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Speciation of arsenic and selenium compounds by ion-pair reversed-phase chromatography with electrothermic atomic absorption spectrometry
Application of experimental design for chromatographic optimisation

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Abstract

An off-line system is proposed consisting of ion-pair reversed-phase liquid chromatography, collections of fractions at the outflow of the column and furnace atomic absorption spectrometry. The so-called system allowed determination of both arsenic and selenium species mainly found in the environment and in mammals (arsenite, arsenate, monomethylarsonate, dimethylarsinate, selenite, selenate, selenocystamine, selenocystine, selenomethionine and selenoethionine). In order to study the retention behaviour of these compounds and to estimate the optimal conditions for the chromatographic separation, central composite designs were used to evaluate the influence of the eluent parameters such as pH, tetrabutylammonium phosphate (TBA) concentration and sodium hydrogenphosphate amounts. The retention factors of each species and the selectivity were established as response criteria. Response surfaces and isoresponse curves were drawn from the mathematical models and enabled one to determine the optimal conditions and to visualise the method robustness. The predicted optimal zone was situated at pH 5.5–6.5, 4.0 m*M* Na_2HPO_4 and 3.0–4.0 m*M* TBA. Regression models suggested linearity for the studied compounds in the range 25–200 μg selenium and arsenic per litre investigated.

Keywords: Speciation; Experimental design; Atomic absorption spectrometry; Optimisation; Arsenic; Selenium

1. Introduction

Selenium and arsenic are generally considered to be nutritionally essential ultra-trace elements for mammals [1]. Yet, these elements may be toxic at only moderately higher levels of intake. Indeed, increase intake of selenium converts overt signs of deficiency to overt signs of toxicity [2,3]. As for arsenic, the high toxicity levels of arsenic are well known. But as an additional complication, the bioavailability of these elements to an organism depends on their chemical forms [1,3,4]. The descending

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order of toxicity of arsenic compounds has been found to be the following: arsenic, arsenite [As(III)], arsenate [As(V)], monomethylarsonate (MMA) and dimethylarsinate (DMA) [4]. Therefore, although the concentration total of these elements is still useful to know, and indeed is essential in many analytical schemes, the determination of species is an important task.

Hence, a number of methods have been investigated for the speciation of arsenic and selenium compounds. Many of these have involved coupling a gas (GC) or liquid (LC) chromatography technique with an element-specific detector [5,6]. Both GC and high-performance liquid chromatography (HPLC) were used as methods of separation, and the detectors were based on furnace atomic absorption spectrometry (FAAS) [7,8], inductively coupled plasma atomic emission spectrometry (ICP-AES) [9,10] and ICP mass spectrometry (MS) [11–13].

HPLC followed by hydride generation with cryogenic trapping with AAS and ICP-MS were the most sensitive and simplest methods for higher levels of arsenic compounds [7–15]. For differentiation of the oxidation states and of several species of selenium, the usual method has been the formation of hydrides prior to chromatographic separation and then detection by AAS using a heated atomiser [16].

ICP-AES and ICP-MS do not authorise high sensitivity for selenium detection because of its high ionisation potential. Moreover, the most abundant isotope of selenium (Se^{80}: 49.8%) cannot be used for mass detection because of an interference due to a molecular ion of argon (Ar_2^+) at m/z 80. Therefore, the sensitivity of selenium is limited with this detection mode because the isotopes amenable to ICP-MS are present in low natural abundance (<10%) [17]. By the way, the potential of chloride to produce isobaric interferences with ^{75}As and ^{77}Se, such as $^{40}Ar^{35}Cl$ and $^{40}Ar^{37}Cl$, respectively, has been reported [17].

Due to the ionisable character of arsenic and selenium species, ion-exchange and ion-pair chromatography were mainly employed to separate non volatile compounds [18–21]. Arsenic species separation was successfully performed by ion chromatography [21] unlike selenium, that only inorganic forms were individualised by this technique [22–24]. As for selenoamino acids, ion-pair reversed-phase chromatography was applied for studying selectively selenocystine (Secystine), selenomethionine (SeMet) and selenoethionine (SeEt) using the trifluoroacetate anion as ion-pair reagent [5]. With the aim of the determination of SeMet in complex matrixes, some authors have turned to transfer of charge chromatography after previous derivatisation of this species to a dinitrophenyl compound. Yet, except capillary electrophoresis techniques coupled with ICP-MS for selenium speciation described by Michalke and Schramel [25], very few chromatographic techniques have proposed simultaneous analysis of both organic and inorganic forms. Moreover, to our knowledge, based on the literature, few methods have described the simultaneous speciation of arsenic and selenium including analysis of organic and inorganic compounds at the same time [26].

Therefore, the objectives of the current study were to develop simultaneous chromatographic separation mode followed by a sensitive and specific detection mode of arsenic compounds [As(III), MMA, DMA and As(V)] and of selenium species mainly found in natural surroundings [Selenite or Se(IV), Selenate or Se(VI), Secystine, Secystamine or Secystamine, SeMet and SeEt). This analytical technique consisted of an off line coupling of ion-pair reversed-phase chromatography with tetrabutylammonium (TBA) as ion-pair reagent, to graphite furnace AAS detector. This detection mode provides very sensitive and specific determination of studied compounds without lack of sensitivity towards selenium species and interferences previously described. However, it had to be preceded by a fraction collection step suggesting a more time-consuming technique and a good resolution between the compounds of interest in order to minimise the possible mixture of them in the same fraction.

Consequently, based on the above, the chromatographic step based on retention factors and selectivity of different species was optimised according to the eluent parameters. A full second-order polynomial model was chosen to approximate the region of the multifactor surface. The method of the experimental design chosen in order to estimate the parameters of the model was an approach using central composite design (CCD) which allowed all operating variables to be investigated individually as squared terms and to consider interaction effects. The results of the

design were evaluated using multiple linear regression analysis.

2. Experimental

2.1. Instrumentation

A HPLC system consisting of a TSP HPLC pump (TSP Constametric 4100 MS) with a 5 ml min^{-1} stainless steel pump head, a module sample processor and a Rheodyne six-port sample injector with 100-μl sample loop, and a HPLC column was used. A reversed-phase C_{18} column (250 mm×4.6 mm, 5 μm particles; Machery-Nagel, Hoerdt, France), linked to a C_{18} guard column (15 mm×4.6 mm I.D.; Interchim, Montluçon, France) was used for the separation.

Detection was achieved by a furnace atomic absorption spectrometer 5100 PC from Perkin-Elmer (CT, USA). It was equipped with a pyrrographite furnace containing a platform and two electrode-less discharge lamps each specific for arsenic and selenium analysis, respectively. Zeeman effect allowed correction of spectral disruptions. Analytical conditions are summarised in Table 1. Samples were dropped by an autosampler.

Collection of fractions was performed with a collector from Gilson (Villiers le Bel, France).

2.2. Reagents

Sodium dimethylarsinate, selenomethionine, selenocystine, selenocystamine, selenoethionine, sodium hydroboride, palladium nitrate, nickel nitrate and phosphate tetramethylammonium were obtained from Sigma–Aldrich (St. Louis, MO, USA). Sodium monomethylarsonate was synthesised by Seratec (Epinay, France). Sodium selenite, sodium selenate, disodium hydrogenphosphate and phosphoric acid were purchased from Merck (Nogent sur Marne, France) and acetonitrile (HPLC grade) from Carlo Erba (Reuil, France).

Deionised water from an ultrapure water system Milli-Q (Molsheim, France) was used for the preparation and the dilutions of all reagents, samples and calibrators.

Stock solutions of arsenic and selenium compounds (1000 μg l^{-1}) were prepared separately by dissolving appropriate amounts and were stored in polypropylene flasks at 4°C [27]. The standard solutions were freshly prepared by dilution of the stock solutions before use.

2.3. Selection of relevant ion-pair LC factors

The retention behaviour of the arsenic and selenium species was studied and the separation efficiency expressed by the chromatographic selectivity between species closely eluted, was optimised according to the eluent variables. The retention mechanism of arsenic and selenium species was mainly governed by their hydrophobic character and their apparent charges (ACs) due to the presence of ionisable functions. Therefore, salt concentration, pH and TBA amount used as ion-pair reagent, could be the factors considered in this study. The selected factors and their corresponding ranges were determined after preliminary experiments (Table 2). Some initial experiments, using the classical single-factor-at-a-time method, have shown insignificant effect of salt amount inside a large range of concentration

Table 1
Detection conditions of graphite furnace AAS

Wavelength	196 nm
Slit	2 nm
Intensity	6 mA
Integration time	4 s
Background	On Zeeman
Signal processing	Integrated absorbance (Abs s)
Type tube	Pyrolytic graphite coated graphite with platform
Gas-flow	300 ml min^{-1}
Volume of each deposit	20 μl sample + 2 μl modifiers
Number of deposits	2

Table 2
Coded values of experimental factors

Level	−1.41	0	+1.41
X_1	5.1	6.5	7.9
X_2	1.0	2.5	4.0
X_3	1.0	2.5	4.0

X_1: pH, X_2: TBA concentration (mmol l^{-1}), X_3: Na_2HPO_4 (mmol l^{-1}).

from 10^{-3} to 10^{-2} mol Na_2HPO_4 per litre. A minimum of 1:100 (v/v) acetonitrile ratio had to be added to the mobile phase to enhance its elution power towards As(V) whose retention time exceeded 25 min, whatever the composition of the eluent.

Furthermore, interactions between salt amount and the other cited factors were not underlined insofar as the effect of Na_2HPO_4 used was not dependent, neither on pH, nor TBA concentration. Therefore, taking these results into account, we chose to exclude the study of this parameter from the experimental design. It was fixed at 4.0 mmol l^{-1} in order to keep the ionic strength of the mobile phase constant.

Retention behaviour and selectivity of arsenic and selenium compounds were followed and optimised by the use of CCD.

2.4. Statistics

2.4.1. Choice of the experimental design

A CCD (Fig. 1) [28] was chosen to investigate the influence of TBA and pH levels on the retention behaviour of the so-called species (capacity factor and selectivity). Each variable will assume five levels in coded levels ($-\alpha$, -1, 0, 1, α) and $\alpha=1.41$ was chosen with sixfold repetition of the centre point, as shown in Table 3. A k-factor two-level CCD requires 2^k+2k+C experiments, where 2^k points are in the corners of the square representing the experimental domain, $2k$ points are the star points and C points are the replicates in the centre of the square that are necessary to estimate the variability of the experimental measurements. Therefore, considering two factors and six replicates at the centre point, this design involves 14 experiments which were performed in random order.

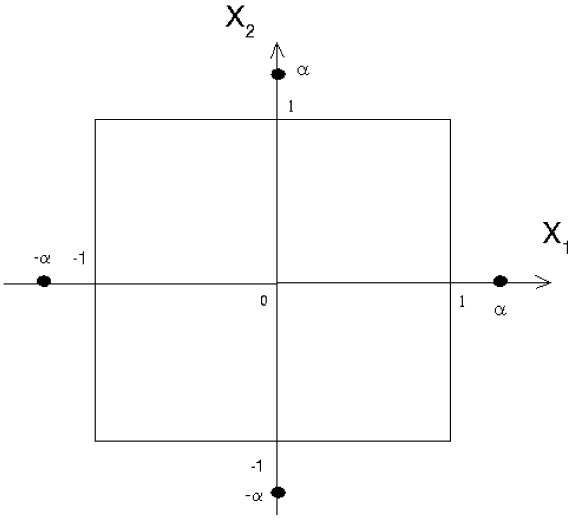

Fig. 1. Representation of a CCD with two factors on five levels. The length of the arm of the star is $\alpha=1.41$.

2.4.2. Regression modelling

Multiple regression gives a mathematical relationship between responses and independent variables.

The CCD provides sufficient data to fit a second-degree expression, such as given below for two factors:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2 \quad (1)$$

where y represents the experimental response, x_i the

Table 3
Experimental matrix of the CCD used for the ion-pair chromatography optimisation

Experiment No.	X_1	X_2
1	−1	−1
2	1	−1
3	−1	1
4	1	1
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	−1.41	0
12	1.41	0
13	0	−1.41
14	0	1.41

independently evaluated factors (in coded variables), b_0 the intercept and b_i the parametric coefficients of the model obtained by multiple regression. The contribution of linear, quadratic and interaction terms to the regression polynoms was calculated an analysis of variance (ANOVA) followed by an F-test. F is calculated as follows:

$$F = \frac{S^2_{\text{source}}}{S^2_{\text{residual}}} \quad (2)$$

This test is a statistical method to determine the practical significance of an effect and demonstrates the extent of variability caused by the imput to be investigated.

In the similar way, the influence of the main factors (TBA and pH) on k' and α was estimated.

3. Results and discussion

3.1. Performance of chromatography

3.1.1. Variation of pH

All of the experiments of the design matrix were performed with UV detection at 200 nm in order to obtain rapid information about the retention behaviour of arsenic and selenium species.

Fig. 2a and b show two chromatograms representing experiment Nos. 9 and 12 of the design matrix, where pH was varied from 0 (6.5) to 1.41 (7.9) by NaOH addition while TBA and Na_2HPO_4 amounts were kept constant.

At the pH value of 5.0, As(III) was at neutral charge and was eluted in the dead volume, whereas DMA presented a partial negative AC (>-1) and could yield ionic interaction with TBA to coat the stationary phase or form an ion-pair with the free-form of the ion-pair reagent. It was then eluted according to its AC and hydrophobic character. However, the retention strength of the last compound was similar to the one observed for As(III), and this led to an incomplete separation between the two species with a selectivity value less than 1.3. On the other hand, the ACs of MMA and As(V) were both mainly at 1 and therefore, interactions with TBA were possible (Fig. 3). MMA and DMA were well resolved with a selectivity value equal to 1.6.

As for selenium species, selenocystamine always positively charged within the conditions of the experimental design was eluted in the dead volume. All analytes were relatively baseline-resolved within 22 min.

Changing the pH level to 7.9, MMA and DMA were closely eluted because of the nearness of their ACs on the one hand, and of their hydrophobic properties on the other hand. By the way, the k' of As(V) increased with increasing AC (-2) and caused an extension of the analysis run beyond 30 min. Under the same condition, the chromatographic result was mediocre for the analysis of selenium species, as the chromatogram was compressed resulting in a co-elution of SeMet and Se(IV). This lack of resolution was ascribed to an enhancement of negative AC of SeMet with pH, whereas the AC of Se(IV) was constant whatever the pH inside the experimental domain.

3.1.2. Variation of TBA concentration

The influence of the variation of TBA concentration is shown in Fig. 2a and c. The two chromatograms represent experiment Nos. 9 and 14, where TBA concentration was increased from 0 (2.5 mmol l^{-1}) to 1.41 (4.0 mmol l^{-1}) at a constant content of Na_2HPO_4 (4.0 mmol l^{-1}) and pH (6.5). The TBA concentration range studied is small, 1.0–4.0 mmol l^{-1}. Indeed, some initial experiments, using the classical single-factor-at-a-time method, have shown that As(V) was strongly retained on the stationary phase and therefore, could yield a very long analysis time (>30 min) since TBA concentration exceeded 5.0 mmol l^{-1}. Even though the optimisation relied on the efficiency of the separation between two successive peaks, it had to take into account the feasibility and the applicability of the technique.

At a TBA concentration of 0.5 mmol l^{-1}, all the analytes were separated within 15 min and 22 min for arsenic and selenium, respectively. Very acceptable selectivity was observed for DMA and Se(IV) eluted after MMA and SeMet, respectively. By increasing the TBA concentration to 3.9 mmol l^{-1}, peaks were shifted to larger retention times without a change in the retention order of the analytes.

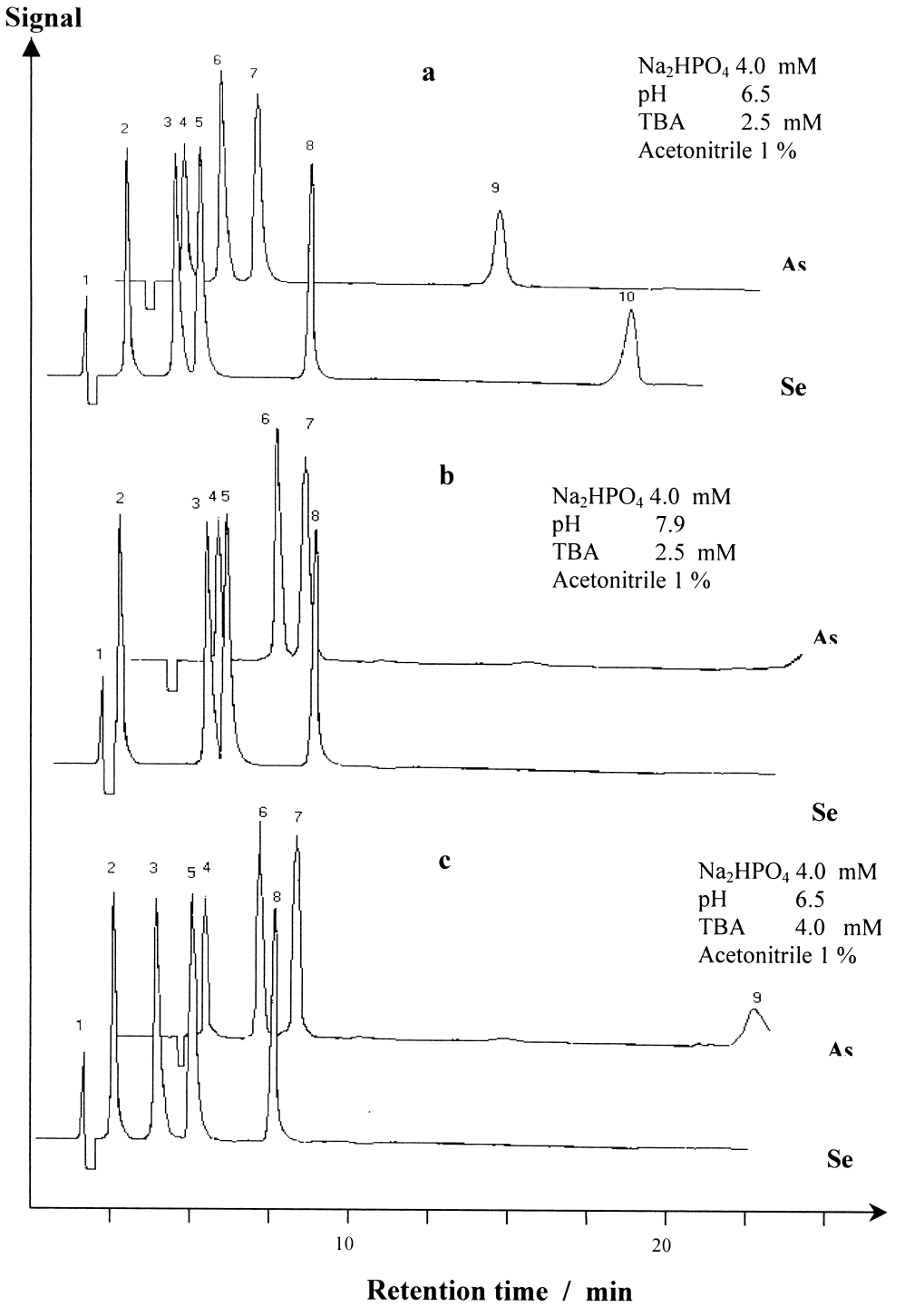

Fig. 2. Retention variations of the selenium and arsenic species investigated in dependence on the pH and the TBA content of the eluent. Analytes: 1=Secystamine; 2=Secystine; 3=SeMet; 4=As(III); 5=Se(IV); 6=DMA; 7=MMA; 8=SeEt; 9=As(V); 10=Se(VI) (5 μg each). UV detection at 200 nm.

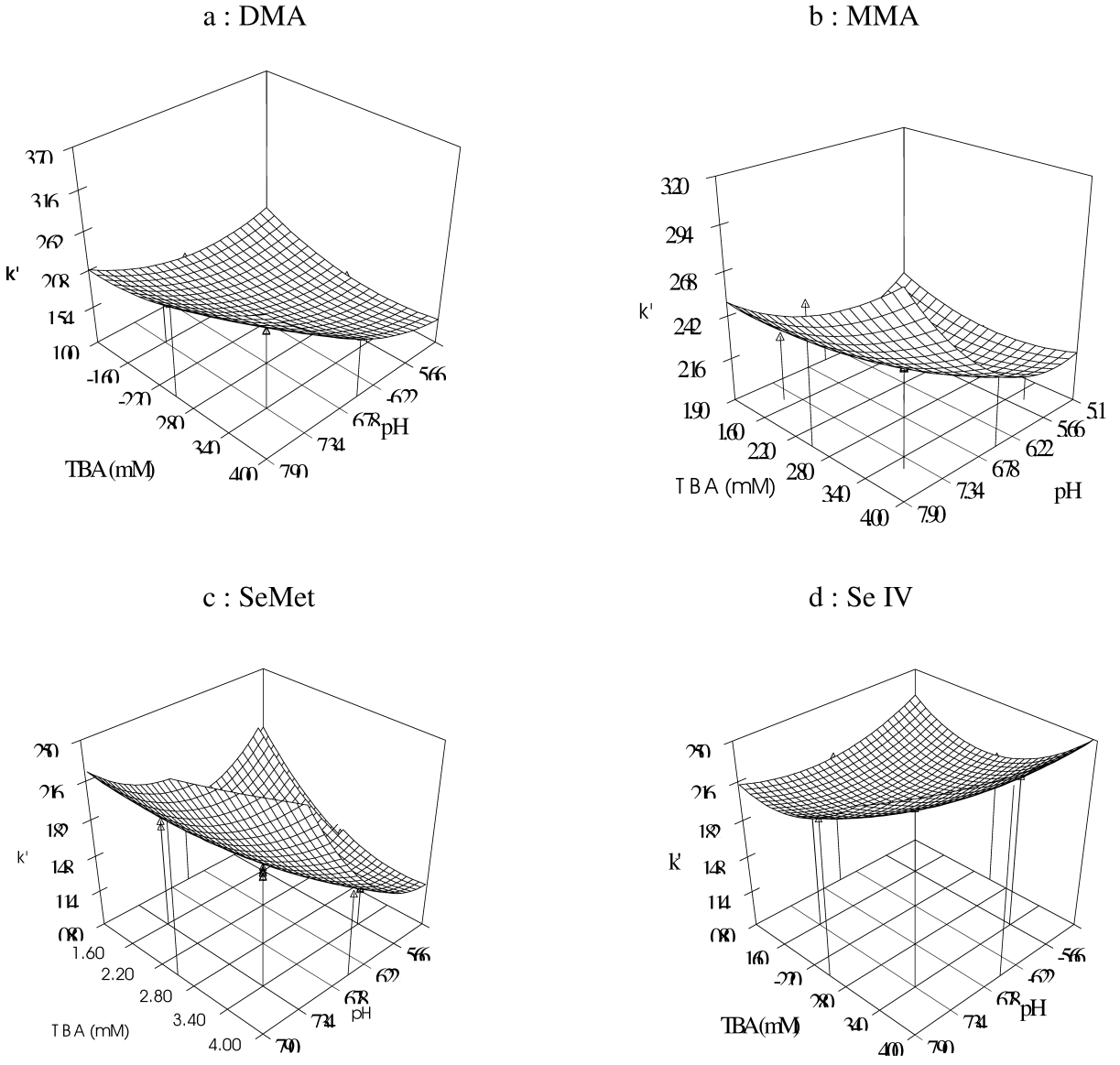

Fig. 3. Response surfaces based on fitted retention factors.

3.2. Statistical analysis of the retention data

3.2.1. Model equations

The retention data for each species investigated was mathematically fitted by means of response surface regression postulated a quadratic model, including interaction terms according to Eq. (1). Table 4 shows the estimated coefficients for retention factor of each specie resulting from the analysis of the retention of the data performed on the coded variables.

As expected, Secystamine was the first compound eluted whatever the location point inside the experimental design and therefore, could be defined as the arbitrary dead volume. This manifested itself by the lack of significance of all their estimated regression coefficients (Table 4). For the other analytes, the ratio of the absolute values of the coefficients X_1

Table 4
Estimated regression coefficients for k' using a full quadratic model including second-order and interaction terms

Species	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
As(III)	*	*	*	*	*	*	*
DMA	1.491	0.292	0.074	0.110	0.110	0.082	0.9949
MMA	2.833	0.288	0.092	0.089	0.122	0.069	0.9843
As(V)	**	**	**	**	**	**	**
Secystamine	***	***	***	***	***	***	***
Secystine	0.305	0.532	0.079	0.148	0.343	0.071	0.9877
Se(IV)	1.96	0.060[a]	0.176	0.060[a]	0.064[a]	0.128	0.9936
Se(VI)	**	**	**	**	**	**	**
SeEt	4.145	0.292	0.156	0.195	0.136	−0.047[a]	0.9411
SeMet	1.334	0.361	0.048	0.180	0.191	0.101	0.9812

R^2 is the quadratic correlation coefficient. *Elution in the dead volume at low coded levels of pH. **At the coded levels >1.0 of both pH and TBA, $t_R>25$ min. ***Elution in the dead volume.
[a] Insignificant terms ($P>0.05$).

and X_2 suggested a higher influence of pH on the retention than TBA amounts except for Se(IV), the explanation of this phenomeon was given before. The quadratic parameters X_{11} and X_{22} describe curvature effects which are to be expected because of the practically observed exponential dependence of k' on the eluent parameters. Apart from As(III), As(V), Secystamine and Se(IV), all X_{11} and X_{22} coefficients are of statistic significance ($P\geq0.95$). The interaction parameter X_{12} is responsible for curvature and twisting effects of the response surfaces. As expected, it is positive in sign because in most cases, the influence of TBA on retention differed when the pH level differed.

The sum of the contributions of linear, quadratic and interaction terms is equal to the quadratic correlation coefficient R^2. The difference to 100%, which would mean the complete fitting of the measured retention data, is the residual error representing the sum of lack-of-fit and pure error. For all cases, the fitting of experimental data is good since the correlation exceeded 94%.

3.2.2. Verification of the model equations

The model equations as presented in Table 4 allow one to calculate retention factors at any eluent composition within the experimental design. The precise prediction of retention factors is possible insofar as the correlation of the experimental data is very satisfactory.

Table 5 shows the result of comparison of experimental and calculated retention factors k' of SeMet and Se(IV) as examples. The results show that with few exceptions, no significant differences between calculated and experimental data are found in the experimental domain.

3.2.3. Examples of response surfaces

Fig. 3 shows the response surfaces for SeMet and Se(IV), and MMA and DMA, based on fitted retention factors. Except for Se(IV), response surfaces show slight twisting, an indication of a non negligible role of interaction terms. For the three so-called organic species, the variation of pH has a higher influence on the retention than the variation of TBA concentration. This translated by the response surfaces since these are asymmetric in shape. For Se(IV), the opposite seems to be true. These results are consistent with the chromatograms (Fig. 2a–c). With the aim of the transfer of this technique to the couplage with furnace AAS implicating collection of fractions, the best eluent condition amenable to the complete separation of all analytes for arsenic on the one hand and selenium on the other hand, must be considered. In one case, the difficulty seems to come from the couple SeMet/Se(IV) and in the other case, it is attributed to the couple MMA/DMA. Therefore, selectivities of MMA and Se(IV), computed from their retention data based on the fitted variables of the CCD, were studied with the same mathematical method employed for k'.

Fig. 4 shows the shape of the isoresponse curves representing the variation of α according to the variation of the eluent factors. The separation be-

Table 5
The observed retention data of SeMet and Se(IV) and their predictive values according to the mathematical model

Experiment No.	k'				α	
	Experimental responses		Predictive responses		Experimental responses	Predictive responses
	SeMet	Se(IV)	SeMet	Se(IV)		
1	1.36	1.97	1.43	1.98	1.04	1.13
2	1.81	1.98	1.75	1.98	1.09	1.22
3	1.16	2.23	1.09	2.18	1.96	1.99
4	2.30	2.47	2.11	2.40	1.07	1.13
5	1.32	1.93	1.33	1.96	1.58	1.54
6	1.33	1.99	1.33	1.96	1.52	1.54
7	1.33	1.94	1.33	1.96	1.57	1.54
8	1.28	1.96	1.33	1.96	1.57	1.54
9	1.38	1.96	1.33	1.96	1.59	1.54
10	1.35	1.99	1.33	1.96	1.58	1.54
11	1.29	2.03	1.21	2.00	1.56	1.53
12	2.38	2.24	2.15	2.17	1.07	0.88
13	1.57	2.03	1.50	1.94	1.27	1.06
14	1.59	2.60	1.51	2.40	1.71	1.70

tween MMA and DMA seems to be efficient when the pH was less than 6.5 or greater than 7.5 and TBA concentration near to 3.0 mmol l^{-1} approximately. On the other hand, for the second couple, the best selectivity of Se(IV) eluted after SeMet was obtained at a pH less than 6.5 but with TBA concentration over 2 mmol l^{-1}.

Hence, in order to simultaneously speciate arsenic and selenium under the same chromatographic conditions, a compromise has to be found. It takes into account the fact that the selectivity of DMA and Se(IV) must be at least greater than 1.3, when separation between two successive compounds is considered to be sufficient. A satisfactory response is found when pH is 5.5; 6.5 and TBA concentration is 2.5; 3.5 mmol l^{-1}. As the response surface shows relative robustness of the analytical method inside the mentioned domain, we chose to work at pH 6.0,

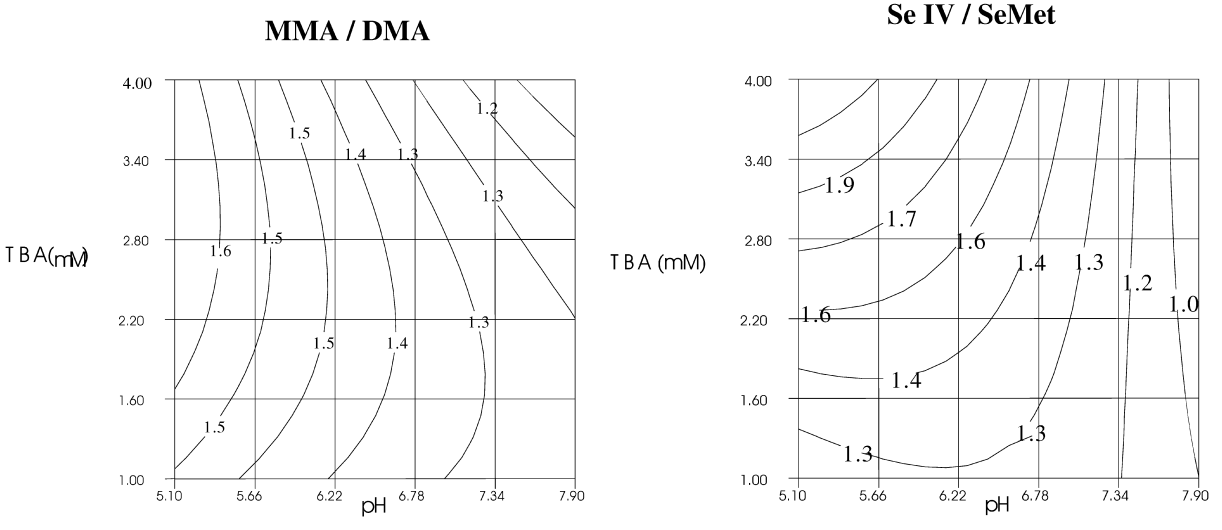

Fig. 4. Selectivity variation of selenium and arsenic species investigated in dependence of pH and TBA concentration.

3.0 mmol l^{-1} TBA and 4.0 mmol l^{-1} Na_2HPO_4 for the hyphenated method.

3.3. Hyphenated technique

As the off-line coupling included collection of fractions at the end of the column, separation between analytes had to be sufficient in order to minimise possible mixture of them in a fraction. Each fraction contained 75 μl of the post-column mobile phase flowing at 1 ml min^{-1}. Each peak of the chromatogram was shaped by successive bars of histograms symbolising the amount of intensity signal obtained by furnace AAS detection (Fig. 5).

Linearity was studied in the range 10–200 μg selenium and arsenic per litre. Calibration curves were obtained by weighted least-square linear regression analysis of the peak-area of the four arsenic species and six selenium species versus their concentrations. The so-called peak-area of each compound was obtained by the sum of intensity signals corresponding to injected fractions, since the signals were at least three times greater than those corresponding to the blank injection.

A statistical test of linearity was performed for each curve separately using an unweighted ANOVA. Analysis of all calibration series showed an excellent linearity. The mean correlation coefficients are given in Table 6.

Table 6
Calibration curves: examples for selenium species since the levels of the HPLC parameters were fixed at 6.0 for pH, 3.0 mmol l^{-1} for TBA and 4.0 for mmol l^{-1} Na_2HPO_4

Species	Linear model	Correlation coefficient (r)
Se(IV)	$y=0.789x-4.304$	0.999
Se(VI)	$y=0.610x-2.478$	0.998
Secystamine	$y=0.914x+1.522$	0.998
Secystine	$y=0.909x-1.739$	0.999
SeMet	$y=0.579x-5.261$	0.992
SeEt	$y=0.583x-1.652$	0.998

The limits of quantification which were evaluated as the minimum injected amount that can be measured routinely with acceptable precision (RSD less than 15%), were found to be about 2.0±0.2 ng for arsenic species, about 1.5±0.2 ng for selenium species and about 800±10 pg for Secystine, when 40 of 75 μl of each fraction were analysed by AAS. The quantification limits are similar for arsenic but lower for selenium comparatively to those obtained by using HPLC–ICP-MS techniques [21,29,30].

The consumption rate of water by ICP is similar to the normal HPLC flow-rate, and therefore the combination of HPLC with ICP is easily achieved by simply connecting the effluent line of the column to the nebulizer of the ICP instrument. To prevent

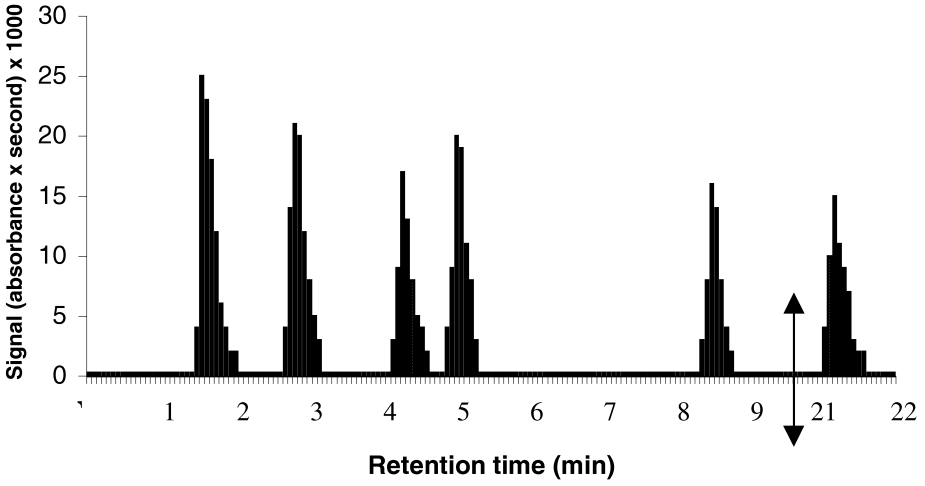

Fig. 5. Separation of inorganic selenium and different selenoamino acids by ion-pair chromatography (pH 6.0; TBA 3.0 mmol l^{-1}; acetonitrile 1% and Na_2HPO_4 4.0 mmol l^{-1}) with 'off line' furnace AAS detection. Analytes: 1=Secystamine; 2=Secystine; 3=SeMet; 4=Se(IV); 5=SeEt; 6=Se(VI) (10 ng each).

Table 7
Number of theoretical plates measured with and without the off-line coupling with GFAAS (TBA = 3.0 mmol l^{-1}, pH 6.0, acetonitrile 1% and Na_2HPO_4 = 4.0 mmol l^{-1})

	UV (200 nm), length of capillary tube: 10 cm	FAAS
DMA	2939	2601
MMA	3589	2953
SeMet	3246	2788
Se(IV)	3817	3595

broadening of the peak, the bore size of the connection line should be kept as small as possible (PTFE tubing of 0.25 mm I.D. in our case). The column conditions are selected primarily to get optimum separation of standards, but the limiting factors (such as the plasma disruptions by the use of organic solvents and the possible interferences with the polyatomic ions) for ICP-MS must, of course, be taken into consideration. Compared with GFAAS that the use in the continuous monitoring is not possible, the sensitivity of ICP-MS is similar for many elements, but ICP-MS is less tolerant to the presence of large amounts of salts in the sample.

Table 7 shows the number of theoretical plates measured with and without the off line coupling with FAAS (TBA = 4.2 mmol l^{-1}, pH 6.5 and Na_2HPO_4 = 2.5 mmol l^{-1}) for four species. The resorting to the collection of the fractions allowing the couplage with the elemental detection, did not contribute much to the post-column dispersion and the lost of the number of the theoretical plates.

4. Conclusions

We have demonstrated that ion-pair chromatography can be interfaced off-line with furnace AAS to allow speciation of both arsenic and selenium species under the same analytical conditions. Although time-consuming, using this described technique, a great number of selenium and arsenic species, mainly encountered in the environment, were determined with high selectivity and sensitivity.

The use of model equations enables one to predict retention times of the analytes in dependence on the eluent composition which is very helpful for solving analytical problems. Response surfaces and isoresponse curves were drawn from the regression models in order to visualise the optimal separation conditions inside the experimental design and the robustness of the separation method. The experimental results were in good agreement with those predicted by the models. Experimental designs afford maximum information about the retention behaviour of the studied analytes in less experiments than an univariate development would have required.

Dual detection of arsenic and selenium compounds will constitute further investigation, by coupling ion-pair HPLC with hydride generation (HG)–ICP-MS. Indeed, HG and the use of membrane-based gas separators can compensate for the loss of sensitivity of ICP-MS towards selenium and minimise interferences. This would yield to a powerful method consisting of an on-line and simpler couplage, allowing simultaneous determination of a great number of arsenic and selenium species with high sensitivity and specificity.

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