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Organic and inorganic selenium speciation using high-performance liquid chromatography with UV irradiation and hydride generation-quartz cell atomic absorption spectrometric detection

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Abstract

We propose a new on-line method for organic and inorganic selenium speciation, consisting of liquid chromatography–UV irradiation–hydride generation-quartz cell atomic absorption spectrometry. Unlike other methods, UV irradiation for the derivatization of all selenium species to selenite, before hydride generation, is a simpler and cleaner method, since it does not require any additional reagent. The reaction time is also much shorter. A good resolution between the four species (selenite, selenate, selenocystine and selenomethionine) is achieved in less than 15 min. The optimisation of operating conditions, including those used during separation and UV irradiation, is described. Detection limits, precision, linear ranges and recovery are reported. The technique can be considered a reliable, straightforward system for selenium speciation.

Keywords: Selenium; Organoselenium compounds; Seleno amino acids; Metals; Selenite; Selenate

1. Introduction

The availability of analytical techniques for the separation and determination of the compounds of an element at trace level has gained considerable importance. In this context, hyphenated techniques are those most frequently used. For selenium, speciation is necessary because of the differing mobility, toxicity and bioavailability of its compounds.

The separation of inorganic selenium species has been achieved by gas chromatography (GC), following derivatization to form a volatile selenium compound [1]. However, liquid chromatography (LC) is the most widely used technique [2]; ion-pair LC [3,4] has been described as a feasible method, but anion-exchange LC is the most widely used because of the anionic charge of both species in a wide range of pH [5,6]. More recently, a variety of other techniques, such as capillary electrophoresis (CE) [7] has been reported for inorganic selenium speciation. Thin-layer chromatography (TLC) [8] and GC [9] have been described for the separation of organic selenium species. However, very few papers deal with the simultaneous separation of organic and inorganic selenium species [2]; in many cases, separation has been achieved by using anion-exchange LC [10–13].

Spectroscopic techniques are those most widely used as a detection system. Molecular fluorescence spectrometry (MFS) has been used [14,15]. More recently, other techniques have been developed for on-line detection. Thus, atomic fluorescence spectrometry (AFS) [5,16], and inductively coupled plasma-mass spectrometry (ICP-MS) [11,17] have

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been incorporated, with very good results. Atomic spectroscopic techniques are the most widely used detectors because of their high selectivity and sensitivity. Similarly, the use of electrothermal atomic absorption spectrometry (ETAAS) to detect previously separated selenium compounds [18,19] has been described; however, including this technique in on-line couplings is complicated by difficulties in the discrete sample introduction. Optical emission spectroscopy (OES) or quartz cell atomic absorption spectrometry (QCAAS), using hydride generation (HG) for sample introduction are the most widely used methods, because of their greater sensitivity. The generation of hydrogen selenide is produced by $NaBH_4$ in an acidic medium. A wide range of concentrations of hydrochloric acid, depending on the coupling system [20–23], is the most widely used medium, although sulphuric acid has also been suggested [24,25].

Unlike selenite, selenate cannot be directly reduced to hydrogen selenide by $NaBH_4$ in an acidic medium. Seleno amino acids are only partially decomposed to form the hydride generation in the acidic–$NaBH_4$ medium. Thus, a derivatization step prior to detection is required. Traditionally, concentrated HCl at high temperatures has been used to reduce selenate to selenite, whereas other reagents reduce selenate to elemental selenium [26]. Before reduction, all the selenium species must be oxidised to selenate. For this purpose, strong oxidising agents have been used, such as concentrated acid mixtures at high temperatures [27] or concentrated H_2O_2 [28]. The dependence of this derivatization method on HCl concentration, reaction time and temperature, as well as the difficulty involved in enclosing this heating step in an on-line coupling, limit its use. More recently, the use of microwave irradiation as a heating system for the derivatization step has been introduced. The simple introduction of a reaction loop into the microwave oven means it is suitable for use in an on-line system, though an ice bath or water bath after the microwave unit is required because of the great increase in sample solution temperature. In many cases the reduction of selenate to selenite can be achieved by using 6 mol l^{-1} HCl [5,16,22,23,29]. The oxidation of selenium compounds to selenate can be achieved by using an additional loop in the microwave unit and using $K_2S_2O_8$ [10]. Other reagents, such as HBr–$KBrO_3$ mixture [30,31] or KBr–HCl mixture [12], are currently being assayed for the simultaneous decomposition of organic selenium and the reduction of selenate to selenite.

This paper presents a new on-line coupling for organic and inorganic selenium speciation, consisting of liquid chromatography–UV irradiation–hydride generation-quartz cell atomic absorption spectrometry (LC–UV irradiation–HG-QCAAS). The studies carried out by Batley [32] suggest that on-line UV–photoreduction of selenate to selenite as a preliminary step to the generation of hydrogen selenide is feasible [6]. Furthermore, the photodecomposition of organic selenium compounds to selenite after UV irradiation has been observed, although the mechanism of this decomposition is still unknown [33]. A new simpler, cleaner chromatographic separation has been developed. Good resolution between the four compounds measured (selenite, selenate, selenocystine and selenomethionine) in less than 15 min has been achieved. The conditions of separation, UV irradiation, as well as the optimisation of hydride generation and the atomisation step are discussed; quality parameters (linear range, detection limits, precision and recovery) are reported.

2. Experimental

2.1. Instrumentation

Fig. 1 shows the complete coupling system.

2.1.1. Liquid chromatography system

A Perkin-Elmer 250 LC binary pump (CT, USA) and a polystyrene–divinylbenzene-based anion-exchange column Hamilton PRP X-100 (Reno, NV, USA) with ammonium quaternary salt with methyl groups as substituents, 10 μm particle size (250 mm×4.1 mm), were used. A Rheodyne 7125 injector (Cotati, CA, USA) with a 100-μl loop was used for sample introduction.

2.1.2. UV photoreactor

The photoreaction system designed can be seen in Fig. 2. It consists of a PTFE tube (I.D. 0.55 mm) from Cole Parmer (Vernon Hills, IL, USA) surround-

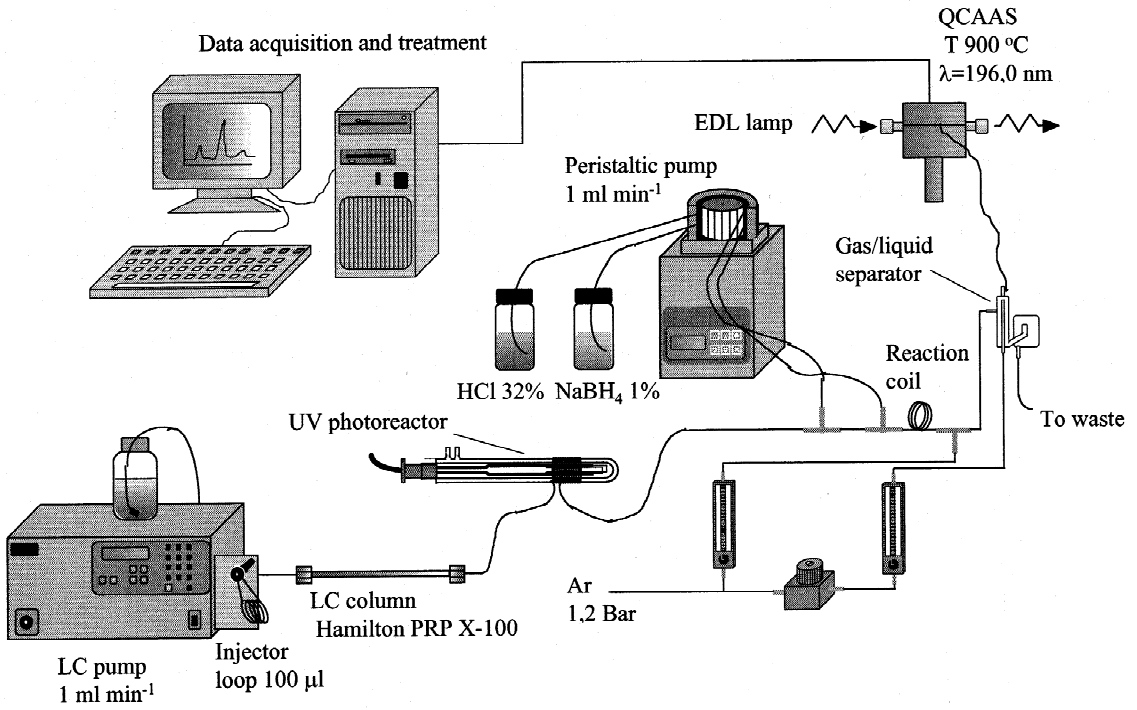

Fig. 1. The complete coupling system.

ing a water refrigerated high-pressure Hg vapour lamp Heraeus TQ 150 (Hanau, Germany).

2.1.3. Hydride generation system

A Gilson Minipuls peristaltic pump (Middleton, WI, USA) was used to add the reagents to the sample solution. The resulting mixture was conducted through a reaction coil (75 cm×1 mm I.D.) to the gas–liquid separator.

2.1.4. QCAAS detection

A Perkin-Elmer 1100B atomic absorption spectrometer, with Se EDL lamp operating at 4.5 W, was used. A deuterium lamp was used for background correction. A Perkin-Elmer AS-90/91 electric heating system and a quartz cell were used for atomisation. Measurement conditions: wavelength: 196.0 nm; slit width: 2 nm.

Data acquisition and processing were performed by a microcomputer [34].

2.2. Reagents

All the solutions were prepared by using doubly-deionized water Culligan Ultrapure GS (Northbrook, IL, USA) 18.3 MΩ cm resistivity.

One thousand mg l^{-1} Se stock solutions were prepared as follows: selenite: 0.2190 g 99% Na_2SeO_3 (Aldrich, Milwaukee, WI, USA) dissolved to a final volume of 100 ml. Selenate: 0.2393 g 99% Na_2SeO_4 (Aldrich) dissolved to a final volume of 100 ml. Selenocystine (SeCys): 52.89 mg seleno-DL-cystine (Sigma, St. Louis, MO, USA) weighed under nitrogen atmosphere and dissolved to a final volume of 25 ml. Selenomethionine (SeMet): 62.10 mg seleno-DL-methionine (Sigma) weighed under nitrogen atmosphere and dissolved to a final volume of 25 ml.

Solutions of HCl ranging from 1.60 to 10.20 mol l^{-1} were prepared from analytical-reagent grade 32% HCl (Merck, Darmstadt, Germany).

Solutions of H_2SO_4 ranging from 1.0 to 5.0 mol

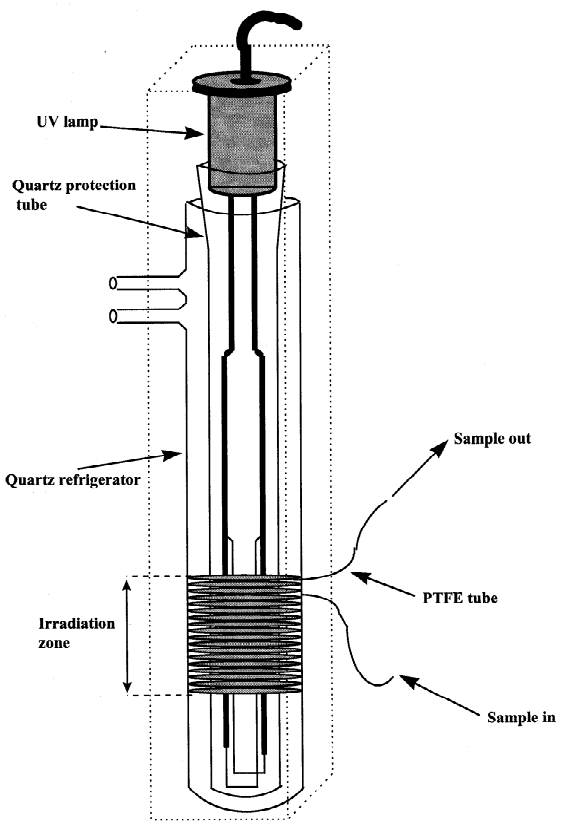

Fig. 2. The photoreaction system.

l^{-1} were prepared from analytical-reagent grade 95–97% H_2SO_4 (Merck).

Solutions of $NaBH_4$ ranging from 0.5 to 2% were prepared from ''purum'' 97% $NaBH_4$ (Fluka, Buchs, Switzerland) in ''suprapur'' 0.5% NaOH (Merck) aqueous solution; the resulting, slightly turbid solution, was filtered off. This solution was prepared daily.

Hydrobromic acid solutions were prepared from ''extrapure'' 47% HBr (Merck).

Phosphate buffer solutions were prepared from 99% $NaH_2PO_4 \cdot H_2O$ and 99% $Na_2HPO_4 \cdot 2H_2O$ (both from Carlo Erba, Milan, Italy) dissolved in water. These solutions were filtered off through a 0.22-μm nylon membrane before use.

''LiChrosolv gradient grade'' 99.8% MeOH (Merck) was used.

Mineral water used in recovery studies had the following composition: residue at 110°C: 128.0, HCO_3^-: 91.0, SO_4^{2-}: 7.1, Cl^-: 7.5, Ca: 25.7, Mg: 3.4, Na: 8.8, SiO_2: 22.4 (all in mg l^{-1}).

2.3. Procedure

A 100-μl aliquot of the sample solution was injected into the LC system. For separation 40 mmol l^{-1} $H_2PO_4^-$–HPO_4^{2-} buffer at pH 7.0 at 1 ml min^{-1} was used as mobile phase. The eluate reached the photoreactor, where the total UV irradiation time was 60 s, and it was then introduced into the hydride generation system, where 32% HCl at 1 ml min^{-1} and 1% $NaBH_4$ in 0.5% NaOH at 1 ml min^{-1} were added for hydride generation. After reaction in a coil (75 cm×1 mm I.D.), the generated hydrogen selenide was driven by an argon flow to the heated quartz cell atomic absorption spectrophotometer (*T*: 900°C), through the gas–liquid separator.

3. Results and discussion

3.1. Chromatographic separation

For the separation of the four selenium species we used a polystyrene–divinylbenzene-based anion-exchange column, as this gave the best resolution between the four compounds. We first studied the dependence of the retention time of the four species on the pH of the phosphate solution used as the mobile phase. The two seleno amino acids showed very high retention times at alkaline pH, whereas at pH values lower than 2–3 retention times were dramatically shortened. In contrast, selenite and selenate required the use of an alkaline solution as mobile phase to obtain low retention times. So, considerable changes in pH during the separation of the four selenium species was needed to achieve the shortest analysis time. By working at pH 7.0 a compromise was achieved and we were able to obtain a good resolution of the four selenium species in a reasonable time, varying the composition and concentration of the mobile phase used.

3.1.1. Effect of the composition of the mobile phase

A study was carried out in order to reduce the retention time of SeCys and SeMet. For this, 100 μl

Table 1
Effect of MeOH in the elution of SeCys and SeMet

MeOH (%)	SeCys		SeMet	
	Peak area	t_R (s)	Peak area	t_R (s)
0	2.436	293.6	1.877	581.3
1	2.595	291.7	1.955	530.5
2	2.439	288.7	1.704	541.5
3	2.487	288.7	1.565	491.7
4	2.524	292.7	1.652	465.8
5	0.726	286.7	1.531	449.9

The LC–UV irradiation–HG-QCAAS system was used. A sample containing 200 μg l^{-1} of selenium as SeCys and 200 μg l^{-1} of selenium as SeMet was used in all the measurements.

of a sample containing 200 μg l^{-1} of selenium as SeCys and 200 μg l^{-1} as SeMet were injected into the LC–UV irradiation–HG-QCAAS system. Several mixtures of a 60 mmol l^{-1} phosphate buffer solution, pH 7.0 and MeOH were used as mobile phases, ranging from 0% to 5% of MeOH. Table 1 shows the results obtained. Major loss of signal was observed for SeCys when using 5% MeOH. On the other hand, there was no significant change in peak area when using up to 4% MeOH. However, the best results were obtained without MeOH or when using 1% MeOH, especially in the case of SeMet. The retention time of SeCys was not affected by changes in the addition of MeOH to the mobile phase, whereas the retention time for SeMet fell as the amount of MeOH in the mobile phase increased. However, the total time for the elution of the two species was always lower than 10 min (Table 1). It was decided not to use MeOH in the mobile phase, since the retention times for SeCys and SeMet were short enough. A good sensitivity was obtained for both species, and the mobile phase was simpler and more homogeneous.

3.1.2. Effect of the concentration of the mobile phase

The concentration of the phosphate buffer solution at pH 7.0 was optimised. For each assay, 100 μl of a mixture containing 200 μg l^{-1} of selenium in each form (selenite, selenate, selenocystine and selenomethionine) were injected into the LC–UV irradiation–HG-QCAAS system. Several concentrations of mobile phase were tested, ranging from 20 mmol l^{-1} to 100 mmol l^{-1}. By way of example, some of the chromatograms obtained are shown in Fig. 3. The retention time for the two inorganic selenium species were affected to quite an extent by changes in the concentration of the mobile phase used, whereas the two organic selenium species were left almost unaffected (Table 2). This can be attributed to the different interaction processes between the stationary phase and the selenium compounds at the pH chosen. Thus, at pH 7.0, both seleno amino acids are present in the solution as neutral zwitterion forms and as such they do not interact with the anion-exchange groups of the stationary column. The retention of these selenium species is only due to a reversed-phase mechanism between the polymeric base of the column and the aqueous phosphate solution. Therefore, a variation in the concentration of phosphate in the mobile phase only slightly affects the retention time of the seleno amino acids (Table 2). On the other hand, inorganic selenium species are present in anionic form in solution at pH 7.0, thus they interact with the anion-exchange groups of the stationary

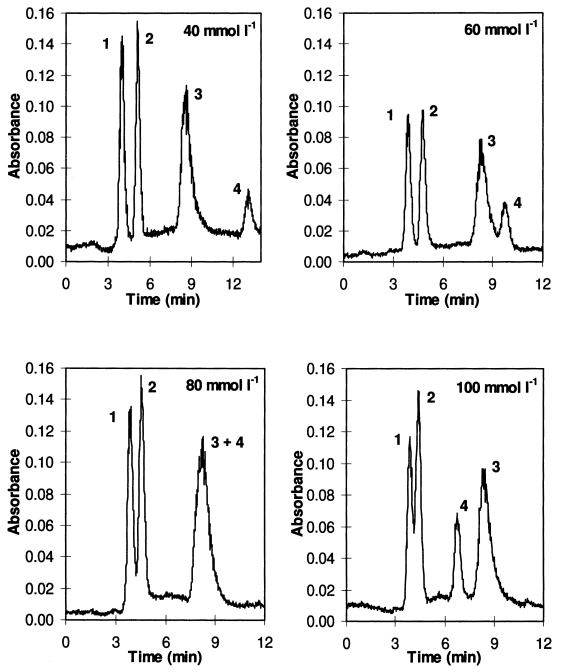

Fig. 3. Chromatograms corresponding to determination of selenite, selenate, SeCys and SeMet at concentrations of 200 μg l^{-1} expressed as selenium, using the LC–UV irradiation–HG-QCAAS system, at several concentrations of mobile phase. 1=SeCys, 2=selenite, 3=SeMet, 4=selenate.

Table 2
Effect of the concentration of mobile phase in the separation of selenium species

Phosphate (mmol l^{-1})	Selenite t_R (s)	Selenate t_R (s)	SeCys t_R (s)	SeMet t_R (s)	R_S SeCys–selenite
20	366.3	1440	256.8	547.5	1.79
40	308.6	789.4	239.9	512.6	1.35
60	287.7	585.3	234.9	498.7	0.88
80	274.7	494.7	233.9	494.7	0.66
100	266.8	406.1	233.9	499.7	0.61

The LC–UV irradiation–HG-QCAAS system was used. A sample containing 200 μg l^{-1} of selenium in each form was used in all the measurements.

phase. As a consequence, a decrease in the ionic strength causes an increase in the retention time of selenite and selenate (Table 2). It can also be observed that the resolution between SeCys and selenite decreases as the concentration of phosphate increases. The best resolution was obtained with a concentration of 20 mmol l^{-1} (Table 2). However, the total time for analysis is too long at this concentration. From this study, 40 mmol l^{-1} phosphate was chosen because this concentration gave sufficient resolution for all selenium species with the shortest total elution time.

3.2. UV irradiation

3.2.1. UV lamp

A study was carried out in order to choose the most appropriate UV lamp for the photoreactions of the selenium species. Molecular absorption spectra of three solutions containing 100 mg l^{-1} of selenium, as selenate, SeCys and SeMet, respectively, were obtained. These spectra were contrasted with the emission spectra of two UV lamps: low-pressure mercury lamp Heraeus TNN 15/32 (15 W) and high-pressure mercury lamp Heraeus TQ 150 (150 W). Fig. 4 shows these spectra, as well as the molecular absorption spectra of the selenium species. The 150 W UV lamp was chosen, since it irradiates effectively over the zone of maximum absorption of the selenium species.

3.2.2. Effect of the addition of HBr

Several assays were conducted to optimise the photoreduction of selenate to selenite, as well as the photodecomposition of the seleno amino acids to selenite, by studying the influence of the presence of HBr at various concentrations in the reaction medium. HBr is proposed as effective reducing agent for selenate, and the Br_2 generated in UV irradiation over the HBr, favours the photodecomposition of organic selenium compounds [35]. Other reducing agents had already been tested for this purpose without any improvement being reported [6]. We carried out the assays by using the LC–UV irradiation–HG-QCAAS system, but leaving out the chromatographic column, i.e., by connecting the LC pump directly to the photoreactor. For each assay 100 μl of a solution containing 200 μg l^{-1} of selenium was injected and pumped at 1 ml min^{-1} to the photoreactor. Solutions of HBr ranging from 0 to 10% were added at the entrance of the photoreactor with a peristaltic pump at 0.2 ml min^{-1} by using a T connection. The irradiation time was 120 s. These assays were applied to the four selenium species, separately. Table 3 summarises the results obtained. The highest values were obtained without adding HBr. We thus decided to work without the addition of any reagent.

3.2.3. Irradiation time

Several irradiation times were assayed in order to obtain the best yield in selenate reduction and seleno amino acids decomposition. The overall coupling was used for this purpose. For each assay, 100 μl of a mixture containing 300 μg l^{-1} of selenium in each form (selenite, selenate, selenocystine and selenomethionine) were injected. Irradiation times ranging from 0 to 240 s were assayed. Without UV irradiation, only three peaks were obtained in the chromatogram, since there was no signal corresponding to

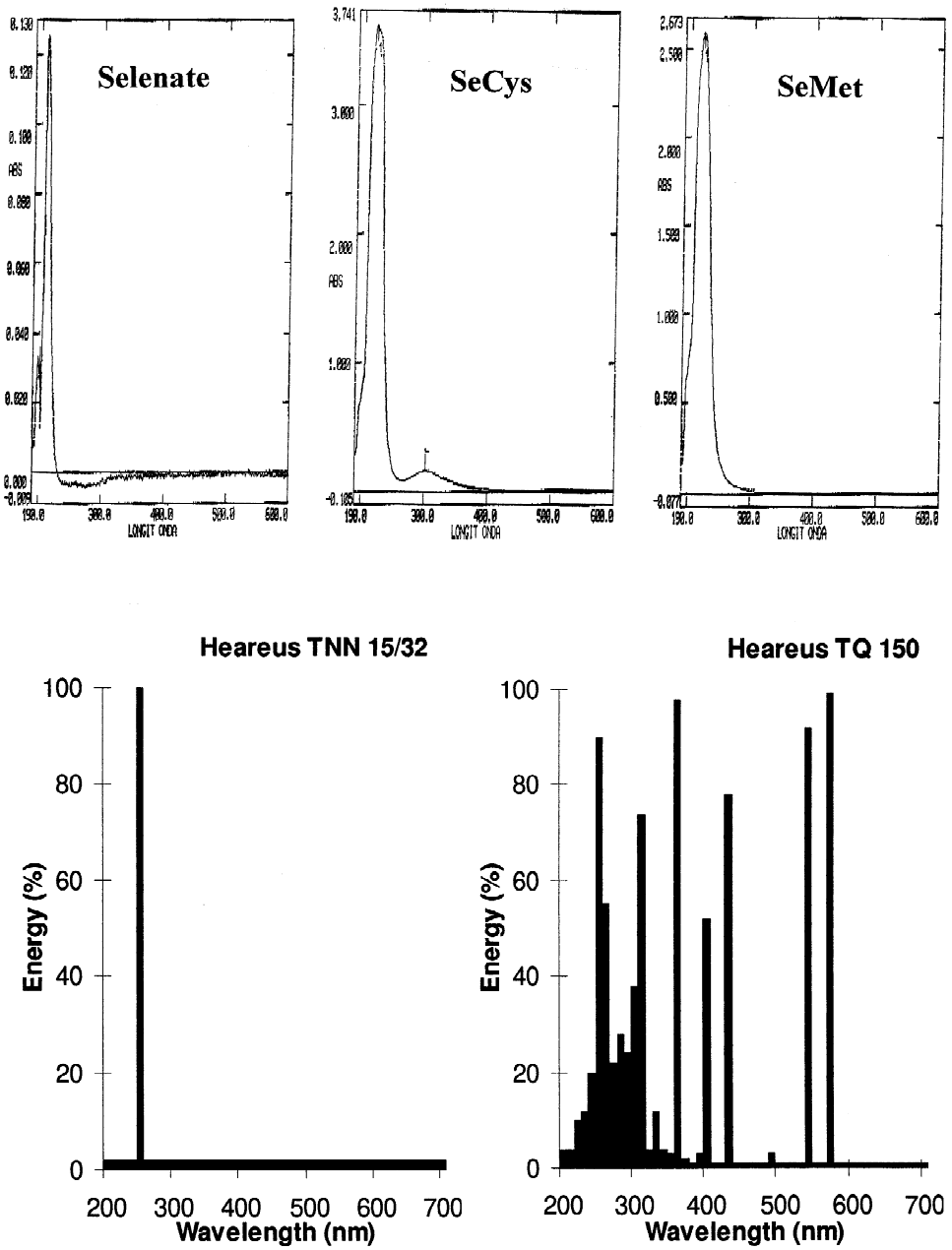

Fig. 4. Molecular absorption spectra obtained from three solutions containing 100 mg l^{-1} of selenium, as selenate, SeCys and SeMet, respectively. Emission spectra of two UV lamps: Heraeus TNN 15/32 and Heraeus TQ 150.

selenate, according to Ref. [6] (Table 4). After 150 s of irradiation, there was a slight decrease in the signal corresponding to selenite, SeCys and SeMet (Table 4). This might have been due to a photooxidation of Se(IV) to Se(VI) [6]. For irradiation times longer than 60 s, no significant signal increase was

Table 3
Effect of HBr concentration on the photoreduction of selenate to selenite and on the photodecomposition of the selenoamino acids to selenite

HBr (%)	Peak area			
	Selenite	Selenate	Selenocystine	Selenomethionine
0	3.727	1.123	3.656	3.305
1	3.510	0.541	2.769	3.269
5	3.750	0.137	2.210	1.132
10	3.762	0.141	1.998	0.928

The UV irradiation–HG-QCAAS system was used. The concentration was 200 μg l^{-1} of selenium in all the measurements.

Table 4
Effect of irradiation time on the photoreduction of selenate to selenite and on the photodecomposition of selenoamino acids to selenite

Irradiation time (s)	Peak area			
	Selenite	Selenate	Selenocystine	Selenomethionine
0	5.068	–	2.875	1.837
30	4.978	0.504	3.029	1.871
60	5.143	0.926	4.487	3.301
90	5.295	0.965	4.468	3.336
120	5.124	0.765	4.425	2.947
150	5.087	0.904	4.387	3.212
180	4.586	0.982	3.573	2.285
210	4.227	0.992	3.465	2.193
240	3.934	1.084	3.399	2.304

The LC–UV irradiation–HG-QCAAS system was used. Mixtures containing 300 μg l^{-1} of selenium, as selenite, selenate, SeCys and SeMet were determined in all the measurements.

observed, thus 60 s were adopted, as it allows the shortest analysis time.

3.3. Hydride generation

Conditions were optimised in order to obtain the maximum yield of hydrogen selenide. The HG–QCAAS system was used. Atomisation was carried out at 900°C. A solution containing 200 μg l^{-1} selenium as selenite, introduced at 1 ml min^{-1}, was used. Solutions of $NaBH_4$ at several concentrations were tested in order to establish the maximum signal/background ratio. One % $NaBH_4$ at 1 ml min^{-1} was chosen, as it allowed the maximum signal stability and sensitivity.

Several HCl and H_2SO_4 concentrations were tested. Table 5 shows the results obtained. Hydrochloric acid gave the best results for the generation

Table 5
Effect of HCl concentration, H_2SO_4 concentration and reaction time on the yield in hydride generation process

HCl			H_2SO_4			Reaction coil	
HCl (mol l^{-1})	Absorbance	R.S.D. (%; $n=5$)	H_2SO_4 (mol l^{-1})	Absorbance	R.S.D. (%; $n=5$)	Loop (cm)	Absorbance
0.00	0.000	–	0.00	0.000	–	0	0.655
1.60	0.696	1.65	1.00	0.693	1.97	28	0.671
3.20	0.714	1.32	2.00	0.677	1.71	75	0.688
4.80	0.711	3.19	3.00	0.642	1.71	103	0.686
6.40	0.716	1.39	4.00	0.626	1.28	150	0.692
8.00	0.730	1.98	5.00	0.604	1.97	178	0.694
10.20	0.735	1.15					

The HG-QCAAS system was used. Two hundred μg l^{-1} of selenium as selenite and 1% $NaBH_4$ were used in each measurement.

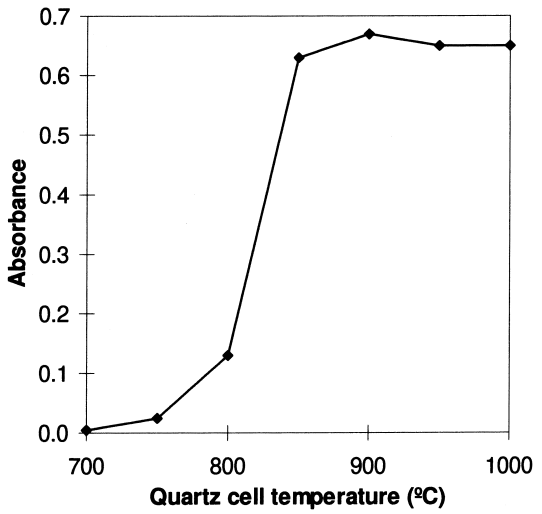

Fig. 5. Effect of temperature of quartz cell on the yield of the atomisation of hydrogen selenide. The HG-QCASS system was used in the measurements. Absorbance signals measured for 200 μg l^{-1} of selenium as selenite.

of hydrogen selenide, with a maximum value when using 10.20 mol l^{-1} HCl (corresponding to 32% HCl).

Several reaction coils (1 mm I.D.) were tested in order to establish the best reaction time for hydride generation, since a variation in the length of the loop led to a variation in the reaction time, for the same flow. Table 5 summarises the results obtained; there was a significant gain in the signal when the loop's length was increased from 28 cm to 75 cm; loops longer than 75 cm did not improve the signal. Thus, a 75 cm loop was chosen.

3.4. QCAAS detection

The influence of the temperature of the quartz cell on the atomisation of hydrogen selenide was studied. A solution of 200 μg l^{-1} of selenium, as selenite, and the HG-QCAAS system were used. Temperatures ranging from 700°C to 1000°C were tested. Fig. 5 shows the effect of the quartz cell temperature on absorbance. A significant increase in the absorption signal above 800°C was recorded. 900°C was adopted for the atomisation, since it corresponded to the maximum sensitivity. The activation of the quartz cell was carried out periodically as described elsewhere [34].

3.5. Quality parameters

Linear range was established for the four selenium species in order to assess the applicability of atomic absorption spectrometry as a detection system. Similar results were obtained by using both peak area and peak height. The linear range for the four selenium compounds was wider than 800 μg l^{-1} (Table 6).

Detection limits were calculated by analysing, in triplicate, several mixtures containing the four selenium species at different concentrations. The regression line for each compound was calculated from the mean values of the peak heights from the chromatograms after applying the Savitzky–Golay filtering method [36]. The concentrations at the detection limits were calculated from 3σ of the background signal and then referred to those regression lines (Table 6).

Precision (Table 6) was calculated as the relative

Table 6
Quality parameters

	Selenite	Selenate	Selenocystine	Selenomethionine
Linear range (μg l^{-1})[a]	800	2000	1000	1500
Detection limit (μg l^{-1})[b]	2.5	46.5	3.6	11.8
Precision (R.S.D., %)[c]	5.5	7.2	3.9	6.3
Recovery (%)[d]	102.4	102.4	98.9	99.3

The LC–UV irradiation–HG-QCAAS system was used in all the measurements.
[a] Calculated both with peak area and peak height and expressed in μg l^{-1} of Se.
[b] Calculated as 3σ of the background signal and expressed in μg l^{-1} of Se.
[c] Short-term precision expressed as R.S.D. of nine peak area measurements obtained from solutions containing about 10-times the detection limits for all the selenium species.
[d] Mean values obtained by spiking mineral water at a concentration level of about 10-times that of the detection limits for the four selenium compounds, in triplicate.

standard deviation (R.S.D.) from nine peak area measurements of a standard solution containing the four selenium compounds at concentrations at about ten-times that of the detection limits.

Recovery, by spiking mineral water, was established. The spiked concentration was at about ten-times that of the detection limits for each selenium compound (Table 6). From the results no influence from the matrix composition of the mineral water used was observed.

4. Conclusions

The method proposed here offers significant advantages for selenium speciation, particularly with regard to on-line separation and derivatization. A good resolution is obtained isocratically, without the use of organic solvents in the mobile phase and within a short overall analysis time. UV derivatization, without the addition of any chemical reagent provides a rapid, straightforward system for the transformation of all the selenium compounds into selenite, which is able to generate the volatile hydride for detection. The method only requires a mobile phase and reducing agents for hydride generation as chemical reagents, thus it is cleaner, simpler and less expensive than other methods reported in the literature for selenium speciation.

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