
Application of a central composite design to the determination of inorganic and organic arsenic species in water by liquid chromatography–hydride generation–atomic absorption spectrometry

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Aplicación de un diseño central compuesto para la determinación de especies de arsénico orgánicas e inorgánicas en agua por cromatografía líquida - generación de hidruros -espectrometría de absorción atómica

Aplicació del disseny compost central a la determinació d'espècies inorgàniques i orgàniques d'arsènic en aigua per cromatografia líquida-espectrometria d'absorció atòmica amb generació d'hidrurs

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RESUMEN

Un sistema acoplado en línea de cromatografía líquida de alta resolución – generación de hidruros y espectrometría de absorción atómica fue empleado para la determinación de [As (III)], [As (V)], ácido monometilarsenioso (MMA) and ácido dimetilarsénico (DMA) en muestras de agua. Se consideraron cuatro variables para un diseño central compuesto 2^{4+} que se utilizó en la optimización de las condiciones analíticas necesarias para desarrollar el método de especiación de arsénico. Las variables investigadas fueron (A) velocidad de flujo de fase móvil, (B) concentración de HCl, (C) concentración de KI, (D) concentración de NaBH_4 . Los factores individuales (A) y (B) fueron los efectos más significativos, siendo las condiciones óptimas para todos los factores $A=1.2 \text{ mL min}^{-1}$, $B=2.8 \text{ mol L}^{-1}$, $C=1.7\% \text{ w/v}$, and $D=1.4\% \text{ w/v}$. La repetitividad, estabilidad, curva de calibración, límites de detección y cuantificación fueron establecidos. El método fue aplicado a diferentes tipos de aguas, se obtuvieron buenas recuperaciones de las muestras adicionadas (92 – 109%) para todos los analitos.

Palabras claves: Especiación de arsénico, diseño central compuesto, cromatografía líquida de alta resolución, generación de hidruros. Espectrometría de absorción atómica, análisis de agua.

ABSTRACT

An on-line high performance liquid chromatography - hydride generation atomic absorption spectrometry coupled system (HPLC–HG–AAS) has been used for the determination of [As (III)], [As (V)], monomethylarsenic acid (MMA) and dimethylarsinic acid (DMA) in water samples. Four variables were taken into account and a central composite

design 2^{4+} was used in the optimization of analytical conditions for the arsenic speciation method. The variables investigated were (A) mobile phase flow rate, (B) HCl concentration, (C) KI concentration, (D) NaBH_4 concentration. Individual factors (A) and (B) were the most significant effects, and the optimum conditions for all factors were $A = 1.2 \text{ mL min}^{-1}$, $B = 2.8 \text{ mol L}^{-1}$, $C = 1.7\% \text{ w/v}$, and $D = 1.4\% \text{ w/v}$. The repeatability, stability, calibration curve and limit of detection were established. The method was applied to different kinds of waters; good recoveries of added spikes (92 – 109 %) were obtained for all analytes.

Keywords: Arsenic speciation; central composite design; high performance liquid chromatography; hydride generation; atomic absorption spectrometry; water analysis.

RESUM

S'empra un sistema acoblat de cromatografia líquida d'alta eficàcia – espectrometria d'absorció atòmica amb generació d'hidrurs (HPLC–HGAAS) per a la determinació de [As(III)], [As(V)], àcid monometilarsònic (MMA) i àcid dimetilarsínic (DMA) en mostres d'aigua. Es consideren quatre variables i s'utilitza un disseny compost central 2^{4+} en la optimització de les condicions analítiques del mètode d'especiació d'arsènic. Les variables investigades són: (A) el cabal del flux de la fase mòbil, (B) la concentració de HCl, (C) la concentració de KI, i (D) la concentració de NaBH_4 . Els factors individuals (A) i (B) són els que produeixen efectes més significatius, essent les condicions òptimes per a tots els factors: $A=1,2 \text{ mL min}^{-1}$, $B=2,8 \text{ mol L}^{-1}$, $C=1,7\% \text{ p/v}$, i $D=1,4\% \text{ p/v}$. S'estableix la repetibilitat,

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l'estabilitat, la corba de calibratge i el límit de detecció. El mètode s'aplica a diferents tipus d'aigües; s'obtenen bones recuperacions de les sembres afegides (92 – 109%) per a tots els analits.

Mots clau: especiació d'arsènic, disseny compost central, cromatografia líquida d'alta eficàcia, generació d'hidrurs, espectrometria d'absorció atòmica, anàlisi d'aigües.

INTRODUCTION

The concentration of total dissolved arsenic in natural water depends on the geological and geochemical composition of natural drainage areas¹. Many geothermal waters and some lakes present relatively high levels of the element^{2,3}. Arsenic distribution is also affected by anthropogenic activities, such as mining and smelting operations, coal-fired combustion, and use of agricultural herbicides, pesticides, and medicinal and cosmetic products⁴.

Chronic ingestion of high levels of arsenic (several hundreds $\mu\text{g L}^{-1}$) in drinking water has been associated with an increased risk of developing skin, bladder, kidneys and lung cancer as well as having other non-cancerous effects⁵⁻⁷. Arsenic has been classified as Group 1 carcinogen in human beings.^{8,9}

It is well known that the toxicity of arsenic is highly dependent on its chemical form. In fact, As (III) is more toxic than As (V) and methylated compounds that contain trivalent arsenic are more cytotoxic and genotoxic than arsenite. Pentavalent arsenicals are significantly less cytotoxic than their trivalent analog.^{8,10-12}

Speciation of arsenic in environmental systems continues to be an important preoccupation, mainly because the toxicity and bioavailability of this element is species dependent. The most abundant arsenic species in groundwater are the inorganic species As (III) and As (V).¹ Through biological activity, the inorganic arsenic present in the water may be partly detoxified and transformed into the organo-arsenic compounds monomethylarsonic acid and dimethylarsinic acid.⁸

In natural water, the concentrations of these two methylated species are found at ultra-trace levels, and in many cases are undetectable.^{13,14} Therefore, the determination of total arsenic content in a sample does not reflect the level of hazard of the element actually present; speciation studies are of critical importance because they provide a better view of the risk associated with exposure to arsenic in the environment.

Low levels of arsenic species in the environment required the use of highly sensitive techniques of analysis. Numerous analytical methods for the speciation of arsenic have been reported, all are based on hyphenated techniques that provide good resolution in separation and powerful detection limits¹. The vast majority of arsenic studies use HPLC coupled to the element selective detectors such as inductively coupled plasma-mass spectrometry (ICP-MS)¹⁵⁻¹⁹, hydride generation atomic absorption (HG-AAS)¹⁹⁻²⁷, or atomic fluorescence spectrometry (HG-AFS).^{24,28} The combination of high performance liquid chromatography^{4,13,23,27} and hydride generation atomic absorption spectrometry is one of the most common techniques used for the speciation of these four arsenic species in environmental samples for their simplicity, sensitivity, precision, speed and low cost.^{1,19,22-24,29,30}

The optimization of the different parameters commonly studied is carried out considering each one of the factors separately using a "one-factor-at-a-time" technique. This approach unfortunately does not pin-point the region of optimum response, since it fails to reveal any interactions that may occur among the factors during the process of separation. An improved scheme is to consider a matrix of all combinations of the operating variables as it would have the advantage of thoroughly exploring the experimental surface. Unfortunately, such an approach would require a large number of experimental measurements. As it is already known, experimental design is the most powerful way to make efficient experiments as we get the information we need with the minimum effort. The response surface method (RSM) is superior as it is able to simultaneously consider several factors at different levels, and may reveal corresponding interactions among these factors; all these using a smaller number of experimental observations.

The central composite design³¹ was used in the optimization of analytical conditions for the arsenic speciation method. The use of a method such as central composite design (CCD) can reduce the number of experiments or provide much more information about the effect of the different variables. The RSM was adopted to locate the optimum levels of the four factors: mobile phase flow rate, HCl concentration, pre-reducing agent concentration (KI) and reducing agent concentration (NaBH_4). Experimental data were then processed by using a multiple regression analysis in order to calculate the mathematical model that represents the relationship between factors and responses and to determine the best experimental conditions for arsenic speciation method in waters samples.

MATERIAL AND METHODS

Reagents

Deionized distilled water (DDW) was used for the preparation of reagents and standards solutions. All chemicals, including standards and solutions, were of pro-analysis quality or better.

The stock standard solutions of As III ($1000 \mu\text{g mL}^{-1}$) were prepared by dilution in water of a Fixanal (Riedel-de Haën, Seelze, Germany). Stock solutions of arsenate, MMA and DMA ($1000 \mu\text{g mL}^{-1}$) were prepared by dissolving appropriate amounts of $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ (Sigma, Australia) of $\text{CH}_3\text{AsO}_3\text{Na}_2 \cdot 6\text{H}_2\text{O}$ (Chem Service, West Chester, PA, USA) and $(\text{CH}_3)_2\text{AsO}(\text{OH})$ (Fluka Chemie, Buch, Switzerland) in water, respectively.

Calibration curves were obtained by adding 0.000, 0.025, 0.050, 0.100, 0.250, 0.500 and $1.000 \mu\text{g mL}^{-1}$ of each arsenic species to the water sample.

Sodium borohydride (Merck, Darmstadt, Germany) was prepared daily by dissolving NaBH_4 powder in 0.5% w/v NaOH (Merck, Darmstadt, Germany), and filtered with Whatman N° 42 paper. A pre-reducing solution for hydride generation KI (Merck, Darmstadt, Germany) was employed. To acidify HCl (Merck, Darmstadt, Germany) was used. The concentrations ranges of reagents tested are summarized in table 1.

The buffered mobile phase was prepared by mixing aqueous solution of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (J.T.Baker, Phillipsburg, NJ, USA) with an appropriate volume of aqueous solution of Na_2HPO_4 (Merck, Darmstadt, Germany). The resulting solutions (10 to

25 mmol L⁻¹, pH 4 to 8) was filtered through a Millipore 0.45 µm membrane filter and degassed before use. All glassware was soaked in 30% v/v HNO₃ for 24 h, and then rinsed three times with deionized water before being used.

Instrumentation

High performance liquid chromatography with hydride generation atomic spectrometry detection (HPLC-HG-AAS) system is shown in figure 1.

HPLC system

The HPLC separation system consisted of a Gilson Model 307 pump (Gilson, Inc., Middleton, USA), vacuum degasser system (Model DG-4400, Phenomenex, Torrance, CA, USA) and a Rheodyne Model 7125 six-port sample injector valve (Cotati, California, USA) fitted to a 200 µL loop (Cotati, CA, USA). Separation of the four arsenic species was carried out on an anion-exchange column Nucleosil SB 100 A (250 mm x 4.6 mm stainless steel, 10 µm particle diameter) from Phenomenex (Torrance, CA, USA). A pre-column containing the same stationary phase was attached to the analytical column.

HG system

Gaseous arsenic hydride generation was obtained by continuous pumping of sample solution, acid solution and reducing agent through a reaction coil and into a gas-liquid separator. The gas-liquid separator employed was made of borosilicate glass tube (height 70 mm, i.d. 14 mm, o.d. 16 mm). All solutions were pumped using a Gilson Model Minipuls 3 (Villiers Le Bel, France) peristaltic pump and Tygon tubing "red/red" (0.8 mm i.d., flow rate of 1.5 mL min⁻¹ for all reagents). The mixing reaction coil (PTFE tubing 3 mm d.i., 75 cm length) was inserted between the mixing point and the gas-liquid separator in order to increase the contact time. The released hydrides were supported by the carrier gas flow (Argon, flow rate of 100 mL min⁻¹) to the atomization cell (Varian, Inc. Palo Alto, CA, USA), heated by an acetylene-air flame (around 900° C) for the determination by atomic absorption spectrometry.

AAS detection

A Model 4200 (Metrolab, Monterrey, N.L. México) atomic absorption spectrometer equipped with microcomputer, background correction incorporated and arsenic hollow-cathode lamp (Photron Pty. Lda, Victoria, Australia) operated at 8 mA, was used. A spectral bandwidth of 1.5 nm was selected to isolate the 193.7 nm wavelength. The spectrometer signal was acquired by an analogue-to-digital converter, using the chromatographic software RIAC-PROCESAR V 2.0 (U.N.L., Santa Fe, Argentina).

Experimental design

The CCD for this study consisted of a 2⁴ = 16 (i.e. four factors) full factorial design augmented by 2 × 4 = 8 axial

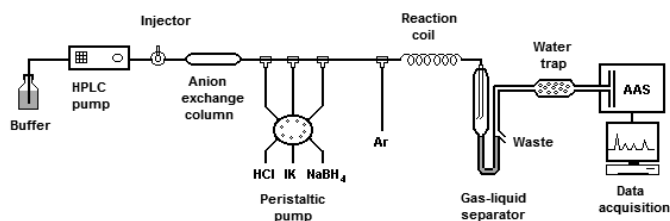


Fig. 1: Schematic diagram of high performance liquid chromatography with hydride generation atomic spectrometry detection (HPLC-HG-AAS) system.

points at ($\pm\alpha, 0, 0, \dots, 0$), ($0, 0, \pm\alpha, \dots, 0$), ($0, \dots, 0, 0, \pm\alpha$) and 7 center points at ($0, 0, 0, \dots, 0$), where α is the distance of the axial point to the center. Random error (standard deviation) can be estimated from the seven center points. The choice of α establishes the rotatability of a central point composite design. Since the distance from the axial point to the center point is given by $\alpha = 2^{n/4}$ (for 4 factors $n = 4$, $\alpha = 2$) α is usually set at 2.0 for a four-factor design.^{31, 32} Therefore, a total of 31 (16 full factorial tests + 8 axial points + 7 center points) batch run tests were performed. The parameter levels were codified in order to set the 31 runs and are presented in table 2.

In this CCD, the influence on the response of the four parameters, buffer flow rate (X_1), HCl concentration (X_2), KI concentration (X_3) and NaBH₄ concentration (X_4), was investigated. Each variable assumed five levels in coded levels ($-\alpha, -1, 0, 1, \alpha$), and $\alpha = 2.00$ was chosen with sevenfold repetition of the center point. Table 1 provides the detail of how each parameter was established for the present study. Model parameters were estimated using a second-order model of the form:

$$E(Y) = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \sum_{j=1}^k \beta_{ij} X_i X_j \quad \text{Eq. (1)}$$

In Eq. (1): $E(Y)$ is the expected value of the response variable, $\beta_0, \beta_i, \beta_{ij}$ are the model parameters, X_i and X_j are the coded factors being studied, and k is the number of factors being studied. $E(Y)$ represents the response related to the concentration in the empirical model.

Data Analysis

An analysis of the residual from the model was calculated in order to determine the adequacy of the least-squares fit. Both the normal probability of the residuals as well as the residuals versus predicted values of the response variable were used to assess the least-squares fit. An analysis of variance (ANOVA) test was used in order to identify the effect of individual factors and their second order interactions.³³ The analysis of variance approach was used to partition the total sum of squares into a portion due to regression and a portion due to error.

Table 1: Quantitative values of the coded parameter levels

Coded factor	Parameter	-2	-1	0	1	2
X ₁ (A)	Mobile phase flow rate (mL min ⁻¹)	0.5	0.8	1.1	1.4	1.7
X ₂ (B)	HCl concentration (mol L ⁻¹)	0.3	1.2	2.1	3.0	3.9
X ₃ (C)	KI concentration (% w/v)	0.1	0.9	1.7	2.5	3.3
X ₄ (D)	NaBH ₄ concentration (% w/v)	0.2	0.8	1.4	2.0	2.6

Table 2: Parameters levels of central composite design (coded value)

Run order	Mobile phase	HCl	KI	NaBH ₄
	flow rate	concentration	concentration	concentration
1	1	-1	1	-1
2	1	-1	-1	1
3	0	0	0	0
4	1	1	1	-1
5	-1	1	-1	1
6	0	0	0	0
7	0	2	0	0
8	-1	1	1	-1
9	-1	0	-2	0
10	-2	0	0	0
11	0	0	0	2
12	0	0	0	0
13	0	0	2	0
14	0	0	0	0
15	-1	-1	-1	1
16	-1	-1	1	1
17	0	0	0	0
18	-1	1	1	1
19	1	-1	1	1
20	0	0	0	0
21	1	-1	-1	-1
22	0	0	0	-2
23	-1	-1	-1	-1
24	-1	-1	1	-1
25	0	-2	0	0
26	-1	1	-1	-1
27	1	1	-1	1
28	1	1	1	1
29	2	0	0	0
30	0	0	0	0
31	1	1	-1	-1

Software for experimental design, statistical analysis, and optimization

Computer program STATGRAPHICS 5.1 (Statistical Graphic Corporation, Manugistics Inc., Rockville, USA) was used in the statistical analysis of the data and the development of the regression equations in the central composite design. A generalized algebraic modeling system was used to optimize the second-order statistical empirical models. A 5 % significant level is used for the statistical model.

RESULTS AND DISCUSSION

Optimization of hydride generation system

When a continuous hydride generator based on NaBH₄ reaction was employed on-line with a post column derivatization reactor, several operational variables may be affecting the observed AAS response. Factors considered for the optimization of hydride generation process were the acid, pre-reducing and reducing agent concentrations. In this case, each interval tested was chosen from those described in the bibliography: HCl concentration (1.2 – 3 mol L⁻¹)^{4, 23, 25, 27, 34}, NaBH₄ concentration (0.8 – 2 % w/v in 0.5 % w/v NaOH)^{4, 23, 27, 34} and KI concentration (0.9 – 2.5 % w/v)^{35, 36}. The sample tested for each parameter was evaluated for each arsenic species and it was formulated as follows: 0.5 µg mL⁻¹ As (III) + 0.5 µg mL⁻¹ As (V) + 1 µg mL⁻¹ MMA + 1 µg mL⁻¹ DMA.

In the first experiments other parameters were evaluated. The carrier gas flow rate (Ar) was assessed within the range 60 – 130 mL min⁻¹. It was found that at a higher flow rate the hydride residence time in the atomizer was low, resulted in signal instability. Nevertheless, when the flow rate was too low the residence time increased.²⁴ The best result was obtained at 100 mL min⁻¹ argon flow rate.³⁷ The atomization temperature was one of the main factors in the determination of arsenic by HG. Optimum atomization was obtained above 900° C.

The efficiency of arsenic hydride generation from arsenic species was evaluated using reaction coil of different lengths (25, 50, 75 and 100 cm). The analytical signal was markedly affected by the coil length. Results show that a reaction coil of 75 cm is necessary to obtain complete volatilization of arsenic species.

Reagents and standard flow rate varied in the ranges 0.50 – 2.5 mL min⁻¹. The best result was obtained at flow rate of 1.5 mL min⁻¹.

Anionic chromatographic separation conditions

Aside from resolution and retention times, which are strictly dependent on the chromatographic separation characteristics, peak height or integrated peak area signals may undergo variations related to the reaction kinetics and the phase separator design. Due to different pK_a values of arsenicals, several retention times are expected, depending upon the pH of the medium. In water, As (V) and the methylated species are partially ionized, while As (III) is mainly in neutral form.⁴ To optimize the chromatographic As (III), MMA, DMA and As (V) separation in the isocratic mode, the use of a phosphate buffer in a pH range from 4 to 8, and phosphate concentration from 10 to 25 mmol L⁻¹ was studied.^{13, 15, 23, 26, 27, 38} Buffer salts based on phosphoric acid are convenient, since phosphoric acid is a three-protic acid resulting in a good buffer capacity over a wide pH range.²²

Better peak separation was achieved by working at a lower phosphate concentration than the optimum proposed for the column, but since As (V) has the longest retention time, significant peak broadening was obtained.²⁷ To buffer concentrations major than the chosen one (17 mmol L⁻¹ pH 7) the opposite effect happens, the times of retention for As (V) and for both organic decreased, provoking an overlapping of chromatographic peaks.

In figure 2 it can be shown how, in a short time (13.30 minutes) with a good resolution, As (III), DMA, MMA and As (V) were resolved on anion-exchange column, with phosphate

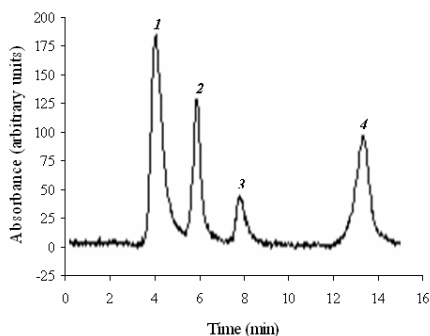


Fig. 2: Typical chromatogram obtained from the HPLC–HG–AAS analysis of a standard solution containing 1: As (III) 0.05 $\mu\text{g mL}^{-1}$; 2: DMA 1 $\mu\text{g mL}^{-1}$; 3: MMA 1 $\mu\text{g mL}^{-1}$; 4: As (V) 0.05 $\mu\text{g mL}^{-1}$.

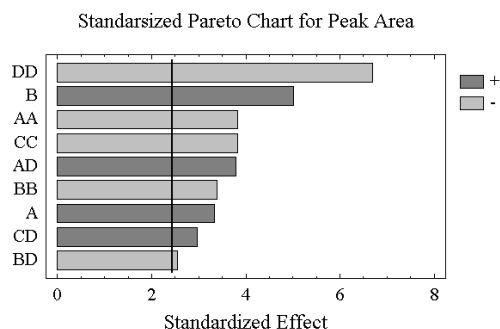


Fig. 3: Standardized Pareto chart for peak area generated for CCD. Each of the estimated effects appear in decreasing order of magnitude were **A:** mobile phase flow rate, **B:** HCl concentration, **C:** KI concentration and **D:** NaBH_4 concentration.

buffer pH 7.00 and 17 mmol L^{-1} concentration. These results remarked the right selection of the run parameter.

Optimization of arsenic speciation: central composite design

An analysis of variance (ANOVA) test was used in order to identify the effect of individual factors and their second-order interactions. The ANOVA partitions the variability in response into separate pieces for each of the effects. Table 3 shows the ANOVA test results, where the statistical significance of each effect is given by comparing the mean square against an estimate of the experimental error. In this case, 9 effects have p-values less than 0.05, indicating that they are significantly different from zero at the 95 % confidence level.

The lack of fit test is performed by comparing the variability of the current model residuals to the variability between observations at replicate settings of factors. Since the p-value for lack-of-fit in the ANOVA test is 0.08, greater than 0.05, the model appears to be adequate for the observed data at the 95 % confidence level.

The R-square statistic indicates that the model as fitted explains 72 % of the variability in the response. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 60 %. The standard error of the estimate shows the standard deviation of the residuals to be 1.2E 07. The mean absolute error of 1.3E 07 is the average value of the residuals. The models were statistically validated using the residual test. The Durbin-Watson statistic tests the residuals to determine if there is any significant correlation based on the order in which they occur in data file. Since the Durbin-Watson value is 2.4, major than 1.4, there is probably not any serious autocorrelation in the residuals.

Data from the 31-batch run were analyzed using the Statgraphics 5.1 software, and the following equation of the fitted model was obtained:

$$\text{Peak area} = -119265.0 + 148412.0 A + 72691.1 B + 19590.0 C + 45272.3 D - 95285.1 A^2 + 63069.8 AD - 9371.49 B^2 - 14103.4 BD - 13390.3 C^2 + 18526.4 CD - 41616.8 D^2$$

Eq. (2)

Eq. (2) shows that the additive combination (+, +, +, +) of the four factors: mobile phase flow rate (A), HCl concentration (B), KI concentration (C), and NaBH_4 concentration (D)

exert an important impact on separation of inorganic and organic arsenic species. The positive coefficients for the four parameters indicate that optimal conditions of separation results from the highest buffer flow rate (A), the highest acid concentration (B), the highest pre-reducing concentration (C), and the highest reducing agent concentration (D). The empirical model also shows interactions between AD, BD and CD. The model also revealed a significant interaction between the mobile phase flow rate and NaBH_4 concentration.

The Pareto chart (Fig. 3), show that the most important effects are the effects of the individual factors of (A) mobile phase flow rate and (B) HCl concentration (p level < 0.05). When the speed flow of mobile phase is too high, it leads to the overlapping of the chromatographic peaks.

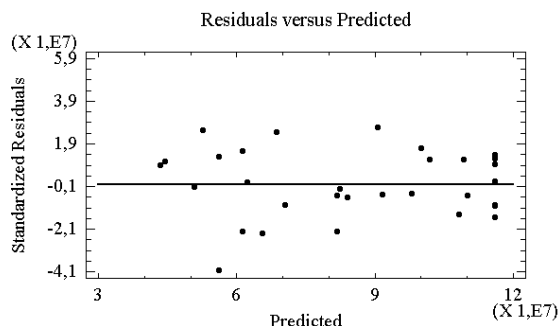
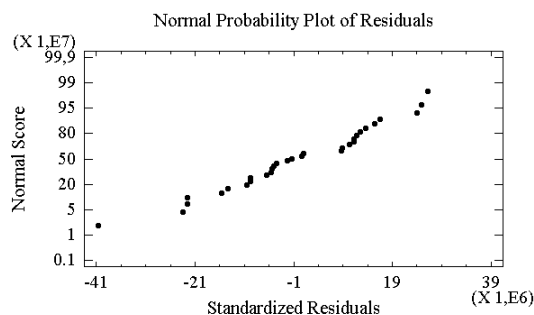


Fig. 4: (a) Normal probability plot of residuals for optimization of separation of four arsenic species. (b) Residuals plot for optimization of separation of arsenic species.

Table 3: Analysis of variance for peak areas in the optimization of speciation of As (III), MMA, DMA and As (V) species

Source	F-Ratio	p-Value
A	11.07	0.0159
B	25.08	0.0024
AA	14.70	0.0086
AD	14.41	0.0090
BB	11.52	0.0146
BD	6.49	0.0437
CC	14.68	0.0086
CD	8.84	0.0248
DD	44.86	0.0005
Lack-of-fit	3.10	0.0850

However, low speed flows of buffer lead to the widening of the peaks decreasing the quality of the resolution of the chromatograms.

The acid concentration had a great influence on the efficiency of hydride generation and therefore on the sensitivity of the analytical method.^{4, 21, 25, 34} The analytical signal for four arsenic species showed an increase in peak area when the HCl concentration is increased.

The most significant effects of the interactions of second order are *AD* (mobile phase flow rate and NaBH₄ concentration), *BD* (HCl and NaBH₄ concentrations) and *CD* (KI and NaBH₄ concentrations). In all of them, the concentration of NaBH₄ plays a determinant role for the complete reduction of different forms of arsenic.

The NaBH₄ concentration is an important parameter for arsine generation because this is formed in the presence of hydrogen generated by NaBH₄ in an acidic medium. Results show that the absorbance, and consequently the peak area, increases with increase in NaBH₄ and HCl concentrations for both inorganic and organic arsenic forms determination. This can be understood considering the higher NaBH₄ concentration, the higher amount of H₂ generated, and consequently the analytical signal increases.³⁴ However, this effect there is not unlimited, because higher concentrations produce foam which results in signal fluctuations.

Fig. 4 a exhibit the normal probability plot for the empirical model for the peak area obtained. The data shows a straight line in the plot of the residuals, which represents a normal distribution and thus supports the adequacy of the least-squares fit. The residual plot (Fig. 4b) which show equal scatter of the residual data above and below the x-axis indicates that the variance was independent of the values of the dependent variable change, again supporting the adequacy of the least-squares fit.

Table 4: Optimum operating parameters estimated generated for CCD for the determination of As (III), MMA, DMA and As (V) in water by HPLC-HG-AAS

Factor	Units	Low	High	Optimum
Mobile phase flow rate (A)	mL min ⁻¹	0.5	1.7	1.2
HCl concentration (B)	mol L ⁻¹	0.3	3.9	2.8
KI concentration (C)	% w/v	0.1	3.3	1.7
NaBH ₄ concentration (D)	% w/v	0.2	2.6	1.4

Surface Response Plots of Peak Area

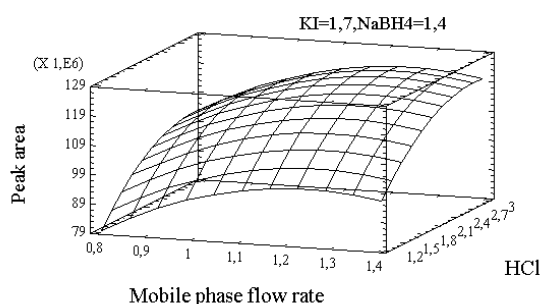


Fig. 5: Surface response plot of the empirical model for optimization of separation of inorganic and organic arsenic species.

Fig. 5 shows the estimated response as a function of two more significant individual factors, mobile phase flow rate and HCl concentration. The height of the surface represents the value of peak area. The other factors are held constant. In this figure the data provided a suitable fit for a first-order regression model, indicating the direction in which the optimum should be sought.

The optimum operating parameters estimated for the determination of inorganic and organic arsenic species in water by liquid chromatography-hydride generation-atomic absorption spectrometry is given in table 4.

Analytical characteristics of the method

Table 5 shows the analytical performance of the presented methods. Using the optimized parameters, calibration curves were carried out by varying the amount of each arsenic species (0.025 to 1.00 µg mL⁻¹) injected on the column and measuring the peak area obtained, and were found linear between 0.025 – 1.00 µg mL⁻¹ for As (III) and between 0.050 – 1.00 µg mL⁻¹ for As (V), MMA and DMA. Higher calibrate concentrations were generally not required for water analyses, and consequently were not tested. The limits of detection (LODs) were calculated by dividing three times the standard deviation of the absorbance area of ten independent reagent blank readings at the time of retention of each arsenical species by the slope of the standard additions curve. The LODs values obtained [0.012 µg mL⁻¹ for As (III), 0.024 µg mL⁻¹ for As (V), 0.042 µg mL⁻¹ for MMA and 0.015 µg mL⁻¹ for DMA] are similar or comparable to some of the reported values of HPLC-HG-AAS methods.^{13, 15, 24, 27, 39, 40}

The precision of the methods, expressed as the relative standard deviation (% R.S.D.), was calculated from a standard solution containing the four As compounds, each one at two different concentrations (0.05 µg mL⁻¹ and 0.10 µg

Table 5: Analytical characteristics and calibration parameters for analytical performance of HPLC–HG–AAS for arsenic speciation.

Parameter	As (III)	DMA	MMA	As (V)
Lineal calibration range ($\mu\text{g mL}^{-1}$)	0.025 – 1	0.050 – 1	0.050 – 1	0.050 – 1
Correlation coefficient (r^2)	0.9976	0.9988	0.9986	0.9992
Linear regression equation	$y=0.2405.c$	$y=0.2101.c$	$y=0.114.c$	$y=0.1902.c$
LOD ($n = 10, \mu\text{g mL}^{-1}$) ^a	0.012	0.024	0.042	0.015
R.S.D. (%) ($n = 10, C_i = 100 \mu\text{g L}^{-1}$) ^b	3.0	2.9	4.7	4.0
R.S.D. (%) ($n = 10, C_i = 50 \mu\text{g L}^{-1}$) ^b	4.1	7.9	4.2	2.2

^a Detection Limit = $3 \sigma_b/m$ (σ_b = standard deviation blank, m = slope).
^b Relative Standard Deviation (%) = $(\sigma/C_i) \cdot 100$ (σ = standard deviation of concentration, C_i = average concentration).

mL^{-1}) and evaluated in terms of repeatability. Repeatability values, established from peak area measurements of ten replicates of each species performed the same day. The precision was good for all the species considered, ranging between 2.2 % for As (V) and 7.9 % for DMA.

Analyses of spiked water samples

Because a certified reference material for the studied species was not available to us, speciation of arsenic in real water samples was evaluated through a standard addition procedure. To perform these experiments we selected water samples collected in low contaminated locations, to assure a possible arsenic content well below the detection limits of the proposed technique. A water sample of drinking quality was also spiked. The composition of those samples, such as common metals and anions, was determined by a standard procedure and it show in table 6. Samples were spiked with a standard mixture of the analyses giving an added concentration of As $0.05 \mu\text{g mL}^{-1}$ each. Recoveries that appear in table 7 were calculated by comparison with a pure water standard of the same

concentration. The four species could be detected in all the water samples with good recoveries, between 92 % and 109 %, demonstrating the reliability of the proposed methods.²⁷

The chromatogram run time of drinking water and fresh groundwater was 13.30 minutes, it was similar or comparable to some of the reported values.^{15,38} Times of the retention of MMA, DMA, and As (V) are lightly higher for the samples of saline ground water analyzed, possibly due to the increment of the saline concentration of those samples.

The results from the validation showed that the proposed methodology is lineal, precise and accurate within the range of study for each species. This fact, would allow to quantify simultaneously As (III), MMAs, DMAs and As (V) in natural water samples.

CONCLUSION

The application of a CCD design allowed optimize the HPLC–HG–AAS method for the determination of the toxicologically relevant arsenic species As (III), As (V), MMA and DMA in water samples. Four factors (i.e. mobile phase flow rate, HCl concentration, pre-reducing agent concentration and reducing agent concentration) were investigated in this study. The central composite design (CCD) was used in order to determine the co-optimum level of the factors, as well as to provide an insight into the interactions amongst these factors. Empirical models obtained through second order regression provided the optimal speciation conditions. Results showed that the most important effects were given by the individual factors: the mobile phase flow rate and the HCl concentration. The most significant effects from the interactions of second order were the mobile phase flow rate and the NaBH_4 concentration, the HCl and NaBH_4 concentrations and the KI and NaBH_4 concentrations. In all of them the concentra-

Table 6: pH and composition of spiked water samples^a

	Drinking water	Saline groundwater	Fresh ground-water
pH	7.9	7.5	7.3
SRD	130.0	3450.5	1060.3
Ca ²⁺	24.0	131.7	52.6
Mg ²⁺	11.3	207.8	19.7
CO ₃ ²⁻	n.d ^b	n.d	n.d
HCO ₃ ⁻	30.0	379.6	384.8
Cl ⁻	87.2	904.2	312.7
NO ₃ ⁻	n.d	n.d	n.d
SO ₄ ⁻	29.0	272.2	208.6

^a Concentrations in $\mu\text{g mL}^{-1}$.
^b Not detected

Table 7: Recovery and retention time of a standard mixture of the arsenic species (As $0.05 \mu\text{g mL}^{-1}$ each) added to water samples.

Sample ^a	Recovery (%) ^b				Retention time (min)			
	As (III)	MMA	DMA	As (V)	As (III)	MMA	DMA	As (V)
Deionizer water	101	100	99	99	3.5	5.5	6.9	12.1
Drinking water	95	95	96	103	4.0	6.0	7.3	13.2
Fresh ground water	109	101	103	97	4.0	5.9	7.3	13.2
Saline ground water	97	100	92	109	4.0	6.2	7.6	14.0

^a Average of three replicates.
^b Calculate from peak heights.

tion of NaBH₄ played a determinant role for the complete reduction of the different forms of arsenic.

The proposed method was demonstrated to be suitable for the detection and quantification of the four arsenic species in different water samples, showing good resolution with elution times shorter than similar methods generally proposed for arsenic speciation. In Argentina, the official regulations imposed by the Código Alimentario Argentino⁴¹ (CAA: Food Argentinean Code) from the year 2007 indicates that the recommended maximum level of total arsenic in tap water should be 0.01 µg mL⁻¹. As a consequence, future research will be done in order to lower the LODs values found in the present work which were higher than the newly recommended value.

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