

Optimization and validation of a methodology to determine total arsenic, As(III) and As(V), in water samples, through graphite furnace atomic absorption spectrometry

Otimização e validação de metodologia de determinação de arsênio total, As(III) e As(V), em amostras de água por espectrometria de absorção atômica com forno de grafite

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Abstract

The Graphite furnace atomic absorption spectrometry (GF AAS) was the technique chosen by the inorganic contamination laboratory (INCQ/ FIOCRUZ) to be validated and applied in routine analysis for arsenic detection and quantification. The selectivity, linearity, sensibility, detection, and quantification limits besides accuracy and precision parameters were studied and optimized under Stabilized Temperature Platform Furnace (STPF) conditions. The limit of detection obtained was 0.13 $\mu\text{g.L}^{-1}$ and the limit of quantification was 1.04 $\mu\text{g.L}^{-1}$, with an average precision, for total arsenic, less than 15% and an accuracy of 96%. To quantify the chemical species As(III) and As(V), an ion-exchange resin (Dowex 1X8, Cl⁻ form) was used and the physical-chemical parameters were optimized resulting in a recuperation of 98% of As(III) and of 90% of As(V). The method was applied to groundwater, mineral water, and hemodialysis purified water samples. All results obtained were lower than the maximum limit values established by the legal Brazilian regulations, in effect, 50, 10, and 5 $\mu\text{g.L}^{-1}$ para As total, As(III) e As(V), respectively. All results were statistically evaluated.

Keyword: arsenic; water; graphite furnace atomic absorption spectrometry; speciation; validation.

Resumo

A técnica de espectrometria de absorção atômica com forno de grafite (GF AAS) foi a técnica escolhida pelo laboratório de contaminantes inorgânicos do Instituto Nacional de Controle de Qualidade em Saúde (INCQS/FIOCRUZ) para ser validada e aplicada em análises de rotina para detecção e quantificação de arsênio. Os parâmetros de validação seletividade, linearidade, sensibilidade, limite de detecção e quantificação, exatidão e precisão foram estudados e otimizados usando as condições STPF (Stabilized Temperature Platform Furnace). Os resultados encontrados apresentaram limites de detecção 0,13 $\mu\text{g.L}^{-1}$ e quantificação de 1,04 $\mu\text{g.L}^{-1}$, uma precisão média para arsênio total inferior a 15% e uma exatidão de 96%. Para quantificar as espécies químicas As(III) e As(V), utilizamos uma resina de troca iônica (Dowex 1X8, forma de Cl⁻) e os parâmetros físico-químicos foram otimizados, obtendo-se uma recuperação de As(III) de 98% e de As(V) de 90%. O método foi aplicado em amostras de águas subterrâneas, águas minerais e água purificada para hemodiálise. Todas as amostras apresentaram resultados abaixo do limite máximo permitido pelas legislações em vigor: 50 $\mu\text{g.L}^{-1}$, 10 $\mu\text{g.L}^{-1}$ e 5 $\mu\text{g.L}^{-1}$, respectivamente. Todos os resultados foram avaliados estatisticamente.

Palavras-chave: arsênio; água; espectrometria de absorção atômica com forno de grafite; especiação; validação.

1 Introduction

The toxic substances resulted from industrial activities are among the major sources of water contamination. More specifically, arsenic contamination, the purpose of this research, has been verified in the last years in many parts of the world (ANDREW; MEHARG; RAHMAN, 2003; BENNETT et al., 2003; VIRARGHAVANT; SUBRAMANIAN; ARULDOSS, 1999). Human exposition to this element, of proved carcinogenic effects, can occur in different ways, and one of the most important is through water. Due to the danger that this element represents to human health and to the environment, its analysis has become more frequent. Considering this fact, the laboratory of inorganic contaminants of the Chemistry Department of the *Instituto Nacional de Controle de Qualidade em Saúde of Fundação Oswaldo Cruz (DQ/INCQS/FIOCRUZ)* has decided

to study and optimize a method to detect and quantify total arsenic, and the species, As(III) and As(V), in water samples from different origins. The Graphite furnace atomic absorption spectrometry (GF AAS) was chosen to be used because its low detection limits and due to the availability of such equipment at DQ/INCQS-Fiocruz. In addition, this technique presents good selectivity once operational conditions are specific for the studied element (WELZ; SPERLING, 1999; FRANCESCONI; KUEHNELT, 2004). To perform the separation of the species As(III) and As(V), was used a mini-column filled with an ion-exchange resin which is a simple, selective, and easy method to be implemented in routine analyzes. The validation methodology, used in this study, includes many stages: method optimization, study of working range, linearity, sensitivity, accuracy and preci-

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sion, and detection and quantification limits (ISO/CD 13812, INMETRO). An internal monitoring of the quality of the whole analytical process was also done, using a control chart (FUNK; DAMMANN; DONNEVERT, 1995). The water samples studied were: 1) groundwater from the city *Cruz Alta/RS/Brazil* which was suspected to be contaminated by a dormant factory which used a toxic substance known as creosote and whose formula presents arsenic trioxide (0.4%); 2) purified water used in the treatment of substitute renal therapy (hemodialysis) collected from different hemodialysis units in the state of Rio de Janeiro; and 3) samples of commercialized mineral water from Rio de Janeiro/Brazil.

2 Materials and methods

The total arsenic was determined by graphite furnace absorption spectrometer using a Perkin Elmer system SIMAA 6000 equipped with a Zeeman - effect background correction system, an electrothermal atomiser with transversely heated graphite tube and an AS-72 autosampler. An arsenic electrodeless discharge lamp (Perkin Elmer) was used, as the radiation source. The lamp current was set at 380 mA, and a primary resonance line of 193.7 nm was used for all measurements with spectral bandwidth of 0.7 nm. The analytical measurements were based on the peak area. The volume of the sample and the chemical modifier injected in the furnace were 20 and 5 μL , respectively. These volumes were used throughout the study for all different samples. The purge gas was argon with 99,996% purity and the gas flow was stopped during atomisation (BEATY; KERBER, 1993).

Table 1 shows the temperature program for the total arsenic determination in water by GF AAS.

2.2 Reagents and solutions

All reagents were of analytical grade unless otherwise stated. Ultrapure water from Milli-Q/Millipore purification system (18 $\text{M}\Omega\cdot\text{cm}^{-1}$) was used to prepare all solutions. The arsenic stock solution was prepared with 1000 $\text{mg}\cdot\text{L}^{-1}$ of Titrisol standard (Merck). The calibration solutions containing 1 to 10 $\mu\text{g}\cdot\text{L}^{-1}$ of arsenic were prepared daily through serial dilutions of the stock solution with the addition of 0.2% (v/v) of nitric acid with suprapur quality (Merck). The NIST CRM 16438d trace element in water was used as a certified reference material and magnesium nitrate was used as the chemical modifier. The final concentration of the matrix modifier solution in de furnace was 0.06% $\text{Mg}(\text{NO}_3)_2$. The material used for the analysis (plastic flasks, auto sampler cups, and glassware) was soaked for 24 hours in 10% (v/v) HNO_3 , and, after this, it was rinsed

Table 1. Graphite furnace temperature program.

Step #	Temperature (°C)	Ramp (seconds)	Hold time (seconds)	Gas flow rate ($\text{mL}\cdot\text{min}^{-1}$)	Read
1	110	1	30	250	
2	130	15	30	250	
3	700	10	20	250	
4	2300	0	3	0	*
5	2450	1	3	250	

three times with deionised water. After the cleaning process, the material was let to dry at room temperature.

2.3 Column packing and conditions for separating As(III) and As(V)

The resin Dowex 1-X8 (100-200 mesh; Cl^- form; Bio-Rad Labs, Richmond, CA, USA) was loosely packed into a glass column (5 cm x 10 mm i.d.). The method consists of the separation of As(III) from As(V) on acetate form of Dowex 1-X8 ion-exchange resin. Before running the sample, the resin was converted into the acetate form, by passing 8 mL of 1.0 mol sodium hydroxide, followed by 10 mL of 4.0 mol acetic acid, at a flow rate of 1.0 $\text{mL}\cdot\text{min}^{-1}$. Then, the mini-column was washed with 10 mL of deionized water. No column degradation was observed after several weeks of usage. An aqueous solution of 5 mL containing As(III) and As(V) was passed through the column at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$, using a peristaltic pump. As(V) was retained in the column while As(III) was collected from the effluent. A solution of 1 $\text{mol}\cdot\text{L}^{-1}$ of HCl was used to eluate As(V) from the column. The eluate was collected in other flasks. After each run, the column was washed with 2.0 mol HCl and then, with 5 mL of water. The arsenic concentration was determined in two fractions by GF AAS. The difference in dissociation constants between arsenous acid and arsenic acid allows separating As(III) and As(V) on the basis of ion-exchange. All experiments were performed in triplicate.

2.4 Analytical sample procedure

All samples were collected in polyethylene flasks and analyzed within 24 hours after collection.

3 Results and discussion

3.1 Optimization – pyrolysis curve and atomization curve

Firstly, a study of the pyrolysis temperature through a curve, which was established through temperature variation, was performed. Afterwards, the atomization temperature, established at 2300 °C, was studied. Figure 1 shows the pyrolysis

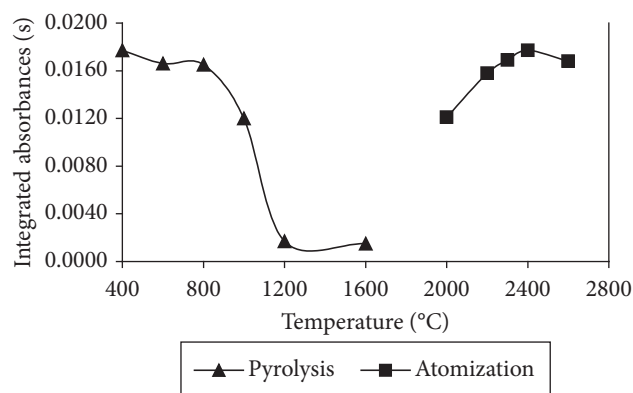


Figure 1. Pyrolysis and atomization curves using 10 $\mu\text{g}\cdot\text{L}^{-1}$ of arsenic solution.

and atomization curves. The chosen modifier for the water analysis was magnesium nitrate, because the water samples studied were simple, and did not present a matrix interference. The use of $\text{Mg}(\text{NO}_3)_2$, as a chemical modifier, was done only to guarantee that arsenic was not lost by volatilization before the atomization step.

3.2 Validation parameters

Working range

The working range is calculated by multiplying the maximum value, allowed by the regulation agency, by 0.1 and by 2. The reference limit for water is $10 \mu\text{g.L}^{-1}$, according to the official Brazilian Sanitary Surveillance Agency -RDC n° 518 from March 25, 2004. Therefore, the chosen working range varied from $1 \mu\text{g.L}^{-1}$ to $10 \mu\text{g.L}^{-1}$, considering the highest limit established by the regulations.

Linearity

In order to verify linearity, we took into consideration the least square method and the certification test of ISO/CD 13812, which established that the linearity (L) must be higher or equal to 0.7, which certifies the value found in our study (ISO/CD 138121). The Statistic Cochran Test was also applied to confirm linearity (MILLER; MILLER, 1998).

Sensitivity

Sensitivity corresponds to the tangent of the calibration curve. The sensitivity found was $0.0034 \text{ L} \cdot \mu\text{g}^{-1}$, and it can also be expressed through the result of characteristic mass of $26.4 \text{ pg}/0.0044 \text{ abs}$ (ISO/CD 13812; BEATY; KERBER, 1993).

Detection limit and quantification limit

The detection limit (LOD) of $0.13 \mu\text{g.L}^{-1}$ and the quantification limit (LOQ) of $1.04 \mu\text{g.L}^{-1}$ were calculated from the standard deviations (s) of the blank solution (mostly nitric acid 0.2% of HNO_3), using the criterion of three times the standard deviations and ten times the standard deviations, respectively. According to United Kingdom Accreditation Service (UKAS) patterns, the minimum concentration point in the calibration curve was prepared to confirm the obtained quantification limit values.

Accuracy

Reference samples certified by the National Institute of Standards and Technology (NIST) were used in this study. Accuracy was evaluated by statistically comparing the values obtained for the samples with the certified values through the hypothesis test (test *T* student). The method proved very accurate showing a recovery of 96%.

Precision

The method precision was characterized by the relative standard derivation. It was studied analyzing the solution with

three different concentrations (1, 3, and $10 \mu\text{g.L}^{-1}$). The repeatability was always studied through the same conditions. The reproducibility was analyzed in different days, and the results were expressed by the relative standard deviation. The precision result for total arsenic was less than 15% RSD.

3.3 Quality internal control of results

A shewhart chart allowed the internal monitoring the quality of the whole analytical quality process. The control chart was established analyzing the same solutions in different days up to a total of 20 results. With these values, the average and the standard deviation were calculated to create the control chart. The calculated average value was used to represent the central line of the graphic, and the control limits and warning limits were calculated through the standard deviation using the following equations: Upper control limit - $\text{UCL} = \bar{X} + 3s$; lower control limit - $\text{LCL} = \bar{X} - 3s$; upper warning limit - $\text{UWL} = \bar{X} + 2s$; lower warning limit - $\text{LWL} = \bar{X} - 2s$. The distance from the $+2s$ line to the $-2s$ line covers 95% of the area underneath the curve, i.e. the probability of a "false alarm" is 4.5% and a single exceeding of these limits is tolerable. The probability of a value exceeding the $-3s$ limit is 0.3%, i.e., its occurrence would be considered an out of control situation. It was established a control chart for each water sample studied and the analysis was monitored using $10 \mu\text{g.L}^{-1}$ solution prepared daily. Figure 2 shows the control chart (FUNK; DAMMANN; DONNEVERT, 1995).

3.4 Study of the analytical methodology for the separation of As(III) and As(V)

To verify the retention and elution of As(V) in the column, the different parameters such as flow rate speeds, volumes and concentrations of HCl, were studied. This study was done by univariate analysis (simplex), which is the study of one single variable while keeping the others fixed. The concentration of HCl was of 1 mol.L^{-1} and the minimum volume of HCl necessary to quantitatively dislocate As(V) from the glass column filled with ion-exchange resin is 3 mL. The volume of the sample, which passes through the column, was also studied. The minimum volume to obtain a good retention is 3 mL, and the ideal volume is 5 mL. Once the ideal conditions for the study were defined, the method precision was studied through the repeatability and reproducibility of As(III) and As(V). The

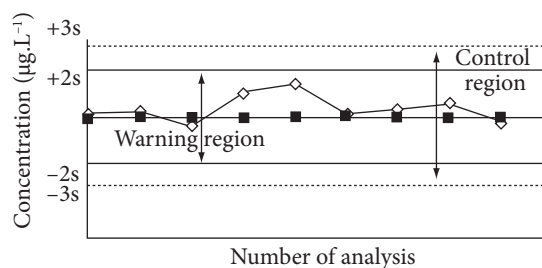


Figure 2. Sheward chart prepared from a $10 \mu\text{g.L}^{-1}$ solution prepared for every new reading in different days to monitor arsenic water determinations.

obtained results for repeatability and reproducibility of the studied species presented a recuperation range of 88-104%. After each species of arsenic had been studied separately, the two species were mixed and the obtained recuperation results ranged from 88 to 105%.

3.5 Application

Total arsenic from real samples of groundwater, hemodialysis, and mineral water were analyzed. All samples presented total arsenic content lower than the quantification limit, $1 \mu\text{g.L}^{-1}$. It can be said that the arsenic content in the water real samples presented at least 10 times lower concentration of arsenic in every sample analyzed than the maximum limit allowed of this element. If the arsenic concentrations were found above the limit stipulated by law, it would be necessary to investigate the possible sources of contamination. Considering that the studied samples presented very low arsenic contents, a speciation study of these samples was not conducted.

4 Conclusions

The developed methodology proved simple and easy, and so it can be very useful for routine laboratories. Under STPF conditions in the graphite furnace, it was possible to obtain precise and accurate results and quantification limits that are necessary to control the quality of water used for different purposes. The separation method chosen to determine As(III) e As(V) species was very efficient presenting excellent precision and accuracy although the efficiency of such separation depends on the matrix, mainly on its acid concentration. The studies of the separation of arsenic species III and V in water may help in the speciation of different samples such as blood, liquid food, and others as well as help with the elucidation of toxicological studies, which may be interesting for health.

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