

Determination of As (III) and As (V) in grape juice samples by differential pulse cathodic stripping voltammetry

Determinação de As (III) e As (V) em amostras de suco de uva por voltametria de redissolução catódica

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Juliana Machado de CARVALHO, Katia Christina LEANDRO*

*Endereço para correspondência: Departamento de Química, Instituto Nacional de Controle de Qualidade em Saúde (INCQS), FIOCRUZ-RJ. Avenida Brasil, 4365. CEP: 21.040-900, Mangueiras, Rio de Janeiro, Brasil. Tel.: + 55 (21) 3865-5180. E-mail: katia.leandro@incqs.fiocruz.br.

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RESUMO

O presente estudo descreve um método voltamétrico simples e sensível para a determinação de As (III) e As (V), em $\mu\text{g.L}^{-1}$. As (III) é determinado pela análise direta, e As (V) é convertido em As (III) por uma solução de redução. A nova concentração de As (III) representa a concentração de As inorgânico total na amostra em análise. As (V) foi determinado pela subtração do As inorgânico total e do As (III). Os limites de quantificação e detecção de As (III) foram, respectivamente, 0,22 e 0,08 $\mu\text{g.L}^{-1}$ para o tempo de deposição de 180 s; a faixa linear foi de 1 a 5 $\mu\text{g.L}^{-1}$ e desvio-padrão ($n = 10$) de 5%, em potencial de -0,68 V. A precisão foi verificada por meio de teste de recuperação em amostras fortificadas [de 98,2% para As (III) e de 96,4% para As (V)]. Estes resultados foram comparados com a análise feita por espectrometria de absorção atômica com geração de hidreto. O método voltamétrico foi aplicado na especiação do arsênio em amostras de sucos de uva contendo concentrações de 51 a 71 $\mu\text{g.L}^{-1}$ de As (III) e 34 a 60 $\mu\text{g.L}^{-1}$ de As (V).

Palavras-chave. especiação de arsênio, suco de uva, voltametria de redissolução catódica.

ABSTRACT

This study describes a simple and sensitive voltammetric method for determining As (III) and As (V) concentrations in $\mu\text{g.L}^{-1}$. Electroactive As (III) is evaluated by direct analysis. As (V) is converted into As (III) using reduction solution; the new As (III) concentration, represents the total inorganic As in the analyzed sample. As (III) and total inorganic As determination showed a work potential peak at -0.68 V; As (V) concentration was determined by subtraction [total inorganic As – As (III)]. Quantification and detection limits for As (III) were 0.22 and 0.08 $\mu\text{g.L}^{-1}$, respectively, for a deposition time of 180 s with the linear range from 1 to 5 $\mu\text{g.L}^{-1}$. Standard deviation for 1 to 5 $\mu\text{g.L}^{-1}$ arsenic concentration ($n = 10$) was 5%. The methodology was successfully applied for identifying and quantifying the inorganic arsenic in grape juices; and the accuracy was assessed by a recovery test on spiked samples, being 98.2% for As (III) and 96.4% for As (V). These results were compared with those from hydride generation atomic absorption spectrometry analyses. The grape juice samples analyzed by voltammetric technique showed As (III) and As (V) concentrations ranging 51-71 $\mu\text{g.L}^{-1}$ and 34-60 $\mu\text{g.L}^{-1}$, respectively.

Keywords. arsenic speciation, grape juice, cathodic stripping voltammetry.

INTRODUCTION

Arsenic is an element with toxic properties, which has been known for centuries¹, and this specie exists in the nature under varied chemical forms² with different biological properties and toxicity. The inorganic forms of arsenic (arsenite and arsenate) are the major elements responsible for the carcinogenic effect; and these inorganic forms are reported to be hundreds times more toxic than the organic arsenic compounds. Inorganic arsenic induces skin cancer by arsenic ingestion³, and the lung cancer is caused by respiratory exposure⁴.

Due to the occurrence of arsenic in the environment under different oxidation states [As (V), As (III), As (0), and As (-III)], and the fact of the inorganic forms being the mostly dangerous for the health than the organic forms, the speciation of arsenic has been gaining increasing importance. The As (III) has been reported to be 25-60 times more toxic than As (V)⁵. Arsenic has been found in water samples, and in foods and beverages as well^{2,6}.

The bentonite, a volcanic clay, is a technological aid widely used for removing or reducing the concentration of undesirable constituents from grape juice, wine and vinegar. Remarkably, it aids by removing proteins; thus, it reduces the risk of protein hazards in those beverages, also it favors the filtration process, decreases the fermentation time, and it preserves the taste and smell. The protein occurrence could impair the product acceptance by the consumer. However, the bentonite contains arsenic as a contaminant^{7,8}.

Hydride generation (HG) coupled to atomic absorption spectrometry (AAS) has been used for determining the total arsenic in orange juice, water, beer and foods⁹.

This study aimed at investigating the presence of inorganic arsenic, As (III) and As (V), in grape juice samples. The arsenic contents were determined by means of cathodic stripping voltammetry at hanging mercury drop electrode (HMDE). In general, the electrochemical methods are employed for determining the arsenic speciation at low concentration, moreover this methodology is simple and of low cost.

MATERIALS AND METHODS

Instrumentation

The voltammetric system – Metrohm Model 757VA Computrace – in the differential pulse cathodic

stripping voltammetry (DPCSV) was used for determining As contents in grape juices samples. Conventional three measuring cell electrodes were employed: the hanging mercury dropping electrode as working electrode, the Ag/AgCl, KCl (3 mol l⁻¹) as reference electrode, and the platinum wire as auxiliary electrode.

All of the used flasks and pipettes were calibrated before performing the analyses.

Solutions and Reagents

All of the used chemical substances and elements were of the available highest purity and being at least of analytical reagent grade; and Milli-Q processed water (Millipore Corporation, Molsheim, France) was employed for preparing the conditioning solutions.

Standard solution of 1000 mg.L⁻¹ As (III) was prepared by dissolving 0.1320 g As₂O₃ (Sigma, 96.7% purity) in 1 mL of 25% NaOH (Merck), and immediately acidified with 2 mL of concentrated HCl and diluted to 100 mL with ultrapure water. A 50 µg.L⁻¹ work solution of As (III) was prepared daily by diluting the standard solution.

The As (V) standard stock solution (1,000 mg.L⁻¹) was prepared by diluting the standard titrisol arsenic of H₃AsO₄ (Merck, Darmstadt, Germany) (1,000 ± 0.002 g As) to 1,000 mL with ultrapure water containing 300 µg.L⁻¹ As (V), which was prepared by diluting the standard reference solution.

The supporting electrolyte was the 0.4 mole.L⁻¹ HCl solution (Merck), Cu (II) solution containing 5 mg.L⁻¹ which was prepared by diluting Cu (II) standard stock solution (1,000 mg.L⁻¹ Cu) (Merck, Darmstadt, Germany), and 0.004 mg.L⁻¹ Se (IV) solution was diluted from 1,000 mg.L⁻¹ Se standard stock solution (Merck, Darmstadt, Germany).

The chemical reducing agent for determining the total arsenic was prepared by dissolving 1.4 g bisulfite sodium (Sigma, Steinheim, Germany) in 10 mL of water and 5 mL of 10% (v/v) H₂SO₄ (Merck, Darmstadt, Germany), and the other solution was set by dissolving 0.14 g of thiosulfate sodium (Merck, Darmstadt, Germany) in 10 mL of water. The second solution was added to the first one and the mixture was filled up to 50 mL in a volumetric flask.

Method

The principle of arsenic speciation using DPCSV is to determine firstly the electroactive As (III) species and then to convert sequentially the other inorganic As species

into As (III) species for performing the measurement. As (V) is usually reduced to As (III) by means of a chemical reducing agent such as thiosulfate and bisulfite, according to Ferreira and Barros¹⁰. The methodology proposed for determining As (III) and As (V) is based on the determination of As (III), followed by As (V) reduction to As (III), and then the total inorganic arsenic is analyzed. The As (V) is quantified by the difference between the total inorganic arsenic and the As (III) contents.

In spite of arsenic being an electroactive specie that can be measured by cathodic stripping voltammetry, this specie should not be deposited directly onto the mercury electrode. In this study, As (III) reacted with Se (IV) and Cu (II), resulting in an inter-metallic compound ($Cu_xAs_ySe_z$); this might deposited onto the HMDE¹ and then stripped in the cathodic scan, improving the peak shape and enhancing the sensitivity.

The analytical curve (five points) was built by subsequent addition of 0.2 mL of 50 $\mu\text{g}\cdot\text{L}^{-1}$ As (III) standard solution into the electrochemical cell containing 10 mL of supporting electrolyte. All measurements were performed in triplicate.

DPCSV measurements were carried out by using stirred deposition at a potential of -0.4 V, and the deposition time was of 180 s. After the 20 s equilibration time, the potential was scanned from -0.60 to -0.80 V with sweep rate of 25 mV s^{-1} and amplitude of 50 mV.

The solutions were deaerated with nitrogen for four minutes before conducting the voltammetric measurements for preventing the interference of dissolved oxygen in the solutions; and at this stage it was needed to reduce As (V) to As (III) for determining the total inorganic arsenic.

For conducting the total inorganic arsenic determination, the samples were added into the voltammetric cell containing 10 mL of supporting electrolyte and 0.2 mL of chemical reducing agent (sodium bisulfite and sodium thiosulfate).

Validation

The methodology was validated following the pertinent recommendations established in the official guide¹¹⁻¹³. The evaluated parameters were: selectivity, linearity (working range), detection and quantification limits, precision (intra-day and inter-day) and accuracy (recovery).

The matrix interference was assessed by comparing the analyte peak currents in ten samples that were fortified with $60 \mu\text{g}\cdot\text{L}^{-1}$ As (III), ten samples fortified with 60

$\mu\text{g}\cdot\text{L}^{-1}$ As (V), and the peak currents of standard solutions at the same concentration prepared with pure water.

Linearity was determined by employing the least-squares regression. Acceptable linearity was achieved when the coefficient of determination was at least 0.999.

The limits of detection (LOD) and of quantification (LOQ) were calculated as $(3.3 \sigma/S)$ and $(10 \sigma/S)$, respectively, where σ is the standard deviation of the intercepts and S is the slope of the calibration curve. Subsequently, the LOQ was validated by analyzing ten samples with known quantification limit. Both LOD and LOQ values confirm the sensitivity of the proposed methodology¹⁴.

Precision (intra-day and inter-day), accuracy (recovery) and matrix interference were examined through the methodology analytical range with low-, medium, and high-concentration (30 , 60 and $90 \mu\text{g l}^{-1}$) of As (III) and As (V) standard solutions.

Intra-day precision was computed by quantifying seven replicates of each As (III) and As (V) concentrations of two validation days, and calculating the percentage of relative standard deviation (%RSD) for that day; and the highest %RSD was reported. Inter-day precision was calculated by using the variance analysis with F-Test for determining whether a statistically significant difference was occurred in the concentrations which were calculated at each day.

Recovery was calculated from the mean concentration of seven replicates compared to the nominal concentration.

Interference study

Interference caused by substances commonly detected in grape juice, as Pb (II), Cu (II), Zn (II) and Cd (II), were investigated. The DPCSV response to a grape juice solution containing $100 \mu\text{g}\cdot\text{L}^{-1}$ As (III or V) and different levels of Pb (II), Cu (II), Zn (II) and Cd (II) up to $50 \mu\text{g}\cdot\text{L}^{-1}$ was surveyed.

Sample collection

Twelve different brands of commercially available grape juice (S-1 to S-12 samples), collected from Rio de Janeiro (Brazil) were analyzed.

Sample analysis

The grape juice samples were analyzed, after performing the methodology validation.

For determining As (III) in grape juices, 0.5 mL of sample and 10 mL of electrolyte were added into the electrochemical cell. The total inorganic As was determined

by adding 10 mL of supporting electrolyte, 0.5 mL of sample and 0.2 mL of chemical reducing agent into this cell.

The S-1 sample was analyzed by means of hydride generation atomic absorption spectrometry (HG-AAS), and the detected results were compared with those found in the differential pulse cathodic stripping voltammetry using F-test.

RESULTS AND DISCUSSION

The determinations of As (III) and total inorganic showed a work potential peak in at -0.68 V.

The methodology was validated for linearity (working range), detection and quantification limits, matrix interference, precision (intra-day and inter-day) and accuracy (recovery).

The working range was from 1 to $5 \mu\text{g.L}^{-1}$ (y (nA) = $7.775x$ ($\mu\text{g.L}^{-1}$) - 0.221) and r -value of 0.9999. The relative standard deviation of analytical curve for all arsenic concentration ($n = 10$) was approximately 5% (Figure 1). The calculated LOD and LOQ for As (III) were $0.08 \mu\text{g.L}^{-1}$ and $0.22 \mu\text{g.L}^{-1}$, respectively. The LOQ was subsequently validated by analyzing ten samples with known contents and at the quantification limit; and the recovery was of $95.45 \pm 1.78\%$. In the course of the methodology processing, the matrix interference was assessed in samples fortified with As (III) and As (V) standard solutions. No interference of matrix that could affect on the As (III) and total inorganic arsenic quantification was noted. Intra-day and inter-day precisions were evaluated by calculating the %RSD of seven determinations of As (III) and As (V) at each concentration (30, 60, and $90 \mu\text{g l}^{-1}$) in grape juice samples; and the precisions were less than 1.7 and 1.6%, respectively. Intra-day and inter-day recoveries, which were assessed by calculating the estimated concentrations as a percent of the nominal concentrations, showed as

better than 94.3% and 96.0%, respectively (Table 1). The interference caused by Pb (II), Cu (II), Zn (II) and Cd (II), which commonly occur in grape juice, was minimal and the concentrations were below $50 \mu\text{g.L}^{-1}$. The accuracy was investigated by using a recovery test in spiked samples. For this purpose, 0.2 mL of $50 \mu\text{g L}^{-1}$ As (III) were added into all of samples. The recovery was better than 96.5% for As (III), 94.2% for As (V) (Table 2), and 99.7% for total As in S-1 sample by means of HG-AAS. The F-test was used for comparing the results from S-1 sample when analyzed by both methodologies (DPCSV and HG-AAS), and no statistically significant difference was found.

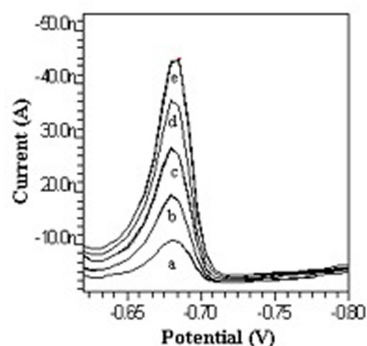


Figure 1. Voltammograms of increasing concentrations of As (III) [0.4 mole.L^{-1} HCl in the presence of 5 mg.L^{-1} Cu (II) and 0.004 mg.L^{-1} Se (IV) as supporting electrolyte, 50 mV amplitude, 25 mV s^{-1} sweep rate]. As (III) concentrations: (a) $1 \mu\text{g.L}^{-1}$, (b) $2 \mu\text{g.L}^{-1}$, (c) $3 \mu\text{g.L}^{-1}$, (d) $4 \mu\text{g.L}^{-1}$, (e) $5 \mu\text{g.L}^{-1}$

The analytical curve shown in Figure 1 was used for analyzing the data from 12 samples of grape juice of different brands assessed by differential pulse cathodic stripping voltammetry. The concentration ranges for As (III) and As (V) were of $51\text{-}71 \mu\text{g.L}^{-1}$ and $34\text{-}60 \mu\text{g.L}^{-1}$, respectively, in an average of seven determinations. All of the samples analyzed in the present study showed

Table 1. Intra-day and inter-day precision and recovery for As (III) and As (V) in grape juice samples

Inorganic arsenic	Concentration ($\mu\text{g.L}^{-1}$) ^a	Intra day			Inter day		
		Found ($\mu\text{g.L}^{-1}$)	Precision %RSD	Recovery (%)	Found ($\mu\text{g.L}^{-1}$)	Precision %RSD	Recovery (%)
As (III)	30	29.4	1.6	98.0	29.8	1.6	99.3
	60	59.6	1.2	99.3	60.3	1.3	100.5
	90	88.9	1.3	98.8	89.2	1.1	99.1
As (V)	30	28.3	1.7	94.3	28.8	1.6	96.0
	60	58.4	1.4	97.3	59.1	1.3	98.5
	90	90.2	1.6	100.2	90.1	1.4	100.1

^anumber of replications ($n = 7$).

to be satisfactorily safe, being in compliance with the Brazilian legislation – Instructive Norm no. 18, July 28, 2004 – Brazilian Ministry of Agriculture, Livestock and Supply. This norm establishes a limit of 200 mg.L⁻¹ of total inorganic arsenic as the maximum concentration allowed in beverages. The samples containing arsenic concentration lower than the detection limit were those based on soybean, and it could be linked to the grape juice clarification step before being bottled, because this step is not carried out for this product fabrication (Table 2).

Table 2. Concentrations (µg.L⁻¹) and recovery (%) found for As (III) e As (V) in grape juice samples by DPCSV

Samples	As (III) ^b		As (V) ^b	
	µg.L ⁻¹ (S.D.)	Recovery (%)	µg.L ⁻¹ (S.D.)	Recovery (%)
S-1	71.0 (1.8)	97.8	49.9 (2.2)	95.4
S-2	66.8 (2.3)	96.5	34.4 (2.6)	96.8
S-3	61.2 (1.9)	98.8	45.9 (2.2)	95.8
S-4	< 0.08	–	< 0.08	–
S-5	67.5 (1.4)	97.2	58.7 (1.8)	96.3
S-6	69.3 (2.0)	97.6	47.5 (2.3)	96.6
S-7	63.9 (2.1)	98.9	54.2 (1.4)	97.2
S-8	57.2 (1.2)	99.4	36.7 (1.7)	97.7
S-9	50.9 (1.9)	98.6	42.9 (2.2)	95.9
S-10	66.3 (1.7)	98.2	60.1 (1.9)	94.2
S-11	69.7 (2.3)	99.0	46.6 (1.9)	96.6
S-12	< 0.08	–	< 0.08	–

^bnumber of determinations (n = 7).

CONCLUSION

The voltammetric methodology showed a favorable outcome for identifying and quantifying the inorganic arsenics contents, As (III) and As (V), in grape juices. Grape juice samples were analyzed by means of differential pulse cathodic stripping voltammetry, and the As (III) and As (V) concentrations ranged 51-71 µg.L⁻¹ and 34-60 µg.L⁻¹, respectively. These results were compared with those from hydride generation atomic absorption spectrometry analyses. All of the samples analyzed in the present study showed to be safe, being in compliance with the Brazilian legislation – Instructive Norm no. 18, July 28, 2004 – the Brazilian Ministry of Agriculture, Livestock and Supply. This legislation establishes the maximum concentration of 200 mg.L⁻¹ of total inorganic arsenic, as being the limit permitted for beverages.

The significant advantage of using this methodology for determining the arsenic contents is the fact of performing the effective and sequential analyses of both arsenic species.

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REFERENCES

1. He Y, Zheng Y, Ramnaraine MM, Lock DC. Differential pulse cathodic stripping voltammetric speciation of trace level inorganic arsenic compounds in natural water samples. *Analytica Chimica Acta*. 2004;511:55-61.
2. Herce-Pagliai C, Moreno I, Gonzalez G, Repetto M, Camean AM. Determination of arsenic, inorganic and organic arsenic species wine. *Food additives and contaminants*. 2002;19:542-6.
3. Hsueh YM, Cheng GS, Wu MM, Yu HS, Kuo TL, Chen CJ. Multiple risk-factors associated with arsenic-induced skin-cancer: Effects of chronic liver-disease and malnutrition status. *British Journal of Cancer*. 1995;71:109-14.
4. Engel RR, Hopenhaynrich C, Receveur O. 1994. Vascular effects of chronic arsenic exposure: A review. *Epidemiologic Reviews*. 1994;16:184-209.
5. Carvalho LM, Nascimento PC, Bohrer D, Pilau EJ, Stefanello R. Voltammetric behavior of arsenic (III) in the presence of sodium diethyl dithiocarbamate and its determination in water and highly saline samples by adsorptive stripping voltammetric. *Electroanalysis*. 2006;18:1081-9.
6. Uneyama C, Toda M, Yamamoto M, Morikawa K. Arsenic in various foods: Cumulative Data. *Food Additives and Contaminants*. 2007;24:447-534.
7. Catarino S, Madeira M, Monteiro F, Rocha F, Curvelo-Garcia AS, Sousa B. Effect of bentonite characteristics on the elemental composition of wine. *Journal of Agricultural and Food Chemistry*. 2008;56:158-65.
8. Weiss KC, Bisson LF. Effect of bentonite treatment of grape juice on yeast fermentation. *American Journal of Enology and Viticulture*. 2002;53:28-36.
9. Cervera ML, Lopez JC, Montoro R. Determination of arsenic in orange juice by dry ashing hydride generation atomic absorption spectrometry. *Microchemical Journal*. 1994;49:20-6.
10. Ferreira MA, Barros AA. Determination of As (III) and Arsenic (V) in natural waters by cathodic stripping voltammetry at a hanging mercury drop electrode. *Analytica Chimica Acta*. 2002;459:151-9.
11. EURACHEM. The fitness for purpose of analytical methods, a laboratory guide to method validation and related topics. Teddington: LGC; 1998.
12. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Validation of analytical procedures: methodology. Geneva: ICH/IFPMA; 1996.
13. Instituto Nacional de Metrologia, Qualidade e Teconologia (INMETRO). Orientação sobre Validação de Métodos de Ensaio Químicos: DOQ – CGCRE – 008. Rio de Janeiro; 2007.
14. Miller JC, Miller JN. Statistics for analytical chemistry. New York: Ellis Harwood; 1993.