se}$  or  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios were calculated and corrected for mass bias using an exponential model.<sup>24</sup> The mass bias factor was calculated using the same standard solution as for  $\text{SeH}^+$  correction. Finally the on-line isotope dilution equation<sup>26</sup> was applied to each point of the chromatogram

obtaining a mass flow chromatogram. The amount of selenium in each chromatographic peak was calculated by integration of the chromatographic peaks and the concentration of selenium was calculated by dividing the Se amount found by the injection volume (75  $\mu\text{L}$ ).

## Results and Discussion

### *Semiquantitative analysis*

Analysis of industrial wastewaters by IC-ICP-MS is not easy because competition between matrix and analyte anions in the chromatographic separation and also spectroscopic interferences during the detection by ICP-MS are common place. In order to investigate the extent of such interferences in the wastewater samples, a semi-quantitative analysis was first performed for every sample. The waste water samples from the petroleum refinery contained appreciable amounts of chloride, sulfur and bromide (*ca.* 2  $\mu\text{g g}^{-1}$ ) and also high amounts of sodium, magnesium, calcium, and potassium (*ca.* 200, 14, 10 and 4  $\mu\text{g g}^{-1}$  respectively). The high saline content of this complex matrix may affect the analytical performance of the chromatographic separation. Moreover, some major matrix anions (e.g. chloride, sulfate) also cause chromatographic interferences, as will be discussed later. Also, spectroscopic interferences due mainly to the high amount of bromide in the matrix may occur. As previously published,<sup>16</sup> alkaline and alkaline-earth elements are selectively removed from the chromatographic eluent by the use of a suppressor column (so we do not expect significant spectral or matrix interferences in the plasma derived from the high saline content of the sample).

### *Total Se determination by IDA-ICP-MS*

For an accurate isotope dilution analysis (total Se determination and quantification of the different Se species after separation by IC), all the chosen Se isotopes should be free of spectral interferences. In previous work<sup>22</sup> no detectable interferences at  $^{77}\text{Se}$  by chlorine up to concentration of 400  $\mu\text{g g}^{-1}$  was observed in the determination of total selenium in biological samples by IDA-ICP-MS. However, the interference of  $^{79}\text{BrH}^+$  on  $^{80}\text{Se}$  was important at these bromide levels even with the employ of the octapole reaction system (ORS) pressurized with  $\text{H}_2$ . This interference was corrected by monitoring also  $^{79}\text{Br}$  and  $^{81}\text{Br}$ , at the same time as the analyte isotopes, and then using mathematical corrections.

The total Se amount in the petroleum refinery wastewater samples was determined using IDA-ICP-MS after 5-fold

dilution of the sample with ultra pure water using  $^{77}\text{Se}$  standard as spike. The total Se concentrations obtained using  $^{78}\text{Se}/^{77}\text{Se}$  and  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios respectively are listed in Table 2. Three replicates of each sample were analyzed. As can be seen from the table, the results found by measuring both isotope ratios after adequate correction of  $\text{SeH}^+$  and  $\text{BrH}^+$  interferences were in good agreement.

**Table 2.** Analysis of total selenium in wastewater samples from petroleum refinery by IDA-ICP-MS (uncertainty expressed as std. dev.,  $n = 3$ )

Sample	Concentration found / ( $\text{ng g}^{-1}$ )	
	$^{80}\text{Se}/^{77}\text{Se}$	$^{78}\text{Se}/^{77}\text{Se}$
CAC	$48.7 \pm 0.3$	$48.4 \pm 0.4$
CAO	$55.6 \pm 0.2$	$55.4 \pm 0.3$
BA	$31.4 \pm 0.7$	$31.2 \pm 0.6$

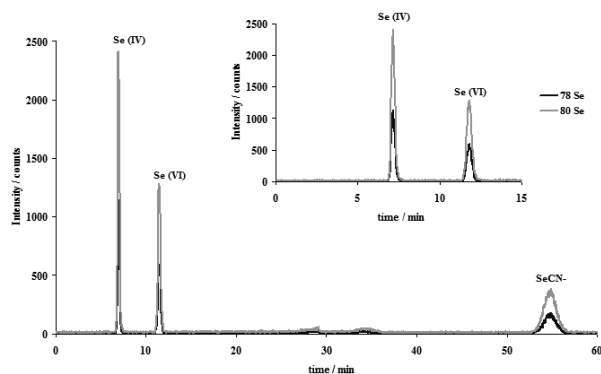
This demonstrates that the spectral interferences (due mainly to the high amounts of bromine in this type of environmental matrix) are satisfactorily corrected using the mathematical corrections, as previously reported for biological materials<sup>22</sup> It is worth mentioning that Se level found in the BA sample complies with the ranges given in the Brazilian regulation for effluents discharge to freshwaters ( $< 50 \mu\text{g L}^{-1}$  of total Se).<sup>27</sup>

### *Analytical performance characteristics for Se speciation in wastewaters*

Prior to the quantification of inorganic selenium species in wastewaters by IDA-ICPMS, the on-line ion chromatographic separation was investigated. The selected chromatographic conditions are listed in Table 1. To achieve a high resolution for inorganic selenium species, an isocratic elution with 20  $\text{mmol L}^{-1} \text{Na}_2\text{CO}_3$  / 2  $\text{mmol L}^{-1} \text{NaHCO}_3$  was applied. With this mobile phase, a complete separation of the Se (IV), Se (VI) and  $\text{SeCN}^-$  is achieved (60 min are necessary due to the high retention of the  $\text{SeCN}^-$ ). In spite of the long elution time for  $\text{SeCN}^-$ , the long-term stability of this method is not affected for clogging problems caused by the highly saline eluent thanks to the use of a membrane salt suppressor. The chromatogram of the three species in aqueous standard solution (10  $\text{ng g}^{-1}$  per species) is shown in Figure 2. The observed detection limits of  $^{80}\text{Se}$  (calculated as  $3\sigma$  of the baseline noise, based on peak height) were ( $0.18 \pm 0.01$ ), ( $0.13 \pm 0.01$ ) and ( $1.0 \pm 0.08$ )  $\text{ng g}^{-1}$  for Se (IV), Se (VI) and  $\text{SeCN}^-$  respectively. The detection limits obtained are comparable with those previously reported with sample volumes commonly used in IC-ICP-MS for Se (IV) and Se (VI), which eluted at shorter retention times.<sup>11,12,28</sup> The observed higher detection limit for  $\text{SeCN}^-$  could be described



to the broad peak obtained for  $\text{SeCN}^-$  due to its comparatively long retention time under the selected chromatographic conditions. Detection limits of *ca.*  $0.2 \text{ ng L}^{-1}$  for these three species by IC-ICP-MS have been recently reported by Wallschlager and London<sup>14</sup> but using an additional hydride generation step in order to increase the analyte introduction efficiency. Of course, such additional step increases the complexity of the setup and can be recommended only for the analysis of uncontaminated waters at  $\text{ng L}^{-1}$  levels. The detection limits obtained in our work are adequate for the precise and accurate quantification of Se species in petroleum refinery wastewater. Reproducibility of replicate analyses of standard solutions was evaluated at  $10 \text{ ng g}^{-1}$  and RSD between 3.4% and 7.6% ( $n = 3$ ) were obtained.



**Figure 2.** IC-ICP-MS chromatogram of Se (IV), Se (VI) and  $\text{SeCN}^-$  in aqueous standard solution ( $10 \text{ ng g}^{-1}$  per specie). Chromatographic conditions are listed in Table 1.

Besides the spectroscopic interferences previously discussed, major matrix anions could also cause chromatographic interferences in the analysis of petroleum refinery wastewaters by IC-ICP-MS. In fact, Wallschlager and Roehl<sup>13</sup> reported chromatographic interferences by chloride and sulfate in estuarine waters. This problem was avoided by diluting 20-fold the estuarine waters and using large sample volume injected (1-11 mL sample loop) that resulted in higher matrix tolerance than using smaller volume of the undiluted sample.<sup>13</sup> Unfortunately, for refinery wastewater samples (with an extremely complex matrix and low concentration of Se species) the use of large volumes of diluted samples turned out to be inadequate to obtain suitable chromatographic peaks for on-line accurate quantification of Se species by IDA IC-ICPMS. Tirez *et al.*<sup>12</sup> found optimal separation of inorganic Se species in industrial wastewaters by combining low sample volume injection with high flow rates. In the analysis of such petroleum refinery wastewaters, chromatographic interferences by chloride, bromide and sulfate on the Se (IV) peak were observed under the selected chromatographic

conditions, due to retention times overlapping. In our work, a sample loop volume of  $75 \mu\text{L}$  (undiluted samples) with a flow rate of  $1 \text{ mL min}^{-1}$  of the mobile phase offered a compromise between matrix tolerance and good resolution of the Se chromatographic peaks allowing the accurate quantification of Se (IV) in wastewaters by IDA IC-ICP-MS as discussed in next section.

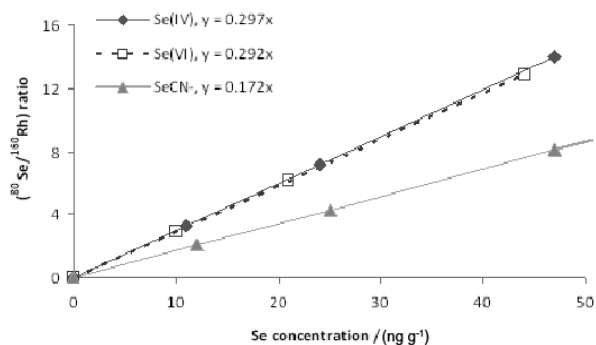
#### *Determination of Se species by post-column IDA*

Accurate Se concentration results by post-column isotope dilution analysis are obtained provided that the elemental response in the ICP is independent of the nature of the Se chemical species entering the ion source. Thus, nebulization, atomization and ionization efficiencies should be species independent. However, for certain sample introduction systems, such as ultrasonic nebulizer with membrane desolvator, significant differences have been observed.<sup>29</sup> In our case, using a standard nebulization system (Meinhard nebulizer coupled to a double path spray chamber). Figure 3 shows typical calibration graphs obtained by direct nebulization of standard solutions of Se (IV), Se (VI) and  $\text{SeCN}^-$ . The anomalous behavior for  $\text{SeCN}^-$  could be reproduced on different days directly in the ICP-MS and also after separation of the species by ion chromatography. As the total Se concentration in  $\text{SeCN}^-$  standard solutions was also confirmed by IDA-ICP-MS (after microwave-assisted digestion with  $\text{HNO}_3/\text{H}_2\text{O}_2$ ) it appears to be an analyte transport problem to the plasma.

As the post-column spike  $^{77}\text{Se}$  used is in the form of Se (VI) a systematic error in the determination of  $\text{SeCN}^-$  would be expected if this difference in ICP-MS response is not taken into account. Hence, for the calculations by post-column isotope dilution analysis the intensities obtained for  $\text{SeCN}^-$ , after the chromatographic separation, were corrected by an adequate factor, corresponding to the ratio of the slopes of the calibration graphs obtained for Se (VI) and  $\text{SeCN}^-$  respectively (0.291/0.172). Of course, this correction procedure increases the uncertainty of the results for  $\text{SeCN}^-$  and creates some concerns about the advantages of post-column isotope dilution analysis for the determination of the selenium species  $\text{SeCN}^-$ .

#### *Analysis of the refinery wastewater*

Wastewater samples (three replicates) were injected undiluted in the chromatographic system and ion chromatography (IC) was used for the Se species separation. The instrumental setup is shown in Figure 1 using optimised parameters, listed in Table 1. Then, the IC-IDA-ICP-MS approach was applied to the determination



**Figure 3.** Calibration curves of selenium species using conventional nebulisation ICP-MS.

of inorganic Se species in 3 petroleum refinery wastewater samples, including internal stream waters (CAC and CAO samples) and treated effluents (BA sample) from the oil refinery. The analytical results are summarized in Table 3 for the  $^{78}\text{Se}/^{77}\text{Se}$  isotope ratio. Again, the concentrations found by measuring the  $^{78}\text{Se}/^{77}\text{Se}$  and  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios were in good agreement, demonstrating that the spectral interferences were satisfactorily corrected using the mathematical equations used here.

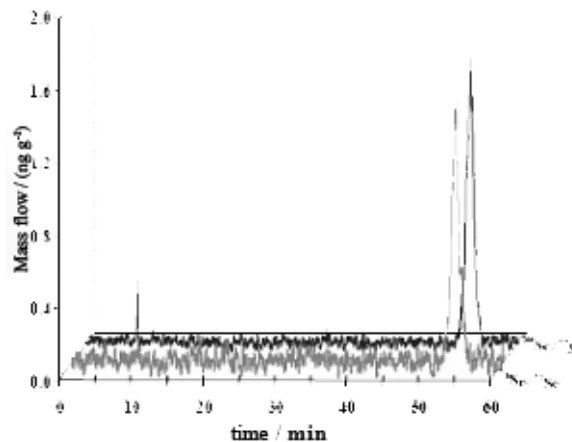
**Table 3.** Determination of inorganic selenium species in petroleum refinery wastewater by IC-IDA-ICP-MS and of total selenium concentration, determined by direct ICP-MS (uncertainty expressed as std. dev.,  $n = 3$ )

Sample	Se concentration / ( $\text{ng g}^{-1}$ )			
	$^{78}\text{Se}/^{77}\text{Se}$ isotope ratio			Total
	$^{\text{a}}\text{Se(IV)}$	$^{\text{a}}\text{Se(VI)}$	$^{\text{a}}\text{SeCN}^-$	
CAC	<sup>b</sup> n.d.	n.d.	$46 \pm 2$	$48.4 \pm 0.4$
CAO	n.d.	n.d.	$56 \pm 1$	$55.4 \pm 0.3$
BA	$12 \pm 1$	n.d.	n.d.	$31.2 \pm 0.6$

<sup>a</sup>Normalized to injection sample volume. <sup>b</sup>Not detected.

In the internal stream waters from the petroleum refinery (CAC and CAO samples), which are essentially an aqueous extract of the crude oil,  $\text{SeCN}^-$  was the only Se species detected. The  $\text{SeCN}^-$  concentrations in those two samples (Table 3) were  $(46 \pm 2)$  and  $(56 \pm 1)$   $\text{ng g}^{-1}$ , respectively after the correction for the  $\text{SeCN}^-$  differential response. As can be seen in Table 3, there is a good agreement between the speciated content of Se found by IC-IDA-ICP-MS and total Se found directly by IDA-ICP-MS. The average recoveries were  $(95 \pm 6)\%$  ( $n = 3$ ) for CAC sample and  $(101 \pm 5)\%$  ( $n = 3$ ) for CAO sample.

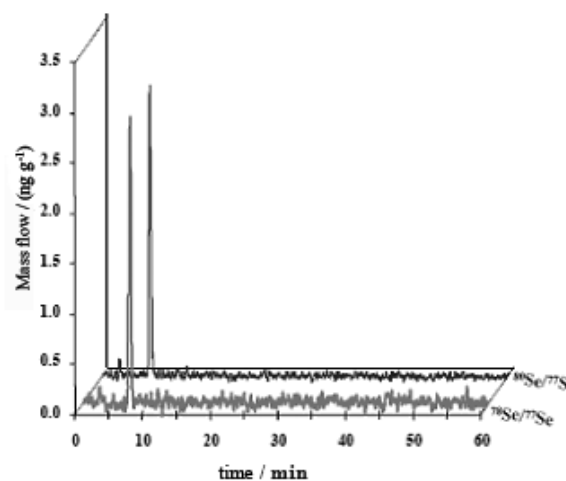
As an example, intensity chromatograms by IC-IDA-ICP-MS obtained at masses 77, 78 and 80 and the corrected final mass flow chromatograms at the  $^{78}\text{Se}/^{77}\text{Se}$  and  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios respectively (shifted for clarity) for the CAO sample are shown in Figure 4.



**Figure 4.** (a) Intensity chromatograms for  $^{77}\text{Se}$ ,  $^{78}\text{Se}$  and  $^{80}\text{Se}$  isotopes and (b) corresponding mass flow chromatograms obtained for  $^{78}\text{Se}/^{77}\text{Se}$  and  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios during separation in the internal stream water (CAO sample) from the petroleum refinery by IC-ICP-IDA-MS.

The integration of the corresponding mass flow chromatograms for  $^{78}\text{Se}/^{77}\text{Se}$  and  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios showed in the Figure 4 provided virtually the same results (within the reported standard deviation of the measurements).

Along the water treatment process, the  $\text{SeCN}^-$  was effectively removed and the total Se concentration decreased as can be observed in Table 3 for BA sample. The corrected final mass flow chromatograms for the BA sample are given in Figure 5.



**Figure 5.** (a) Intensity chromatograms for  $^{77}\text{Se}$ ,  $^{78}\text{S}$  and  $^{80}\text{Se}$  isotopes and (b) corresponding mass flow chromatograms obtained for  $^{78}\text{Se}/^{77}\text{Se}$  and  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios during separation in the treated effluent (BA sample) from the petroleum refinery by IC-ICP-IDA-MS.

Although the detector response is specific for Se, the retention times of chloride, bromide and sulfate peaks are very close to Se (IV) affecting the peak shape and thereby the precision of the integration of this peak.

Spectroscopic interferences of  $^{79}\text{BrH}^+$  (on  $^{80}\text{Se}$  isotope) also affected the precision of the quantification of Se (IV)

(to a lesser extent than for  $\text{SeCN}^-$ ), which is reflected in the values showed in Table 3 obtained by IDA-IC-ICP-MS. However, precise quantification for Se (IV) could be achieved using both isotope ratios.

As can be seen in Table 3, in the analysis of BA sample low overall recoveries of Se (IV) related to total Se were observed ( $40 \pm 8\%$ ,  $n=3$ ), indicating that other unknown selenium species seem to be present in this sample but they are either removed by the suppressor column (cationic species) or not eluted from the column, as already pointed out by Miekeley *et al.*<sup>23</sup>

## Conclusions

The development of robust methods for reliable quantification of Se species in waters is most important today due to the lack of suitable certified reference materials. Of course, using IDA techniques is a powerful tool for validation of quantitative speciation results.<sup>17</sup> The potential of the coupling of ion chromatography with a suppressor system and on-line isotope dilution ICP-MS for the quantification of selenium species in wastewater samples has been demonstrated here.

The use of an ORS pressurized with  $\text{H}_2$  allows the quantification of the main isotope  $^{80}\text{Se}$  and the elimination of polyatomic interferences. The detection limits obtained by the coupling IC-ICP-MS for Se (IV), Se (VI) and  $\text{SeCN}^-$  in the ICP-MS are adequate for Se speciation determinations in industrial or contaminated waters. Moreover, the concentrations found by measuring the  $^{78}\text{Se}/^{77}\text{Se}$  or the  $^{80}\text{Se}/^{77}\text{Se}$  isotope ratios were in good agreement.

The difference in analytical response observed for Se (IV) and Se (VI) with respect to  $\text{SeCN}^-$  in the ICP-MS is a cause of concern for an accurate determination of  $\text{SeCN}^-$  using post-column isotope dilution analysis. The correction factor applied seems to be adequate in the particular cases studied (admittedly it might cause general uncertainties in other cases where unknown selenium species should be determined).

Finally, although the aim of this work was not the study of the wastewater treatment process to eliminate  $\text{SeCN}^-$ , the proposed methodology has proved the effectiveness of the industrial oxidation process of  $\text{SeCN}^-$  (the only species found in untreated waters) to Se (IV) after the industrial effluent treatment.

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