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Arsenic speciation by gradient anion exchange narrow bore ion chromatography and high resolution inductively coupled plasma mass spectrometry detection

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ABSTRACT

Based on gradient anion exchange chromatography (AEC), a new strategy in As-speciation was evaluated. A narrow bore chromatographic system with lower flow rates (≤300 μL) well suitable for the low flow requirements of higher efficiency nebulizers was splitless coupled to a high resolution sector field ICP MS. The AEC system takes full advantage of the detector sensitivity allowing more diluted samples (50–100 times) to be injected, delivering substantially less sample matrix to the column and a lower eluent load to the plasma. The unique plasma compatibility of the NH_4NO_3-eluent salt used in this study enabled high linear salt ramps in gradient applications, highly reproducible retention times (±1%) and detection limits in the low ng/L range. The separation conditions were applied on two different polymeric anion-exchangers: a low capacity, weakly hydrophobic material (AS11, Dionex) and a more frequently used higher capacity, higher hydrophobic material (AS7, Dionex). On both columns, As-species (As(III/V), MMA, DMA, AsB) and Cl^- were separated in less than nine minutes and co-elution was circumvented by adapting the separation pH to the optimal column selectivity. The key-advantage of the NH_4NO_3-eluent is that it can adopt any separation pH without compromising the eluent strength which is not possible with all other eluents used so far. The influences of chloride and methanol were investigated and found not to affect the chromatographic performance. Column deposits caused strong reversible As(v) adsorption which reduced As(v) to As(III). A corresponding phosphate excess in the injected sample eliminated the adsorption and prevented artefacts in As(v)/As(III) ratios. The method applied to ground water samples provided robust separations and is compatible with any sample preservation procedure.

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1. Introduction

Fresh water shortage and new findings on multiple toxicity [1,2] of several arsenic species have intensified the As-problem in recent years. Many investigations have been initiated and regulatory limits (WHO, US-EPA) were lowered down to 10 μg/L As. All aspects of As-related problems such as toxicity, surface adsorption, mobility in the ground [3] and successful elimination strategies in drinking water production [4] depend on reliable As-speciation. A large number of As-species have already been identified however not all of them are toxic. The most abundant arsenicals in environmental waters are the inorganic arsenite (As(III)) and arsenate (As(V)) which still show the highest acute toxicity. These inorganic species are converted into numerous organic As-compounds in biologically mediated transformations [5–7]. The diverse classes of As-compounds formed (alkylated organo arsenicals [5,8], arsenolipids [9], arseno-sugars [10] and thio-arsenicals [11]) reflect the broad chemical reactivity of arsenic to readily adapt to a variety of chemical environments. Therefore, it is not surprising that the most frequently encountered As-species cover the whole range of molecule polarities, e.g. anions (As(V), monomethylarsonate (MMA), dimethylarsonate (DMA)), cations (arsenocholine (AC), tetramethylarsonium (TMA)) and, depending on the pH, neutral molecules (arsenobetaine (AsB), As(III)). Strategies for arsenic speciation have been presented by Larsen [12] and Feldmann et al. [6]. Not only the separation of numerous diverse As-species in short retention times (t_R) is required, but the method should be robust in routine speciation analysis [13], provide the lowest detection limits (<0.1 μg/L) and be fully compatible with sample preservation methods. ICP MS has shown to be the most versatile and most sensitive [14] detector in general speciation analysis [15], as well as in arsenic speciation [16]. Simple quadrupole ICP MS instruments are the most widely used detectors, whereas high resolution sector field ICP MS (HR ICP MS) provide 10–100 times higher sensitivities [17].

The diversity of As-compounds and its growing number are a permanent challenge to ion chromatography (IC) as the over whelming part of As-speciation is done by this method. Different types of IC methods [18] have been applied. While cation exchange chromatography (CEC) can separate cationic arsenicals

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[19], it does not retain and separate the most toxic and most abundant species, As(III) and As(V). This observation has been confirmed in many investigations. A recently developed cation exchange (CEX) gradient method (0–20 mM $^{+}NH_4$, pH 2.5 [20]) improved the separation somewhat, but also confirmed the separation problems: As(III), MMA, As(V) and Cl^- (interfering as $^{40}Ar^{35}Cl^+$ on $m/z = 75$ in $^{75}As^+$-detection by ICP MS [21]) eluted within 3 min in the front and AC with trimethylarsineoxide co-eluted at 15 min. Therefore, CEX remains as recommended [6,19] a secondary option, to separate the less toxic organic cationic arsenicals not resolved on an anion-exchanger. Anion exchange (AEX), however, separates well As(III), As(V) and other ionisable arsenicals. The eluent pH controls the ionisation degrees of both, the eluent anion and those of the analytes [12]. So far, higher capacity columns were almost exclusively used, as they require high eluent concentrations that can convert on-column the pH of the injected sample to the eluent pH. Synthetic polymer columns are typically applied, combining AEX and hydrophobic interaction sites which aid in the separation of neutral As-species [22]. This separation was realised on a multimode ion exchanger (AS7) containing anion and cation exchange sites. Applying strong acidic nitric acid eluent and an ion pairing reagent [23], anionic, cationic and neutral As-species were separated and the procedure was developed for a high resolution IC method [24,25]. However, ion pairing reagents are not gradient compatible [23] and on-column deposition of matrix components and loss of chromatographic performance after a few sample injections have been reported [23]. The procedure may be advantageous for acidic samples or if nitric acid is used to preserve samples. Such a medium, however, seems not to be optimal for As-species [26], since oxidation of As(III) to As(V) in nitric acid preserved samples has been observed [27,28]. This oxidation was found to be catalysed by metals [29] and also mediated by light [30,31].

Alternatively, phosphate eluents have been applied within a broad pH-range (3–8). Depending on the degree of ionisation ($pK_{a1} = 2.1$, $pK_{a2} = 7.2$, $pK_{a3} = 12.6$), the charge of the phosphate anion varies between one and two. With increasing pH, the phosphate eluent strength increases and counteracts the selectivity gained by deprotonation of As-species. At pH higher than 6, more HPO_4^{2-} is formed, significantly reducing t_R of other arsenicals [32,33]. This problem was recently addressed [34] by combining phosphate (2.5 mM) and SO_4^{2-} (10 mM) at pH 6, where sulphate increased the ionic strength without increasing the pH. Compared to other eluents, phosphate is a good buffer in the neutral range and is able to adjust on-column the pH of the injected sample to the eluent pH.

Phosphate in the plasma produces polymeric depositions on MS cones [22] and damage of expensive cones was reported [35]. Therefore, the phosphate burden to the instrument should be as low as possible, restricting its applicability drastically and precluding it from long term usage and gradient application.

Carbonate and hydroxide, or a mixture of the two, are other commonly used eluents with a pH above 9 which substantially increases the dissociation of protonated As-species and increases their affinity to the anion-exchanger. The advantages of this type of AEX were discussed by Larson [12]. Under oxic basic conditions, oxidation of As(III) to As(V) is fast and was repeatedly reported to occur during chromatography [36–38]. Such a high eluent pH might be suitable for strongly basic samples, eliminating the need to adjust the sample pH.

In trace anion analysis by AEX, the sample matrix has a dominant influence on separation conditions. The higher concentrated matrix components, including preserving agents, can easily distort the separation. They outcompete eluent anions and the eluent buffering capacity, altering the pH in a substantial column segment. This leads to irreproducible t_R and loss of selectivity which hamper reproducibility, identification, detection limits and quantification. For these reasons, the sample matrix including preserving additives should be compatible with the column capacity and separation conditions, particularly the pH-difference between sample and eluent should be as minimal as possible. Sample dilution prior to injection is the most simple and convenient remedy. Many sample matrices, especially digestions, require dilutions by a factor 20–100. However, analyte concentrations and instrument detection limit restrict dilution factors. Clearly, the detector sensitivity is linked to the chromatographic performance, as a more sensitive detector allows for greater sample dilution or larger dilution factors, enabling a more robust separation.

AEX coupled to HR ICP MS was previously used to achieve lowest detection limits in As-speciation. Using non-ICP-compatible phosphate eluents on normal bore columns, the phosphate salt load to the instrument had to be reduced either by an eluent split (1:7.5) [39], or a low amount of phosphate together with a polycarboxylate [40] to maintain the elution strength. In both cases, due to the higher instrument sensitivity, lowest detection limits (DL, 2 ng/L) were obtained detecting in the low resolution (LR) mode. In the high resolution (HR) mode, DL (100–200 ng/L) were comparable to simple quadrupole instruments. Higher DL (50–90 ng/L) were obtained [41] with a sodium carbonate eluent (70 mmol/L) that was split (1:10) and diluted with a make up flow (1:3), detecting in LR with a HR ICP MS. However, carbon in the plasma is known to modulate the As-response [42] in a concentration dependent manner, which varies during chromatography.

The new strategy in As-speciation proposed in this work envisages a highly efficient anion exchange separation and a good compatibility with the sample and the plasma as well. The highest sensitivity gained by HR ICP MS detection is utilised to optimally dilute samples, keeping analytes still above detection limits. Minimising the eluent load to the detector and the sample matrix load to the column, provide a more robust chromatography. This was accomplished by a narrow bore separation system, reducing at least three times the salt load to the plasma compared to normal bore systems. The lower flow rates of the narrow bore equipment are also well compatible to higher efficiency nebulizers for a splitless direct coupling to the detector. The eluent must be well plasma-compatible so that large gradients can be applied, achieving a broad separation window and a good selectivity in the shortest possible run times. Following this strategy, results obtained from two separation columns with different affinities are presented. Investigations were conducted on the influences of possible interferents like chloride, methanol and effects of column coating on As-species integrity.

2. Experimental

2.1. Chemicals and solutions

Eluents was prepared from supra pure conc. HNO_3 and conc. ammonia (Merck).

All commercial chemicals were used without further purification. Sodium arsenite (As(III)), sodium arsenate $Na_2HAsO_4{\cdot}7H_2O$ (As(V)), dimethylarsinic acid sodium salt (DMA), monosodium methane arsonate (MMA) and arsenobetaine (AsB) BCR Reference Material #626 were purchased from Fluka (Buchs, Switzerland).

2.2. Instrumental

An all PEEK narrow bore separation system (DX-500, Dionex) was used as described in [43]. The column effluent was introduced without splitting into a μ-flow PFA50 nebulizer (ESI, Elemental Scientific Instrumentation) mounted on a Peltier-cooled (2–5 °C) Scott-type quartz spay chamber of an Element2 (Thermo Fisher,

Basel), high resolution double focusing sector field ICP MS (HR ICP MS). Arsenic was tracked at 1.33 Hz in low resolution on $m/z = 75$. Plasma conditions were as follows: power forward 1150 W (3 W reflected); argon gas flows: 15.5 L/min cooling, 0.9 L/min plasma and 0.9 L/min sample. The automated batch operation was controlled by the MS software. Injection-loop filling (25 μL) was performed by an auto sampler (ASX-510, CETAC). The chromatography pump was programmed to run for a longer lasting time cycle with self-triggered chromatogram restarts which forced the MS detector to wait for the acquisition start from the pump.

Identical instrumental response was obtained for the two As oxidation states, As(III) and As(V), in 140 mM HNO_3 and in 100 mM NH_4NO_3. Both species showed identical AsO^+-formation rate in pure water (0.3–0.4%) and in 100 mM NH_4NO_3 (0.5–0.6%) at an uranium oxide formation rate of 5.0%. This identical response and stabile AsO^+-formation rate independent of pH, eluent concentration and species oxidation states are prerequisites for gradient elution and As-mass balance measurements. The species integrity during chromatography was controlled by mass balances. A change found in the response ratio during chromatography compared to the non-chromatographed single-analyte solutions was indicative for on-column adsorption or transformations.

2.3. Ion chromatography

Details of the chromatographic setup are published in [43]. Briefly, As-species were separated on a low hydrophobic (AG11/AS11, 50 mm × 2 mm/250 mm × 2 mm, 13 μequiv., Dionex) or on a mixed mode (AG7, 50 mm × 4 mm, 25 μequiv., Dionex) anion exchange column. The capacity values used herein are overall column exchange capacities as given by the manufacturer. Prior to injection, samples were diluted 10–100 times to obtain As-species concentration below 5 μg/L As. Samples not containing enough phosphate (2–5 μmol/L after dilution), were diluted in 2 μmol/L phosphate solution (pH = eluent pH), an amount determined to prevent strong adsorption of As(V) to a coated column. The eluent pH was adjusted to the selected separation pH (ammonia/HNO_3) and kept under He pressure (1 bar). Oxygen depletion prevents As(III) from being oxidized to As(V) at elevated pH. Gradients (flow: 0.3 mL/min) were linearly mixed from two NH_4NO_3-solutions, A (0.5 mM) and B (100 mM), both at the same pH. The mixing proportions for gradient 1 were 100% A from start to 0.2 min, linearly increasing to 30% A and 70% B until 5 min, maintained for 2 min and from 7 to 15 min re-equilibration to the initial concentration. Gradient 2 was programmed as follows: 95% A and 5% B at 0 min, 30% A and 70% B at 4 min throughout 7 min and re-equilibration from 7 to 10.5 min. Chromatograms were exported from MS software in ASCII format and peak area integrals were calculated in Excel. Calibration and sample quantification were based on peak area.

3. Results and discussion

3.1. Separation performance

On normal bore AS11 columns, strongly basic sodium hydroxide eluent, either isocratic [44] or as a gradient [45], have been applied so far. The narrow bore format of this solid phase in combination with a NH_4NO_3-eluent has not been exploited for As-speciation, and this eluent has rarely been utilised despite its several useful advantages. Martinez-Bravo et al. [46] and Watts et al. [47] used two concentration steps of NH_4NO_3 at basic pH (8.7), but on a different column material. Acidic NH_4NO_3 (pH < 3) was used on a cation-exchanger [20] and on a multimode phase [48]. Thus, these authors clearly demonstrated the advantages of this eluent such as its excellent plasma compatibility [43,46] and the ability to vary

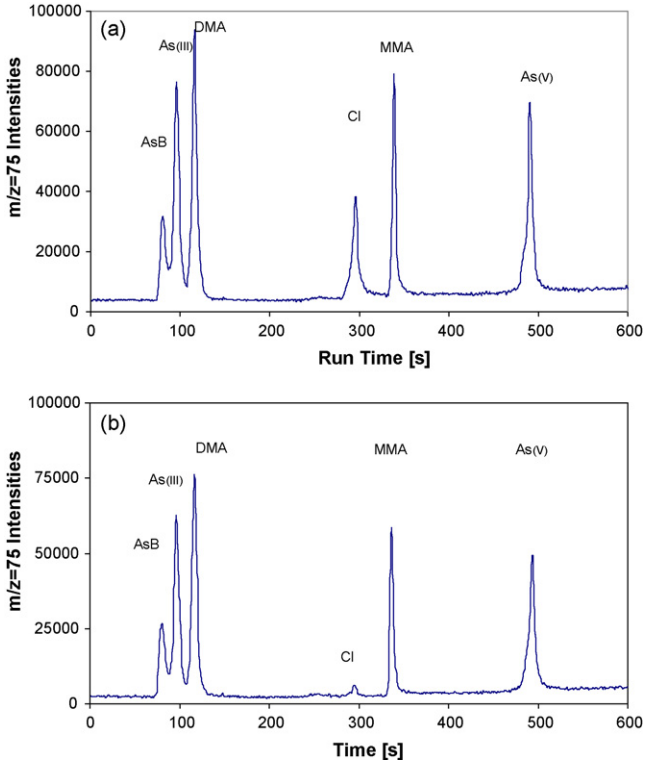

Fig. 1. (a) Separation of standard compounds (1 μg/L each, except AsB 0.4 μg/L) on AS11 by gradient 1. The solution contains 10 mg/L chloride. (b) Same as in (a), except that the eluent contains 2% methanol and the spray chamber is at 20° (switched off).

the separation pH without affecting the eluent strength, making it the ideal choice for gradient elution.

A particular advantage of the AS11 column material is its hydroxylated quaternary ammonium exchange groups located on nano-sized latex beads, which are agglomerated on a nonporous support [18]. The exchanger sites provide a low hydrophobicity around the cationic exchange center and a high affinity for hydrophilic, polarizable anions. This provides short retention times and a good selectivity.

In this work, the narrow bore chromatographic system allowed a splitless direct coupling to the HR ICP MS via a high efficiency nebulizer. The initial eluent concentration affects the separation of species eluting near the front. Running a low initial concentration (0.5 mM NH_4NO_3, gradient 1) separated the two neutral species As(III) (the most toxic) and AsB (non-toxic), as well as the di-methylated DMA. The more anionic species, MMA, Cl^- and As(V), are eluted later at higher eluent concentrations (compare Fig. 1a) all in less than 9 min. Most stable separation conditions were obtained when the pH of the injected solution was identical to the eluent pH, since a low initial eluent concentration is not effective in altering the pH of the injected sample within the column.

In case organic As-compounds are absent, a higher initial concentration and a steeper gradient ramp (gradient 2) can be applied to elute As(V) faster and reduce the chromatogram run time by 3 min.

Contrary to all other eluents used so far, the NH_4NO_3-eluent pH is not determined by a proton dissociation equilibrium that is not compatible with the one of the analyte. Consequently, a separation pH can be chosen according to the As-species deprotonation equilibrium to improve the selectivity [12]. Generally, increasing the eluent pH to the pK_a-value of the As-species increased their negative charge and increased the t_R. Hence, running gradient separations at increasing pH-values prolonged the t_R of de-protonable species, As(III), MMA and As(V). For comparison, the separation

Table 1
Retention and calibration characteristics of arsenic species separated by gradient anion exchange on two different columns and at different eluent pH.

Species	Retention factors k'[a]					t_R RSD[b]	Calibration[b]		Det. limits[b,c]
	AS11		AS7			%	Range[d]	Sensitivity per μg/L As	ng/L
	pH 7.6	pH 8.3	pH 4.6	pH 7.6	pH 8.5		μg/L	$\times 10^6$ counts	
AsB	0.61	0.61	2.25	0.32	0.26	1.0	0–5	1.6	10
As(III)	0.91	1.3	0.34	0.60	0.71	0.7	0–10	1.8	5
DMA	1.3	1.3	0.87	4.1	4.4	0.5	0–10	1.9	5
MMA	5.8	6.0	5.1	5.9	5.7	0.4	0–10	1.8	5
Cl^-	4.9	4.9	9.0	8.3	8.3	–		–	
As(V)	8.8	8.9	6.0	8.2	8.9	0.5	0–10	1.7	5

[a] $k' = (t - t_0)/t_0$.
[b] Results obtained from AS11 column.
[c] DL for 25 μL injections, lower values can be reached by larger injection volumes.
[d] Calibration range for diluted samples measured in the low resolution. Higher resolution settings would lower the sensitivity and increase this range by a factor 10–100.

conditions were applied to the AG7 column containing twice the exchange capacity and an often used, more hydrophobic exchanger material. Retention factors (k'), calculated from t_R which were obtained on the two columns, are presented in Table 1. Dead-volume independent k'-values characterise the retention behaviour and show that AS11 provides similar (MMA) or better (AsB, As(III)) retention than AG7, whereas DMA and Cl^- were more retained on AG7 (compare Fig. 2a). On both columns, a pH-dependent co-elution of two species occurred. On AS11, AsB and As(III) at pH < 7.0 and the couple As(III) and DMA at pH > 8.2 were not sufficiently separated. On AG7, at pH < 8.2, As(V) and Cl^- co-eluted (see Fig. 2b). However, the same gradient at pH 8.6 on this column produced a better shaped DMA-peak (DMA more anionic), and a separation of Cl^- and As(V) was obtained (see Fig. 2b). This demonstrates how separation problems can be solved by choosing an optimal pH, provided the elution strength of the eluent anion is not affected.

The higher affinity of DMA and Cl^- for the AG7-phase is due to its higher hydrophobicity and its higher overall column capacity, respective. The latter attracts better the non-polarizable Cl^- whereas the organic DMA interacts better with the denser hydrophobic sites. The higher affinities of the other more polarizable arsenicals on the AS11 column are due to the lower hydrophobicity of this solid phase, compensating its lower capacity. A possible difference in the local charge density alone cannot explain this different behaviour of polarizable and non-polarizable ions. Because Cl^- is preferentially retained by the higher charge density the latter, therefore, must be located on AG7. Other chromatographic interference could arise from arseno-sugars [47] and thio-arsenicals, but remain to be elucidated by such a gradient on these columns.

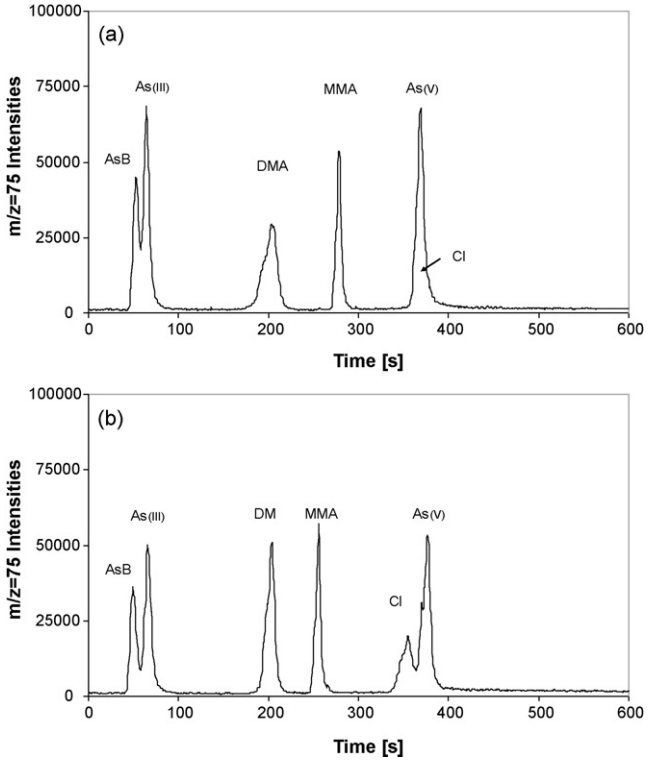

Fig. 2. Separation of As-species on AG7 (same conditions as in Fig. 1a). (a) pH 7.6 and (b) pH 8.2.

The hyphenated system was continuously operated over several days and nights and no signs of deposition on the HR ICP MS were observed. Retention times of diluted samples under the applied gradient regime were highly reproducible within and between batches. In extended kinetic As(III)-oxidation studies [49], two dozens batches comprising of 15–60 diluted (f_{dil} = 20) groundwater samples were analyzed. Typically, t_R varied within ±2 s (RSD 0.5% and 1% for As(v) and As(III) resp.). Matrix dilutions as well as phosphate in the injected sample contributed to the stabile and reproducible gradient separation conditions. Phosphate, eluting in front of As(V) but separated from As(V), did not affect the retention time of As-species, as it was also observed by Manning and Martens [44]. Detection limits for 25 μL loop injections of As(III) and As(V) (50 ng/L As each) were calculated (3σ) to be 16 ng/L for a high background (4000 cps) from a coated column with a high noise (600 cps) and DL = 5 ng/L for a freshly washed column with a lower background (300 cps) and lower noise (150 cps). This allowed for a raw sample containing only 1 μg/L As per species to be diluted 50–100 times before injection, drastically reducing matrix influences on the separation. It is worth noting that no efforts have been undertaken to reach lowest detection limits which can be easily lowered further by larger loop injections.

3.2. Method validations

3.2.1. Ar-plasma based interference

Chloride forms $^{40}Ar^{35}Cl^+$ in the plasma, so Cl^- moving through the column generates a peak on mass m/z = 75. Detection in the HR-mode is required to separate the two masses. However, detecting in low resolution provides the highest sensitivity and, therefore the chromatographic behaviour of Cl^- had to be elucidated. Diluted samples containing less than 5 mg/L Cl^- did not show a $^{40}Ar^{35}Cl^+$ peak. Higher concentrations produced a peak which did not interfere with As-species (compare Fig. 1a) and gave a 20,000 times lower response than arsenic in this organic carbon free chromatography (e.g. a 20 mg/L Cl-solution produced a peak integrating to a 1 μg/L As).

Heavy interference problems were observed for HCl preserved samples, which were insufficiently neutralized prior to injection. They produced acidic conditions on the column that accumulated HCl and increasingly released it from the stationary phase. Over several injections, the background steadily increased up to 100-fold.

Several effects of organic carbon have been described. Organic solvents have often been applied [40,41,50] since they can increase the nebulizer efficiency and enhance the degree of As-ionisation in the plasma [42]. The carbon load from organic solvent was also found to efficiently reduce $ArCl^+$ by the competing formation of ArC^+ and thus, minimize the interference from $ArCl^+$. Adverse effects from low amounts of methanol (MeOH) have been reported to cause variations in the response from As-species [25] and unpredictable effects in the spray chamber such that MeOH was not recommended in a gradient [12]. As sensitivity is not an issue detecting in low resolution with a HR ICP MS, MeOH-free conditions were preferred. However, a constant amount of MeOH (2% in eluent A and B, no spray chamber cooling) efficiently suppressed the formation of $ArCl^+$. A reduction by a factor of 13 was observed, whereas As-peaks were only reduced by 15–30% (see Fig. 1b). A cooled spray chamber partially removes MeOH so that 8% MeOH in the eluent was required to reduce the $ArCl^+$-signal by the same factor. This can be used to subtract the Cl-signal in case Cl^- is coeluting with the As-species as described above.

Other possible interferents like $^{59}Co^{16}O^+$, ^{74}GeH, $^{150}Sm^{2+}$ and $^{150}Nd^{2+}$ have been considered as irrelevant in these samples and were not measured.

3.2.2. *Influence of column coating on As(V)-integrity*

Within a batch of samples not containing phosphate, it was observed that the As(V) peak height from matrix-free standard solutions decreased by 60–70% compared to As(III). The decrease was linearly correlated with the number of previously injected groundwater samples (data not shown). Freshly prepared and immediately injected As(V) standards showed the same decreased response as the last standard within the batch. According to published findings [7,51], reduction of As(V) in purified water was found to occur within days, but not hours. Therefore, the column was suspected to cause the effect. Injecting directly a As(V) standard solution onto the separator column without the guard column connected in-line, a much higher As(V)-response was observed. However, this was not the case injecting onto the guard column immediately after the manufacture's column cleaning procedure was applied. Hence, the effect occurred within a zone on the column top, where most likely sample matrix components were deposited on the guard column upon the first contact. Because phosphate was shown to be effective in preventing As(III/V)-conversion [52], PO_4-additions to samples prior to injection were tested. Expecting PO_4 to mask adsorption sites, experiments were performed on a coated column to elucidate the fate of As(V). Injections of pure As(V) solutions containing no PO_4 produced strongly broaden peaks at the retention time of As(III) and As(V), and the sum of peak areas was reduced to 30–60% of that obtained from PO_4-containing solution. Missing As(V) was remobilized from the column by a immediately injected pure PO_4-solution. These findings are explained by adsorption of As(V) and subsequent reduction during the initial period of the gradient when As(V) is not, or only slowly, migrating through a reductively active zone on the column. Adsorbed and not jet reduced As(V) was remobilized by PO_4. The reduction was further evidenced by stop-flow experiments. After a pure As(V)-solution reached the column top, the pump was stopped. Different stop durations retained As(V) for different residence times in the coated zone at the column top. The amount of As(V) reduced to As(III) increased with longer residence time in the guard column (see Fig. 3), and less peak broadening occurred. No As(V) was remobilized by PO_4 when all As(V) was completely reduced to As(III). However, if the injected solutions contained PO_4, the reduction of As(V) (1 μg/L) was efficiently slowed down in stop-flow experiments. For example, after six minutes residence time, only 15% As(III) was formed compared to 100% As(III) after three minutes without PO_4 (see Fig. 3). Evidently, an appropriate PO_4-excess provides sufficient protection during the gradient run. This role of PO_4 parallels the huge effects of PO_4 on As(V)-desorption reported for sediments [44], soil [53] and for the accuracy of peak area integrals [44]. Dozens of samples within a batch and several subsequent batches can be analyzed without column cleaning, so lengthy column wash and re-equilibration procedures can be circumvented. The PO_4-enrichment recommended is fully compatible with sample preservation based on optimized phosphoric acid addition [52], and has no adverse effect to the MS since the PO_4-load to the plasma occurs only intermittently at more than 10,000 times lower total amount.

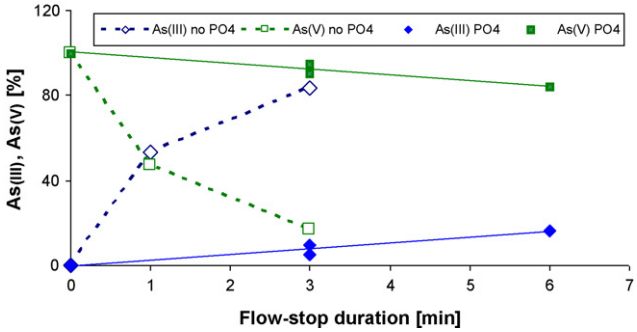

Fig. 3. Percentage of As(III) and As(V) detected after injection of a pure As(V)-solution staying during different contact times on a column top that is coated by sample matrix components. Calculated in % of the unaffected As(V)-peak area.

4. Conclusions

So far, higher capacity columns (>0.1 mequiv.) and eluents with a pH fixed to a non-favourable proton dissociation equilibrium have almost exclusively been applied in As-speciation by AEX. Correspondingly, these separations must be performed under either strongly acidic (HNO_3) or basic (OH^-, CO_3^{2-}) conditions and are prone to species transformations. Around natural pH, PO_4 eluents can be applied, however, they have severe limitations with respect to plasma compatibility and pH flexibility.

Alternatively, the strategy proposed here is based on sample dilution, the adaption of the eluent pH to the sample pH or to any pH providing an optimal selectivity in combination with a lower capacity column but with comparable or higher affinity to As-species, due to less-hydrophobic exchange sites.

The unprecedented plasma compatibility of the NH_4NO_3-eluent allows drastic concentration changes with minimal ion suppression in ICP MS detection, enabling robust gradient applications which can separate more species in a shorter time. As the pH does not influence the eluent strength, an eluent pH can freely be chosen according to selectivity optimization requirements. Selecting the pH around the ionisation of the analyte allows to adjust and to control the retention time. This largely increases the method's flexibility, as the separation pH can be used as an independent parameter to enhance selectivity [54].

While sample dilution minimizes the matrix load per injection, matrix components can be deposited on the column and accumulate during numerous injections of diluted samples. High sample dilutions resulted in a low absolute amount of As (2–50 pg) injected which was susceptible to adsorption and reduction of As(V) caused by partial column coatings. This is another example of a matrix which can be a relevant source of As-speciation inaccuracy [55]

on a separation column. The results demonstrate that the instability of As-species in the matrix itself is not the only factor that must be accounted for, a fact that has not yet been fully recognized [56]. Different and extended column purifications were no solution to this problem. It is very likely that the problem occurs with other matrices and columns and is, therefore, of general relevance. However, such artifacts are easily recognized by simple mass balance measurements. Adsorption sites were efficiently masked in the presence of phosphate, a close analogue to As(V), which also prevented it from on-column reduction during the run. Depending on dilution factors, matrix concentrations and number of injections, the analyst has to decide whether to clean a column more frequently or to increase the PO_4 concentration in the injected samples.

The strategy in As-speciation presented here works with sufficiently diluted samples, which might require the sensitivity of an MS but, depending on sample concentrations, is not restricted to a HR ICP MS. The method's merits for As-speciation are also beneficial to any MS detector. While the application of the method demonstrated useful advantages improving reliability and robustness in As-speciation, the separation performance with arseno-sugars and thio-arsenicals remains to be elucidated.

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