Journal of Chromatography A, 1217 (2010) 6186–6194

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Optimization of an anion-exchange high performance liquid chromatography-inductively coupled plasma-mass spectrometric method for the speciation analysis of oxyanion-forming metals and metalloids in leachates from cement-based materials

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ARTICLE INFO

Article history:
Received 28 May 2010
Received in revised form 26 July 2010
Accepted 27 July 2010
Available online 11 August 2010

Keywords:
HPLC-ICP-MS
Oxyanions
Concrete
Leaching
Speciation analysis

ABSTRACT

A method was developed for the speciation analysis of the oxyanions of As(III), As(V), Cr(VI), Mo(VI), Sb(III), Sb(V), Se(IV), Se(VI) and V(V) in leachates from cement-based materials, based on anion-exchange HPLC coupled with ICP-MS. The method was optimized in a two-step multivariate approach: the effect of sample pH and mobile phase composition on resolution, peak symmetry and analysis time was studied. Optimum conditions were then identified for the significant experimental factors by studying their inter-dependence. A mobile phase composition of 20 mM ammonium nitrate, 50 mM ammonium tartrate and pH 9.5 was found to be a compromise optimum for the separation of the target analytes using isocratic elution. The optimum condition provided separation of the analytes in less than 6 min, at a mobile phase flow rate of 1.0 mL/min. The signal intensities of the analytes were improved by adding 1% methanol to the mobile phase. The limit of detection of the method was in the range 0.2–2.2 μg/L for the various species. The effect of sample constituents was studied using spiked concrete leachates. The method was used to determine the target oxyanionic species in leachates generated from a concrete material in the pH range 3.5–12.4; CrO_4^{2-}, MoO_4^{2-} and VO_4^{3-} were detected in most of the leachates.

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1. Introduction

Construction and demolition waste are recycled for various applications. In several countries, these materials are used as aggregate substitutes for road construction. The environmentally acceptable application of recycled materials necessitates evaluation of the mobility of their constituents. As the release and transport mechanisms as well as the environmental impact of elements depend on their chemical forms, the leaching of elements from such materials should preferably be assessed based on the determination of their distinct species. The release of oxyanion-forming metals and metalloids from cement-based materials is enhanced when the pH of the materials is lowered as a result of long-term processes such as carbonation. There are few experimental studies on the speciation analysis of this group of elements released from cement-based materials [1]. In our recent studies, the species of As, Cr, Mo, Sb, Se and V leached from such materials were determined using charge-based fractionation [2,3] and redox speciation [4] analyses. Interest in the mentioned oxyanion-forming elements in relation to the land-filling and recycling of waste materials is growing. As and Cr have for a long time been recognized as priority pollutants whereas Mo, Sb, Se, and V came to the environmental agenda more recently [5].

The charge-based fractionation analyses of leachates from a concrete material showed that As, Cr, Mo, Sb, Se and V predominantly exist as anions in the leachates [2,3]; however, the species of the elements were not determined individually in the mentioned studies. The present work aimed to develop a method for the speciation analysis of the anionic species of the above-listed elements in such leachate samples. High performance liquid chromatography (HPLC), in combination with spectrometric techniques, has been much used in elemental speciation analysis because of its capability to separate, and unequivocally identify and quantify species [6–8]. Inductively coupled plasma mass spectrometer (ICP-MS) is a preferred detector for HPLC systems because of its diverse analytical advantages which include high sensitivity, multi-element capability and ease for online coupling [9–11]. Furthermore, due to its element specificity, resolution needs to be monitored only for species of a given element, unless there are interfering substances. Ion-exchange liquid chromatography is suitable for the separation and identification of ionic or ionizable substances.

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0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.07.082

In the present study, an anion-exchange HPLC-ICP-MS method was developed for the speciation analysis of the oxyanions of As, Cr, Mo, Sb, Se and V in leachates generated from cement-based material over a broad pH range (3–13). The target elements may exist as oxyanions in the following oxidation states: As(III), As(V), Cr(VI), Mo(VI), Sb(III), Sb(V), Se(IV), Se(VI) and V(V) [12]. Anion-exchange HPLC-ICP-MS methods have been developed for the determination of some of the analytes considered in the present study in water samples [13–17], and in fly ash extracts [18]. However, none of the methods addressed the simultaneous determination of all the species targeted in this study. In the development of a chromatographic method, selecting an appropriate separation column and identifying the optimum mobile phase composition are key steps. Carbonate and phosphate salts are often used to prepare the mobile phase for anion-exchange liquid chromatography. However, the use of these salts is not advisable in systems utilizing an ICP-MS detector, because carbonate leaves carbon deposits on the sampling and skimmer cones [19] and phosphate causes clogging and rapid erosion of the cones [20]. Thus, instrument- as well as plasma-friendly salts should be used. Studies have shown that ammonium nitrate is a suitable salt because it does not cause clogging and interference problems [16,21,22]. In addition, it has minimal effect on the ionization characteristics of the plasma; such an effect is normally encountered when the mobile phase is prepared from salts of alkali or alkaline earth metals.

Chromatographic methods are often optimized in a univariate approach, i.e. by varying one parameter at a time. Such optimization does not take the interdependence of experimental factors into account, and the approach is also time-consuming [23]. The present method was optimized in a stepwise multivariate approach. A Plackett–Burman experimental design was used to evaluate the effect of sample pH and mobile phase composition (ammonium nitrate and ammonium tartrate concentration, and pH) on resolution, peak symmetry and analysis time. Optimum values were then identified for the factors which had significant effect using a face-centred central composite design (FC-CCD) of experiments. A compromise optimum condition was identified for the separation of the target analytes based on isocratic elution. Isocratic elution requires relatively simple instrumentation, and unlike gradient systems, it needs no equilibration time between sample runs. The statistically found optimum conditions were validated experimentally. In addition, an optimum concentration was identified for methanol in the mobile phase to improve the signal intensities. The effect of matrix constituents on the chromatographic separation of the analytes was also studied. Finally, the oxyanionic species were determined in concrete leachates of pH 3.5–12.4.

2. Experimental

2.1. Instrumentation

An ELAN 5000 ICP-MS (Perkin-Elmer, Norwalk, USA) with cross-flow nebulizer and double pass spray chamber was used. The instrument operating conditions, data acquisition parameters and selected mass-to-charge ratios of the elements are given in Table 1.

The chromatographic system consisted of an anion-exchange column (PRP-X100, Hamilton, see Table 1), a metal-free HPLC pump (Model DXP-1, Dionex), a high pressure pulse damper (Dionex), a pressure monitor (LDC, Milton Roy) and a six-port syringe-loading sample injector (Model 7125, Rheodyne). A guard column (PRP-X100, Hamilton, see Table 1) was fitted to the separation column. Samples were introduced manually using a syringe (Hamilton).

A switching valve was used to connect the nebulizer inlet of the ICP-MS to the HPLC column outlet or to a peristaltic pump which delivered the ICP-MS optimization solution and drained waste from the spray chamber. After optimization, the ICP-MS instrument was connected to the column by switching the valve.

Table 1
Operating conditions of the anion-exchange HPLC-ICP-MS system.

ICP-MS	ELAN 5000
Instrument parameters	
RF power, W	1000
Plasma Ar flow, L/min	15
Auxiliary Ar flow, L/min	1.0
Nebulizer Ar flow, L/min	0.85 - 1.0 (optimized daily)
Sample uptake rate, mL/min	1.0
Data acquisition parameters	
Scanning mode	Peak hopping transient
Data acquisition mode	Graphics (signal intensity *vs* time)
Dwell time per unit, ms	10
Sweeps per reading	1
Estimated replicate time, ms	40,000
Number of replicates	1
Reading per replicate	4000
Isotopes monitored	As (75), Cr (53), Mo (98), Sb (121), Se (82), V (51)
HPLC	**Anion-exchange**
Column[a]	PRP-X100 (250 × 4.1 mm i.d., 10 μm particle size)
Guard column[a]	PRP-X100 (20 × 2.0 mm i.d., 10 μm particle size)
Mobile phase	20 mM ammonium nitrate, 50 mM ammonium tartrate, 1% (v/v) methanol, pH 9.5 (adjusted with NH_3)
Elution mode	Isocratic
Flow rate, mL/min	1.0
Sample injection volume, μL	200

[a] The column has trimethylammonium strong anion-exchange functional group supported on a polystyrene-divinylbenzene resin. Its working pH range is 1–13.

2.2. Chemicals and working solutions

1000 mg/L stock standard solutions of As(III), As(V), Cr(VI), Mo(VI), Sb(III), Sb(V), Se(IV), Se(VI) and V(V) were prepared from $NaAsO_2$ (Fluka), $Na_2HAsO_4{\cdot}7H_2O$ (Merck), Na_2CrO_4 (Merck), $Na_2MoO_4{\cdot}2H_2O$ (Sigma), $KSbOC_4H_4O_6{\cdot}0.5H_2O$ (Merck), $KSb(OH)_6$ (Sigma), Na_2SeO_3 (Fluka), $Na_2SeO_4{\cdot}10H_2O$ (BDH) and Na_3VO_4 (Aldrich), respectively. The solutions were prepared in polyethylene containers and kept at 4 °C. Ultrapure water (18.2 MΩ cm, Millipore, USA), ammonium nitrate (Merck), ammonium-L-tartrate (Alfa Aesar), HPLC grade methanol (BDH), NH_3 solution (25%, Merck), Suprapur HNO_3 (65%, Merck), and pro-analysis NaOH (Fluka) were used.

Three separate solutions (I, II and III), which contained 25.0 μg/L of each of the target elements, were prepared with the following compositions—I: As(III), Sb(III), Se(IV); II: As(V), Cr(VI), Mo(VI), Sb(V), Se(VI), V(V) and III: mixture of I and II. The three solutions were prepared by mixing appropriate volumes of the stock standard solutions of the analytes just before analysis to minimize any change in the speciation of the analytes during storage of their mixtures.

2.3. Mobile phase preparation

The optimum composition of the mobile phase is given in Table 1. For the method optimization, mobile phases of various compositions were prepared (see Section 3.2.1). The mobile phases were pH adjusted with HNO_3 and NH_3, filtered through a 0.45 μm membrane filter and degassed with helium before use.

Table 2
pK_a values of the oxyanionic species of As, Cr, Mo, Sb, Se and V at 25 °C.

Species	Formula	Protonated form	pK_a	Reference
Arsenite, As(III)	AsO_2^-	$HAsO_2$	9.29	[27]
Arsenate, As(V)	AsO_4^{3-}	H_3AsO_4	2.24, 6.69, 11.50	[27]
Chromate, Cr(VI)	CrO_4^{2-}	H_2CrO_4	−0.20, 6.51	[28]
Molybdate, Mo(VI)	MoO_4^{2-}	H_2MoO_4	4.24, 8.24	[28]
Antimonite, Sb(III)	SbO_2^-	$HSbO_2$		
Antimonate, Sb(V)	$Sb(OH)_6^-$	$Sb(OH)_5$	2.7	[29]
Selenite, Se(IV)	SeO_3^{2-}	H_2SeO_3	2.27, 7.78	[28]
Selenate, Se(VI)	SeO_4^{2-}	H_2SeO_4	<0, 1.7	[28]
Vanadate, V(V)	VO_4^{3-}	H_3VO_4	4.0, 8.5, 14.3	[28]

2.4. Leachate samples

Four leachate samples of pH 3.5, 7.6, 10.3 and 12.4 were prepared according to a standard pH-dependent leaching test [24] from the concrete material described in our previous study [4]. The crushed concrete sample (<1 mm particle size) was mixed with leachants in polyethylene containers at a liquid-to-solid ratio of 10. Deionised water was used as a leachant to prepare the pH 12.4 leachate (the pH of the leachate was dictated by the concrete material itself), and deionised water acidified with different volumes of HNO_3 were used to generate the other three leachates. The mixtures were agitated in an end-over-end fashion for 48 h. Following separation of the solid and liquid phases by settling, leachate pH measurements were taken and the suspensions were filtered through 0.45 μm membrane filter using vacuum filtration, and the filtrates (leachates) were kept in a deep freeze.

2.5. Software and data handling

Chromatograms were plotted by exporting the experimental data from ELAN to MATLAB (The Mathworks Inc., USA, 2008). "MINITAB Release 14" software (Minitab Inc., USA, 2003) was used for building the Plackett–Burman and the FC-CCD experiments.

2.6. Identification of analyte species and quantification

In the method optimization, the peaks of the analyte species were identified by separately injecting solution I and II (see Section 2.2), whereas the chromatographic response variables were determined from the chromatograms of solution III. Peaks for the species of an element were identified according to the retention times of the analytes. Calibration curves were plotted on the basis of peak height.

2.7. Chromatographic response parameters

The resolution (Rs) for species 'a' and 'b' of an element was calculated as $Rs = 2(t_{Rb} - t_{Ra})/(w_a + w_b)$, where t_R represents the retention time of a species and w denotes the baseline width of a peak. w was measured as the length of a segment between the intersections of the tangent lines on each side of the peak with the baseline. The complete separation of adjacent peaks is signalled by a minimum Rs value of 1.5 [25].

The asymmetry factor of a peak (A_s) was calculated as $A_s = B/A$, where A and B represent the distance between a perpendicular line passing through the peak maximum and the edges of the peak on the left and right side of the line, respectively, at 10% of the peak height [25]. Symmetrical peaks have $A_s = 1.0$. A_s is greater than 1.0 in case of peak tailing and its value will be less than 1.0 for fronted peaks.

Analysis time (t) was measured as the retention time of the last-eluting peak. To avoid discrepancies which arise due to the delay time and the variation in the length of the tubing connecting the parts of the HPLC system, t was measured relative to the solvent front (t_0) as $t_{R(\text{last})} - t_0$. A response function was set for t based on preliminary studies as used elsewhere [26]: moderate separation of the analytes (with poor Rs and A_s for some of the species) was achieved in about 400 s; hence, analysis time function (t_a) was defined as $t_a = t/400$ s. t_a close to or less than 1 were targeted in the method optimization (see Section 3.2.2).

3. Results and discussions

3.1. Analyte species and their pK_a values

The pK_a values of the oxyanionic species (protonated form) of the target elements are given in Table 2. Only the monomeric species were considered because polymerization of the oxyanions occurs only at high concentration in strongly acidic solutions [12]. According to these values, all the species except As(III) should exist predominantly as anions in neutral and alkaline solutions. As(III) should be 50% ionized at pH 9 and almost not ionized below pH 7.

3.2. Optimization of the chromatographic method

Optimization of a chromatographic method should be carried out to find a condition which separates analytes with well-resolved and symmetrical peaks in a short analysis time [30]. The present anion-exchange HPLC-ICP-MS method was optimized using a two-step multivariate approach. A two-level Plackett–Burman's factorial experimental design [31] was used to study the effect of sample pH and the composition of the mobile phase in the initial screening step. In the subsequent step, optimum conditions were identified for the factors which showed significant effect on the system, using a three-level FC-CCD [31] of experiments. Rs, A_s and t_a were used as response parameters for optimization (see Section 2.7 for the definition of the parameters).

3.2.1. Effect of experimental factors on the chromatographic response parameters

The mobile phase was prepared from ammonium nitrate. Preliminary experiments showed that this mobile phase cannot elute Sb(III) from the anion-exchange column. This was possibly due to the strong adsorption or on-column precipitation of the species [29]. Literature reports show that the problem can be solved by adding tartrate [13,32–34], citrate [35] or phthalate (with [36] or without [37] EDTA) to the mobile phase. Lindemann et al. [13] showed that tartrate gives better elution of Sb(III), thus, this reagent (as its ammonium salt) was used in the present study.

The effect of sample pH and mobile phase composition (ammonium nitrate and ammonium tartrate concentration, and pH) on the chromatographic response parameters (Rs, A_s and t_a) was studied using a two-level Plackett–Burman's factorial design of experiments as shown in Table 3. The low and high (− and +, respectively) values used in the design were: sample pH (3, 13), ammonium nitrate concentration (20, 50 mM), ammonium tartrate concentra-

Table 3
Plackett–Burman's experimental design for studying the effect of sample pH and mobile phase composition (ammonium nitrate and ammonium tartrate concentrations, and pH) on the chromatographic response parameters (Rs, A_s and t_a).

Factor	Analysis*											
	1	2	3	4	5	6	7	8	9	10	11	12
A	–	–	+	+	+	–	–	+	–	–	+	+
B	+	+	+	–	+	–	–	–	–	+	–	+
C	+	+	+	+	–	–	–	–	+	–	+	–
D	–	+	–	+	+	–	+	–	–	+	+	–

(A) Ammonium nitrate concentration, mM: (–) = 20, (+) = 50.
(B) Mobile phase pH: (–) = 4, (+) = 10.
(C) Ammonium tartrate concentration, mM: (–) = 20, (+) = 80.
(D) Sample pH: (–) = 3, (+) = 13.
* Each analysis was carried out two times using a solution containing 25 μg/L of each of the analyte species (solution III, see Section 2.2).

tion (20, 80 mM) and mobile phase pH (4, 10). The boundary values of sample pH reflect the pH range in which leachates are prepared according to the pH-dependent leaching test. The values for the other experimental factors were set based on preliminary studies and literature reports [13,14,16,32–34].

The analyses in Table 3 were carried out in a randomized order using solution III (see Section 2.2) in two replicates. A total of 24 chromatograms were recorded and the chromatographic responses parameters were determined for the analytes from each chromatogram. The data obtained from the Plackett–Burman experiments gave the standardized main effect Pareto charts shown in Fig. 1(a)–(c). The relative significance of the factors on a chromatographic response parameter was evaluated by comparing the length of their bars. The effect of an experimental factor is significant (at 95% confidence level) if its bar length is greater than the critical value, 2.36. The plots show absolute values of the estimated effects of the factors. In the present study, a factor was said to have a positive effect if an increase in its value improves the chromatographic response parameter(s), i.e. if it provides high *Rs*, low t_a or low A_s (no experimental condition gave peak fronting of analytes, hence A_s was ≥1.0 in all cases). A negative effect implied that increasing the experimental factor lowered *Rs* or increased A_s and t_a.

The Pareto charts showing the effects of the experimental factors on the *Rs* for the As, Sb and Se species are presented in Fig. 1(a). The concentrations of ammonium nitrate and ammonium tartrate, and the mobile phase pH were significant on the Rs of As(III)–As(V), Sb(III)–Sb(V) and Se(IV)–Se(VI), except the concentration of ammonium nitrate on the Sb species. The effect of the salts was negative, i.e. increasing the concentration of the salts decreased Rs. This may be because a high salt concentration in the mobile phase decreases the interaction of the ionic analyte species with the ion-exchange sites leading to faster elution and poorer *Rs* of the analytes. The mobile phase pH had positive effect on the *Rs* of the As species and negative effect on the Sb and Se species (the reasons are discussed in Section 3.2.2).

The Plackett–Burman screening experiments showed that none of the four experimental factors had significant effect on the A_s of the analytes except for Sb(III) and V(V). The chromatograms from the experiments showed that the peaks of Sb(III) and V(V) were tailed mostly at low mobile phase pH. As can be seen from Fig. 1(b), the pH of the mobile phase had significant effect on the A_s for both species and the ammonium tartrate concentration on that for Sb(III). The effects were positive; increasing the factors decreased the A_s for both species.

Fig. 1(c) presents a Pareto chart showing the effect of the four experimental factors on t_a. The mobile phase pH was the only factor which had significant effect on this parameter; t_a decreased as the mobile phase pH increased. It was observed in the chromatograms from the Plackett–Burman experiments that either Cr(VI), Mo(VI), Sb(III) or V(V) were the last-eluting species (i.e. which determined t_a). According to the p*K*a values given in Table 2, the deprotonation of the analyte species increases with increasing mobile phase pH. Hence, the interaction of the analytes with the ion-exchanger and in turn their retention times are expected to increase with pH. However, when the mobile phase contains a negatively charged component such as tartrate at a concentration much higher than those of the analytes, the interaction of this component with the ion-exchanger will decrease the elution time of the analytes [38]. The positive effect of the mobile phase pH on t_a was probably due to elevated elution capacity of the mobile phase which resulted from the increased concentration of doubly charged tartrate ions at high pH (tartrate: $pK_{a1} = 3.0$, $pK_{a2} = 4.4$).

As observed in Fig. 1(a)–(c), the concentrations of ammonium nitrate and ammonium tartrate, and the mobile phase pH had significant effect on the chromatographic response parameters of most of the analyte species. The effect of sample pH was not significant for any of the analytes; this may be because the pH of the small sample portion injected into the HPLC system was dominated by the pH of the larger volume of the carrier stream (the mobile phase).

3.2.2. Identifying optimum conditions for the significant experimental factors

Table 4 shows the three-level FC-CCD of experiments used to study the interdependence of the significant experimental factors and to identify their optimal values. The upper and lower values for

Table 4
"2^3 + star face-centred central composite design" matrix used for the optimization of the three significant experimental factors; ammonium nitrate and ammonium tartrate concentrations, and mobile phase pH.

Factor	Analysis*																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	0	–	0	0	+	0	0	0	–	0	0	+	–	+	0	–	0	–	+	+
B	+	0	0	0	0	–	0	0	+	0	0	+	–	–	0	+	0	–	–	+
C	0	0	–	+	0	0	0	0	+	0	0	–	–	+	0	–	0	+	–	+

(A) Ammonium nitrate concentration, mM: (–) = 20, (0) = 35, (+) = 50.
(B) Mobile phase pH: (–) = 4, (0) = 7, (+) = 10.
(C) Ammonium tartrate concentration, mM: (–) = 20, (0) = 50, (+) = 80.
* Each analysis was carried out three times using a solution containing 25 μg/L of each of the analyte species (solution III, see Section 2.2).

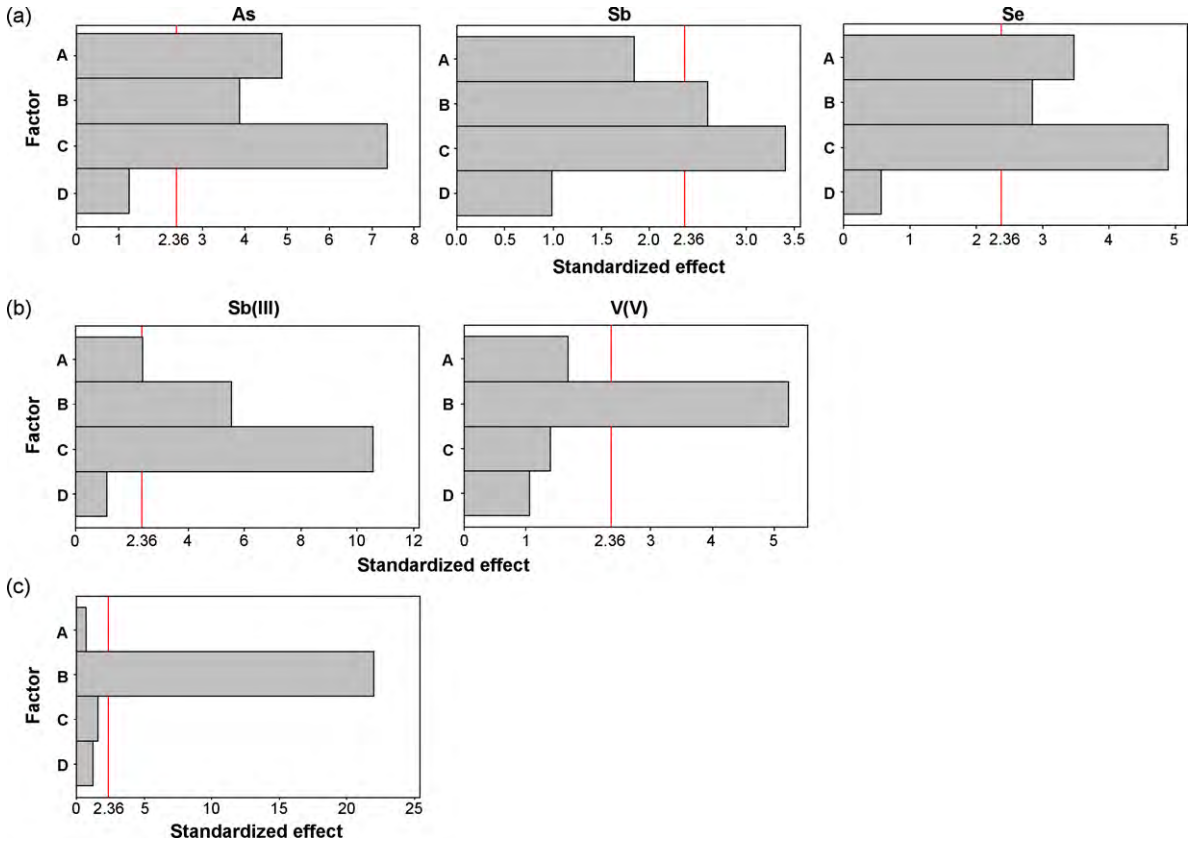

Fig. 1. Standardized main effect Pareto charts showing the effect of (A) ammonium nitrate concentration, (B) mobile phase pH, (C) ammonium tartrate concentration and (D) sample pH on (a) the resolution (Rs) for As(III)–As(V), Sb(III)–Sb(V) and Se(IV)–Se(VI), (b) the asymmetry factor (A_s) for Sb(III) and V(V), and (c) the analysis time function (t_a). The vertical lines in the charts indicate the minimum value for the statistical significance of a parameter at 95% confidence level.

the significant factors were set as in the Plackett–Burman design (see Section 3.2.1), and the sample pH was kept at 7. The experiments were run randomly in three replicates. The experimental data gave two second-order polynomial equations for the analytes where ammonium nitrate and ammonium tartrate concentrations, and mobile phase pH were independent variables, and *Rs* and A_s were responses. A response equation of the same variables was also obtained for t_a. The models showed good agreement with the experimental data as characterised by multiple regression coefficients (R^2) of ≥0.992.

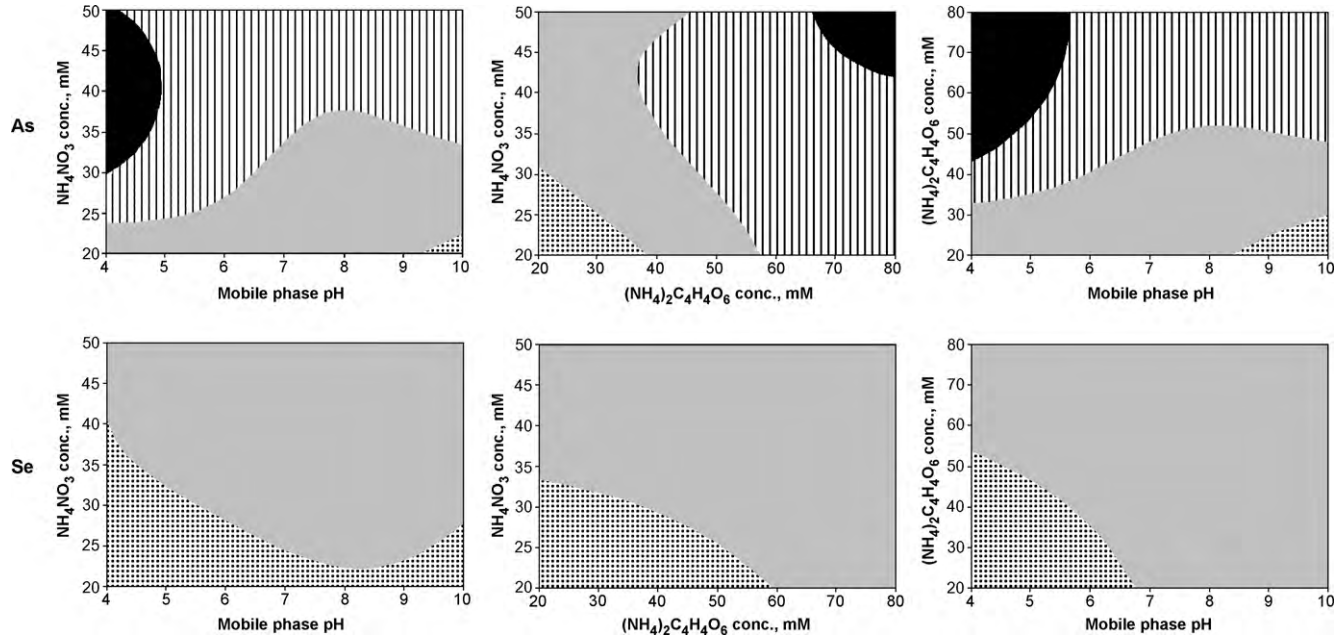

Fig. 2. Contour plots showing the interdependence of a pair of experimental variables and their effect on the resolution (*Rs*) for As and Se species. The third factor was fixed at its middle value (see Table 4). Contour areas: ■ (*Rs* < 0.5), ▥ (*Rs* 0.5–1.0), ▤ (*Rs* 1.0–2.0) and ▦ (*Rs* > 2.0).

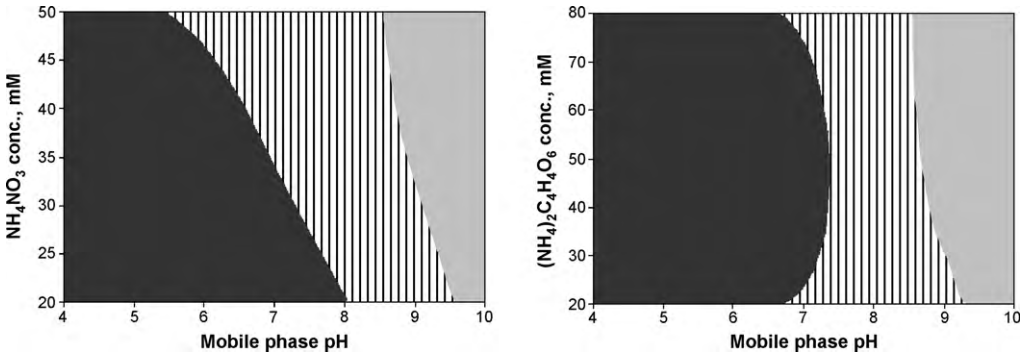

Fig. 3. Contour plots showing the interdependence of a pair of experimental variables and their effect on the asymmetry factor (A_s) for V(V). The third factor was fixed at its middle value (see Table 4). Contour areas: ▭ (A_s 1.0–2.0), ▯ (A_s 2.0–3.0) and ■ (A_s > 3.0). The plots were similar for Sb(III).

Contour plots were used to observe the interdependence between a pair of factors and the predicted responses. Optimum values were identified for the experimental factors by visually inspecting the plots. Representative plots are presented in Figs. 2–4; the plots show interactions between two factors within their experimental ranges keeping the third one at its middle value.

Fig. 2 presents contour plots which show the interaction between a pair of experimental factors and the predicted *Rs* for the As and Se species. All the examined conditions resolved Sb(III) and Sb(V) with Rs ≥ 2.0 (contour plots not shown). As can be seen from the figure, the *Rs* for the As species can be >2.0 if the mobile phase pH is kept above 9, provided that the concentrations of ammonium nitrate and ammonium tartrate are very low, i.e. less than about 25 mM and 30 mM, respectively. In this pH range, As(III) predominantly exists as AsO_2^- and As(V) as $HAsO_4^{2-}$ or AsO_4^{3-} (see Table 2). As the ionic strength of the mobile phase is lowered, the interaction between the two differently charged species and ion-exchange site will be improved and results in better resolution for the species. *Rs* in the range between 1.0 and 2.0 can be obtained over the whole pH range at moderate salt concentrations (the maximum salt concentration depends on the pH). The plots illustrate that *Rs* decreases when the concentration of mobile phase salts increases, because the anionic analyte species will have less interaction with the ion-exchange site when the ionic strength of the mobile phase increases. The pK_a values (Table 2) can be helpful in explaining the behaviour of the species. For example, the low *Rs* for As(III)–As(V) at pH below 6 is probably due to the predominance of singly charged species of As(V), $H_2AsO_4^-$ and uncharged species of As(III) in this pH region. At higher pH values the As(V) species become doubly charged, whereas the As(III) species remain uncharged or singly charged, giving rise to a higher *Rs* value.

The contour plots for Se in Fig. 2 show that all the examined conditions separate Se(IV) and Se(VI) with a minimum *Rs* of 1.0. The *Rs* can be increased above 2.0 if a mobile phase of pH < 6 is used with maximum ammonium nitrate and ammonium tartrate concentrations of 30–40 mM and 40–50 mM, respectively (the values depend on the mobile phase pH). Below pH 6 Se(IV) is singly charged ($HSeO_3^-$) whereas Se(VI) exists as SeO_4^{2-} (see Table 2). The species probably interact differently with the ion-exchange sites resulting in high *Rs*. However, above pH 8, also Se(IV) becomes doubly charged (SeO_3^{2-}). Due to their very similar ionic radii [39], SeO_3^{2-} and SeO_4^{2-} may interact with the ion-exchange sites in the same way, and they will therefore be eluted close to each other.

Fig. 3 presents contour plots showing the effect of the experimental factors on the A_s for V(V); the effects were similar for Sb(III). As no experimental condition gave peak fronting, the A_s values were ≥1.0. As can be seen from the plots, the pH of the mobile phase significantly affects the A_s of V(V) whereas the ammonium nitrate and ammonium tartrate concentrations have less pronounced effect. A mobile phase of high pH (mostly above 9) will lower the A_s of V(V) to the range of 1.0–2.0.

The interaction between the experimental factors and their effect on t_a is shown in Fig. 4. The contour plots show that a mobile phase of pH < 6 results in t_a higher than 2.0 (t > 800 s) at all concentrations of the two salts. t_a can be lowered below 0.75 (t < 300 s) if the concentrations of ammonium nitrate and ammonium tartrate are higher than about 35 mM and 50 mM, respectively, provided that the pH of the mobile phase is above 9.

3.2.3. Compromise optimum condition

When several experimental parameters are optimized for the simultaneous determination of a number of analytes, it may be difficult to find conditions which are equally optimal for all the analytes.

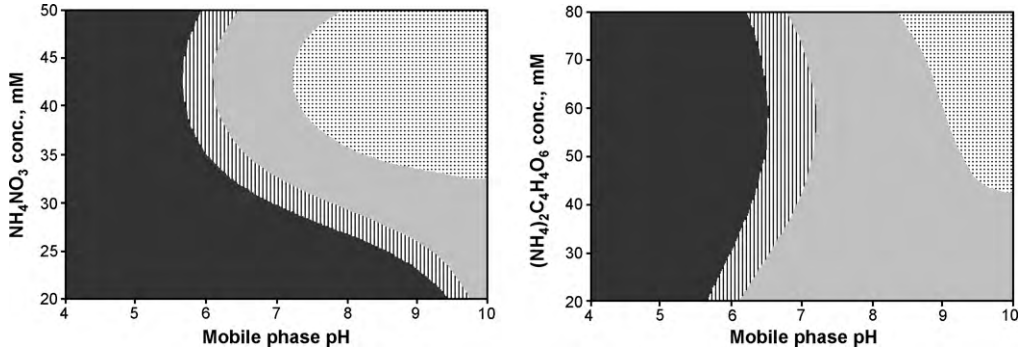

Fig. 4. Contour plots showing the interdependence of a pair of experimental variables and their effect on the analysis time function (t_a). The third factor was fixed at its middle value (see Table 4). Contour areas: ⬚ (t_a < 0.75), ▭ (t_a 0.75–1.5), ▯ (t_a 1.5–2.0) and ■ (t_a > 2.0).

As can be inferred from the results discussed in Sections 3.2.1 and 3.2.2, the optimum conditions predicted to give better chromatographic responses vary among the analytes. For instance, a mobile phase of low ionic strength improves *Rs*, but increases A_s (for some species). A high mobile phase pH decreases A_s and t_a, but had no similar effect on the *Rs* for the As, Sb and Se species. Therefore, it was compulsory to find a compromise condition at which the analytes can be separated simultaneously with acceptable chromatographic characteristics. The compromise optimization was performed using MINITAB software. As observed from the results discussed in Section 3.2.2, a high ionic strength mobile phase lowers the *Rs* for the As species below 1 (the values depend on the mobile phase pH). In addition, the A_s of Sb(III) and V(V), and the t_a are highly affected by the mobile phase pH. The compromise optimization was carried out by setting limit values for these highly affected parameters; the values were *Rs* (1.0–1.5), A_s (1.5–2.5) and t_a (1.0–1.5). The optimality of a condition for each parameter was measured as individual desirability function (d_i) and the suitability of the condition for the simultaneous analysis of the analytes was evaluated from the geometric mean of the d_i values (composite desirability function, *D*) [40]. The compromise optimization of the factors gave an optimum mobile phase composition of 20 mM ammonium nitrate, 50 mM ammonium tartrate and pH 9.5, with d_i and *D* values of ≥0.90 and 0.96, respectively.

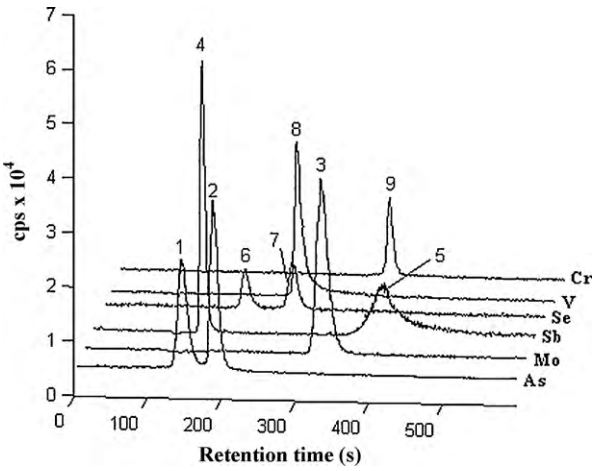

Fig. 5. Chromatograms for the speciation analysis of the oxyanions of As, Cr, Mo, Sb, Se and V using anion-exchange HPLC-ICP-MS. A standard solution containing 25.0 μg/L of each of the species was analysed using the optimum conditions established in this study. Column: PRP-X100 anion-exchange column; mobile phase: 20 mM ammonium nitrate, 50 mM ammonium tartrate, pH 9.5; flow rate: 1 mL/min, injected sample volume: 200 μL. Peaks: (1) AsO_2^-, (2) AsO_4^{3-}, (3) MoO_4^{2-}, (4) $Sb(OH)_6^-$, (5) SbO_2^-, (6) SeO_3^{2-}, (7) SeO_4^{2-}, (8) VO_4^{3-} and (9) CrO_4^{2-}.

3.3. Validation of the compromise optimum conditions

The validity of the predicted compromise optimum conditions was experimentally verified by analyzing a standard solution containing 25.0 μg/L of each of the analyte species (solution III, see Section 2.2). 200 μL of the sample was injected and analyzed using isocratic elution at a mobile phase flow rate of 1.0 mL/min. The chromatogram recorded for the analysis is shown in Fig. 5; the optimum condition provided baseline separation for the species of each element in less than 6 min (t_a < 0.9). The As, Sb and Se species were well-resolved and peaks of acceptable symmetry were obtained for all of the species.

3.4. Sensitivity improvement

In HPLC-ICP-MS systems where samples are introduced by conventional nebulization, the signal intensity will be relatively low owing to the small sample volumes introduced. Water-miscible organic solvents such as methanol and acetonitrile can be added to the mobile phase to improve the signal intensity of analytes [19]. In this study, the mobile phase was treated with 1–3% (v/v) methanol. As shown in Fig. 6, 1% methanol significantly increased the intensities for most of the species. Further increase in intensity with increasing amount of methanol was observed for As, Cr and V, but the trend was opposite for Mo, Sb and Se. A methanol concentration of 1% (v/v) was used in further experiments (except for Fig. 7 where 2% methanol was used). Addition of the organic solvent did not affect the chromatographic characteristics of the analytes apart from increasing the background level for Cr with constant noise (see Fig. 7).

3.5. Effect of matrix constituents

The effect of matrix constituents in the chromatographic separation of the target analytes was evaluated by addressing the composition of leachate samples. Three concrete leachates of pH 3.5, 7.6 and 12.4 were spiked with 25.0 μg/L of each of the analyte species and analyzed using the optimum chromatographic condition established in this study. Fig. 7 shows chromatograms for the unspiked and spiked leachates. The chromatograms of the pH 12.4 leachate are similar to that of the standard solution in Fig. 5; the *Rs* and the retention times of the analytes are similar for the two solutions, indicating no significant matrix effects. This is because this

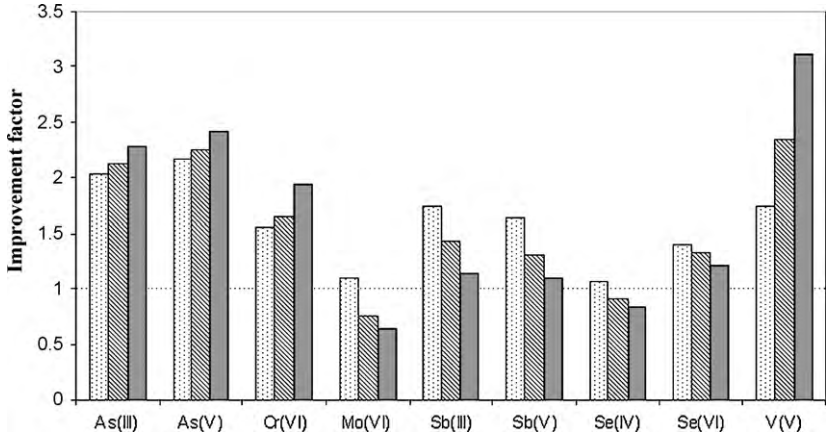

Fig. 6. Effect of methanol on the signal intensities of the analytes. The horizontal dotted line represents the relative sensitivity of the analytes without methanol. Shadings: ⬚ (1%), ▨ (2%) and ■ (3%) (v/v) methanol added to the mobile phase.

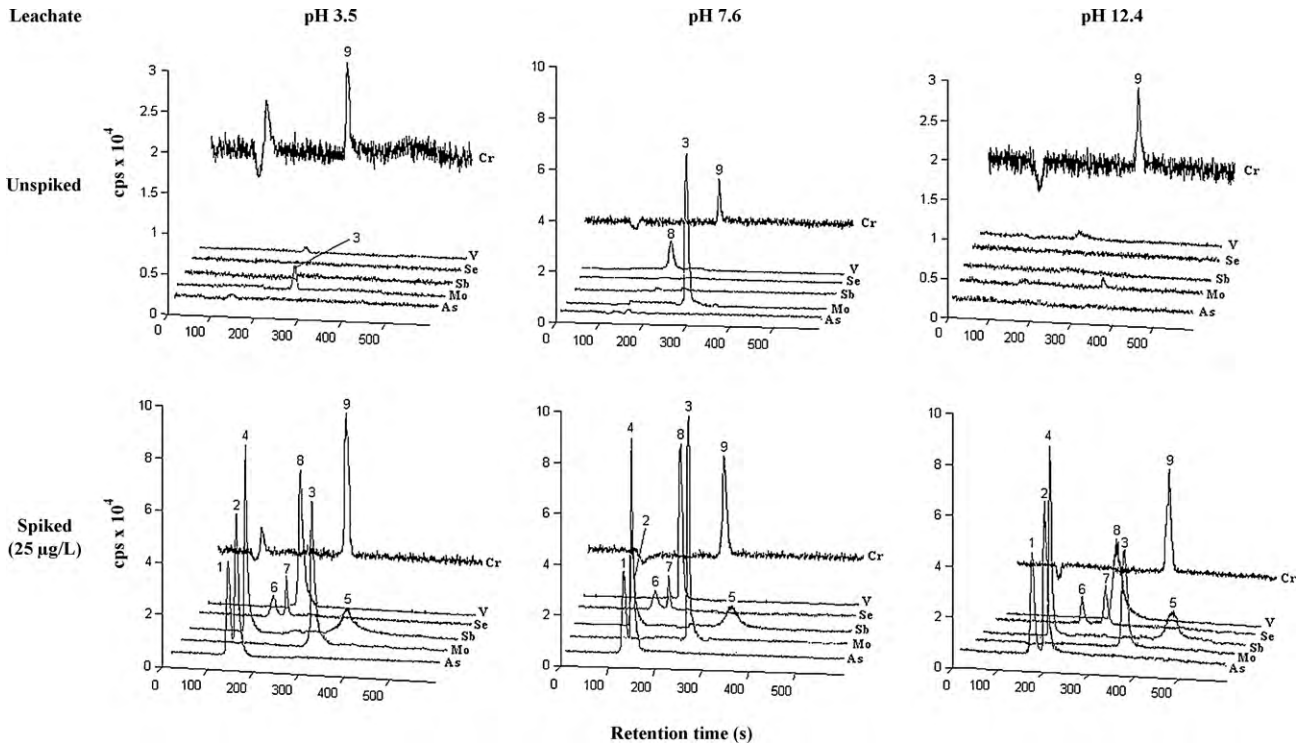

Fig. 7. Chromatograms for the speciation analysis of the oxyanions of As, Cr, Mo, Sb, Se and V in leachates of a concrete material at pH 3.5, 7.6 and 12.4 using anion-exchange HPLC-ICP-MS. Unspiked and spiked (25 μg/L) leachates were analysed using the following condition; column: PRP-X110 anion-exchange column; mobile phase: 20 mM ammonium nitrate, 50 mM ammonium tartrate, 2% (v/v) methanol, pH 9.5; flow rate: 1 mL/min; injected sample volume: 200 μL. Peaks: (1) AsO_2^-, (2) AsO_4^{3-}, (3) MoO_4^{2-}, (4) $Sb(OH)_6^-$, (5) SbO_2^-, (6) SeO_3^{2-}, (7) SeO_4^{2-}, (8) VO_4^{3-} and (9) CrO_4^{2-}.

leachate had a low concentration of interfering anions as it was prepared using only deionised water (see Section 2.4). But even for the pH 3.5 and 7.6 leachates, which were prepared using leachants acidified with HNO_3, relatively small changes in the retention times and *Rs* were observed. The changes observed were possibly due to the high concentration of NO_3^- in these leachates which resulted in faster elution of analytes. Apart from the small changes in retention time and *Rs*, no significant matrix effect was observed for the chromatographic characteristics of the species in these leachates, which were prepared using leachants acidified with HNO_3.

3.6. Limit of detection (LOD)

The LOD of the analytes were determined as the concentration which provides a signal three times the level of the baseline noise [25]. Ten blank solutions were injected and the noise levels were measured for each species at the respective retention times. With 1% methanol in the mobile phase, the following LOD values, given as μg/L of the target element, were obtained for a 200 μL injection volume: AsO_2^- (0.6), AsO_4^{3-} (0.5), CrO_4^{2-} (2.2), MoO_4^{2-} (0.2), SbO_2^- (0.9), $Sb(OH)_6^-$ (0.1), SeO_3^{2-} (1.0), SeO_4^{2-} (1.3) and VO_4^{3-} (0.2).

3.7. Speciation analysis of the target elements in leachates from a concrete material

Four concrete leachates of pH 3.5, 7.6, 10.3 and 12.4 (see Section 2.4) were analyzed using the developed method to determine the target oxyanionic species. The leachates were spiked with 10, 15 and 25 μg/L of each of the species and the analytes were determined based on standard addition calibration. Table 5 presents the total concentrations of As, Cr, Mo, Sb, Se and V and the concentrations of the target species in the leachates. CrO_4^{2-} was detected in all the four leachates, MoO_4^{2-} in all but the pH 12.4 leachate and VO_4^{3-} only in the pH 7.6 and 10.3 leachates. The As, Sb and Se species were all below the LOD. Table 5 illustrates that Cr, Mo and V are present in the leachates predominately as their oxyanions, except for CrO_4^{2-} at pH 3.5, which was much lower than total Cr at this pH.

Table 5
Total concentrations (μg/L) of As, Cr, Mo, Sb, Se and V, and concentrations of the elements in the respective oxyanionic species in leachates of a concrete material. Each data is the average of three determinations. The RSD values were in the range 2.9–7.5%.

pH	Concentration (μg/L)[a]								
	As	Sb	Se	Cr		Mo		V	
	Total	Total	Total	Total	CrO_4^{2-}	Total	MoO_4^{2-}	Total	VO_4^{3-}
3.5[b]	0.6	0.4	2.8	190	32	2.1	1.6	1.8	ND
7.6[b]	1.2	1.4	3.6	17	12	38	29	14	11
10.3[b]	0.9	0.8	2.6	114	102	26	22	11	8
12.4	0.5	0.3	1.8	19	17	1.7	ND	0.6	ND

ND: not detected (<LOD).
[a] The As, Sb and Se species were below LOD.
[b] Leachate prepared using leachants acidified with HNO_3.

A second peak was identified for Cr at pH 3.5 close to the solvent front (see Fig. 7).

The results of this study for the speciation of Cr, Mo and V are in good agreement with the findings of our previous works which identified the predominance of the anionic fractions of the elements in concrete material leachates [2,3]. Studies have identified that cement hydrate phases such as ettringite and calcium silicate hydrate retain CrO_4^{2-}, MoO_4^{2-} and VO_4^{3-} via different mechanisms [1,41,42] (see also the references therein). As the pH of the cement matrix is lowered, these phases are degraded [41] releasing the species from their structures into the leaching medium. The second peak for Cr at pH 3.5 was probably due to cationic Cr species; the charge-based fractionation analysis [2] identified a high cationic Cr fraction in acidic leachates (pH < 4).

4. Conclusion

In this study, an anion-exchange HPLC-ICP-MS method was optimized for the speciation analysis of the oxyanions of As(III), As(V), Cr(VI), Mo(VI), Sb(III), Sb(V), Se(IV), Se(VI) and V(V) in leachates from cement-based materials of a broad pH range (3–13). It is shown that a mobile phase containing ammonium nitrate and tartrate is suitable for the separation and analysis of the species; tartrate is required for the elution of Sb(III). The concentrations of ammonium nitrate and ammonium tartrate, and the pH of the mobile phase were found to affect the selected chromatographic response parameters, i.e. resolution, peak symmetry and analysis time, whereas the sample pH proved to have insignificant effect. The optimum conditions determined by multivariate analysis were verified experimentally, and confirmed the effective separation of the analytes in less than 6 min. The signal intensities of the analytes were improved by a small amount (1%) of methanol in the mobile phase. It is shown that the chromatographic response of the analytes is not much affected by the constituents of the leachate matrix, not even a very high concentration of nitrate affected the retention time and resolution much. The study thus illustrates that relatively complex inorganic matrices can be analyzed with the present HPLC-ICP-MS approach. Analysis of leachates of a wide pH range showed that Cr, Mo and V predominately exist in the leachates as their oxyanions. The analytical method thus provides relevant information regarding the pH-dependent release mechanism and stability after the release of the elements from cement-based materials.

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