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Arsenic species in herbal tea leaves and infusions determination by HPLC-ICP-MS

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ABSTRACT

A simple method for arsenic species As (III), As (V), monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA) were investigated in 18 herbal tea leaves and their infusion by high performance liquid chromatography hyphenated with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The proposed method presented absence of interconversion between the arsenic species and high sensitivity, being limits of detection and quantification 11 and 38 μg L^{-1} for herbal tea leaves and 0.20 and 0.66 μg L^{-1} for their infusions, respectively. About 28% samples present total arsenic levels above Brazilian and MERCOSUR maximum limits (0.6 mg kg^{-1}). The estimative of exposure to inorganic arsenic was made taking into account the benchmark dose lower limit ($BMDL_{05}$) considering the daily ingestion of one cup of herbal tea (200 mL) for adults (70 kg) and children (20 kg). Although this estimative demonstrates low exposure for adults (2.9% of $BMDL_{05}$), for children, however, the daily consumption of a single cup of herbal tea can reach 10.3% of $BMDL_{05}$ for inorganic arsenic.

1. Introduction

Tea and herbal tea are one of the most consumed beverages in world. Their intake have been associated to several health benefits (Dalipi, Borgese, Tsuji, Bontempi, & Depero, 2018; Herrera et al., 2018; Karak & Bhagat, 2010) and their chemical composition also include amino acids, vitamins and minerals, such as iron, magnesium, manganese, potassium and zinc (Martín-Domingo et al., 2017; Szymczycha-Madeja, Welna, & Pohl, 2012).

Besides mineral content, herbal tea may also present trace elements in its composition. Although some trace elements have nutritional importance, such as cobalt, manganese and zinc, others may accumulate in organism and cause several diseases and they are considered inorganic contaminants. Arsenic is an inorganic contaminant that may be present due to natural and anthropogenic activities, such as volcanic eruptions and herbicides that may transfer inorganic contaminants from water and soil to the plant (Lai, Chen, & Chen, 2016). Its toxicity is strictly related to its chemical structure, oxidation state, absorption and elimination rates (Barra, Santelli, Abrão, & de la Guardia, 2000) and implies in different values of LD_{50} (lethal dose for 50% of a population): Arsine (AsH_3) = 3 mg kg^{-1}; As (III) = 14 mg kg^{-1}; As (V) = 20 mg kg^{-1}; MMA (monomethylarsonic acid) = 1800 mg kg^{-1}; DMA (dimethylarsonic acid) = 2600 mg kg^{-1}; arsenbetaine and arsencholine > 10000 mg kg^{-1} (Cornelis et al., 2005). Consequently, the inorganic arsenic species, As (III) and As (V), are the most toxic ones.

Inorganic As species are commonly reported in vegetables samples, especially rice and rice products. A study including hot pepper, bean, rice, shallot, carrot, potato and cabbage indicated the predominance of inorganic specie As (V) in these plants (Bohari et al., 2002). Sun et al. (2009) reported a study with forty rice products (breakfast cereals, rice crackers and Japanese rice condiments) from markets in the UK and the results also shown inorganic species predominance (75.2–90.1%). In the same year, Signes-Pastor et al. (2009) described in their study with 60 samples of Japanese rice drinks and condiments, inorganic levels 2-fold higher than DMA levels. Juskelis, Li, Nelson, and Cappozzo (2013) studied several samples of infant rice cereals from USA and also verified the predominance of inorganic As species. MMA was only detected at trace levels while DMA ranged between 23.0 and 153 μg kg^{-1}. Islam, Rahman, Rahman, and Naidu (2017) investigated total and inorganic arsenic in rice and rice-based diets commercialized in South Australia. The authors verified the presence of total arsenic ranged from 30 to 273 μg kg^{-1}, being 35–100% of this value corresponding to inorganic arsenic.

Some studies of arsenic speciation in wine samples have been

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conducted by different research groups. Moreira et al. (2011) studied fourteen wine samples from South America (Argentina, Brazil and Chile) and verified the majority of inorganic As species in all samples, and found that only two samples had DMA quantifiable levels. Escudero, Martinis, Olsina, and Wuilloud (2013) proposed a method for arsenic speciation analysis in wines using on-line ionic liquid-based dispersive liquid-liquid microextraction and quantification using electrothermal atomic absorption spectrometry. The method provide information about As (III) level in wine samples and As (V) for difference between the total As and As (III). A recently work reported by Grijalba, Fiorentini, Martinez, and Wuilloud (2016) also applied ionic liquid for arsenic speciation in wine. In this study, the authors evaluated different ionic liquids for As (III), As (V), DMA and MMA in wine samples from Argentina. In contrast, Lai et al. (2016) established a method also based on microextraction for As (III) and As (V) in fruit juices by hydride generation-atomic fluorescence spectrometry. Values ranged between ND (not detected) – 3.9 $\mu g\,L^{-1}$ and 0.1–6.6 $\mu g\,L^{-1}$ for As (III) and As (V), respectively.

Our previous studies focused in methods for total content of trace elements in tea leaves and beverages. A direct method was proposed for trace elements determination in tea and herbal beverages by ICP-MS and it was proved to be reliable, with high sensitivity, low time of analysis agreeing with the "green chemistry" concept (Milani, Morgano, Saron, Silva, & Cadore, 2015). For black, green, oolong and white tea samples we found arsenic levels above the thresholds established by Brazilian and MERCOSUR regulations and a remarkable behavior was observed using multivariate analysis, which allowed to classify samples in three tea types: black and green, oolong and white tea (Milani, Morgano, & Cadore, 2016).

Nevertheless, to the best of our knowledge, just a few studies are reported concerning the arsenic species in tea or herbal tea samples. Yuan, Gao, He, and Jiang (2007) were one of the first groups to report a method for arsenic speciation in tea leaves and their infusions. In their study, the authors utilized the microwave assisted extraction to extract the arsenic species from tea leaves and quantified this element using atomic fluorescence detection. Recently, Chen, Li, Lu, and Zhang (2016) proposed an innovative method for arsenic inorganic species determination in tea samples using a dual extraction procedure, based on solid phase extraction and solidified floating organic drop microextraction, and quantification by electrothermal vaporization inductively coupled plasma mass spectrometry. This procedure allowed to determine the total and inorganic species As (III) and As (V), with a preconcentration factor of 500-fold.

Thus, the present work aims: i) to establish and to validate a simple and accurate method for As speciation in herbal tea leaves and their infusions and ii) to estimate the dietary exposure to inorganic arsenic from herbal tea consumption. To provide these important data, herbal tea samples were purchased in southeastern Brazil and total arsenic was quantified by inductively coupled plasma mass spectrometry (ICP-MS) whilst arsenic species were determined using high performance liquid chromatography hyphenate technique (HPLC-ICP-MS).

2. Material and methods

2.1. Instrumentation

All measurement were performed using an ICP-MS (Agilent 7700x, Agilent, Tokyo, Japan) operating with helium as collision cell gas (3 $mL\,min^{-1}$) to avoid isobaric interfering. For arsenic species, a HPLC (1260 Series, Agilent, Tokyo, Japan) equipped with an automatic sample injector was applied and sample was directly introduced into the ICP-MS equipment for quantification. The conditions used are described in Table 1.

Table 1
Instrument conditions for total and arsenic species determination.

Total Arsenic	
RF power	1550 W
Ar and auxiliary flow rate	15 and 0.9 $L\,min^{-1}$
He gas flow rate	10 $mL\,min^{-1}$
Micro-mist nebulizer gas flow rate	1.1 $L\,min^{-1}$
Spray chamber	Quartz, double pass
Sample uptake	0.1 rps
Number of replicates	4
Integration time	0.3–1.0 s
Isotopes	^{75}As and ^{89}Y (internal standard)
ISIS-DS parameters (introduction system applied for infusions)	
Loop volume	150 µL
Uptake time and acquisition delay	20 and 20 s
Rinse time	During data acquisition
Arsenic species	
Analytical column	Hamilton PRP-X100 (250 × 4.1 mm; 10 µm)
Flow rate	1.5 $mL\,min^{-1}$
Injection volume	100 µL
Mobile phase	20 $mmol\,L^{-1}$ $(NH_4)_2HPO_4$ (pH 6.0)
Column temperature	27 °C
RF power	1600 W
Micro-mist nebulizer gas flow rate	1.0 $L\,min^{-1}$

2.2. Reagents

Nitric acid was purified using a sub-boiling distiller (Berghof, Eningen, Germany) and reverse osmosis-purified water (18.2MΩ cm) was employed in the analyses. For total arsenic, the analytical curve was obtained by successive dilutions of 1000 $mg\,L^{-1}$ standard solution (Merck, Darmstadt, Germany) in 0.1–100 $\mu g\,L^{-1}$ range. Yttrium was employed as internal standard and this solution was prepared by dilution of 100 $mg\,L^{-1}$ yttrium standard (Specsol, São Paulo, Brazil) in 2.5% (v/v) isopropyl alcohol (Merck, Darmstadt, Germany).

For arsenic species, stock solutions of arsenic species were prepared by dissolution of their respective salts: Arsenic (III) oxide (As_2O_3 99.995%, Sigma-Aldrich, Milwaukee, USA); Arsenic (V) oxide (As_2O_5 99%, Sigma-Aldrich, Milwaukee, USA); Disodium methyl arsonate hexahydrate (MMA, $CH_3AsNa_2O_3.6H_2O$ 99.5%, ChemService, West Chester, EUA); Cacodylic acid (DMA, $C_2H_7AsO_2$ 99.0%, Sigma-Aldrich, Buchs, Switzerland). These solutions were prepared considering the FDA recommendations (FDA, 2012) to preserve As species and real concentrations were measured by ICP-MS. Analytical curves were prepared in 0.25–10.0 $\mu g\,L^{-1}$ range in mobile phase (20 $mmol\,L^{-1}$ phosphate buffer solution ($(NH_4)_2HPO_4$, Synth, Diadema, Brazil) in pH 6.0). A solution of 2.0% (v/v) acetone (Synth, Diadema, Brazil) was employed to increase the method sensitivity.

2.3. Samples

Eighteen samples of herbal tea leaves (Flowers and fruits, n = 9 and Strawberry, n = 9) were purchased from markets in southeastern Brazil between 2013 and 2015. Flowers and fruits consisted in a mix with apple fruits *(Pyrus malus, L)*, gooseberry *(Ribes nigrum)*, blueberry *(Vaccinium myrtillus, L.)*, hibiscus flowers *(Hibicus sabdariffa L.)*, rosehips *(Rosa canina, L.)* and chicory *(Chichorium intybus,L.)* whilst Strawberry contains a mix with apple fruits (*Pyrus malus L.*), strawberry (*Fragaria spp.*) and hibiscus flowers (*Hibiscus sabdariffa L.*).

Sampling considered three different batches from the three main manufactures, totalizing 9 samples of each type of herbal tea. Data concerning the origin or age (i.e., young or old) of the herbal tea leaves were unavailable. Infusions were prepared by brew the herbal tea leaves, considering the proportion recommended by manufactures: 1 bag (approximately 1.5 g) for a 200 mL cup. The leaves were kept in contact with boiling purified water for 3 min and then filtrated through a 0.25 mm polymeric membrane.

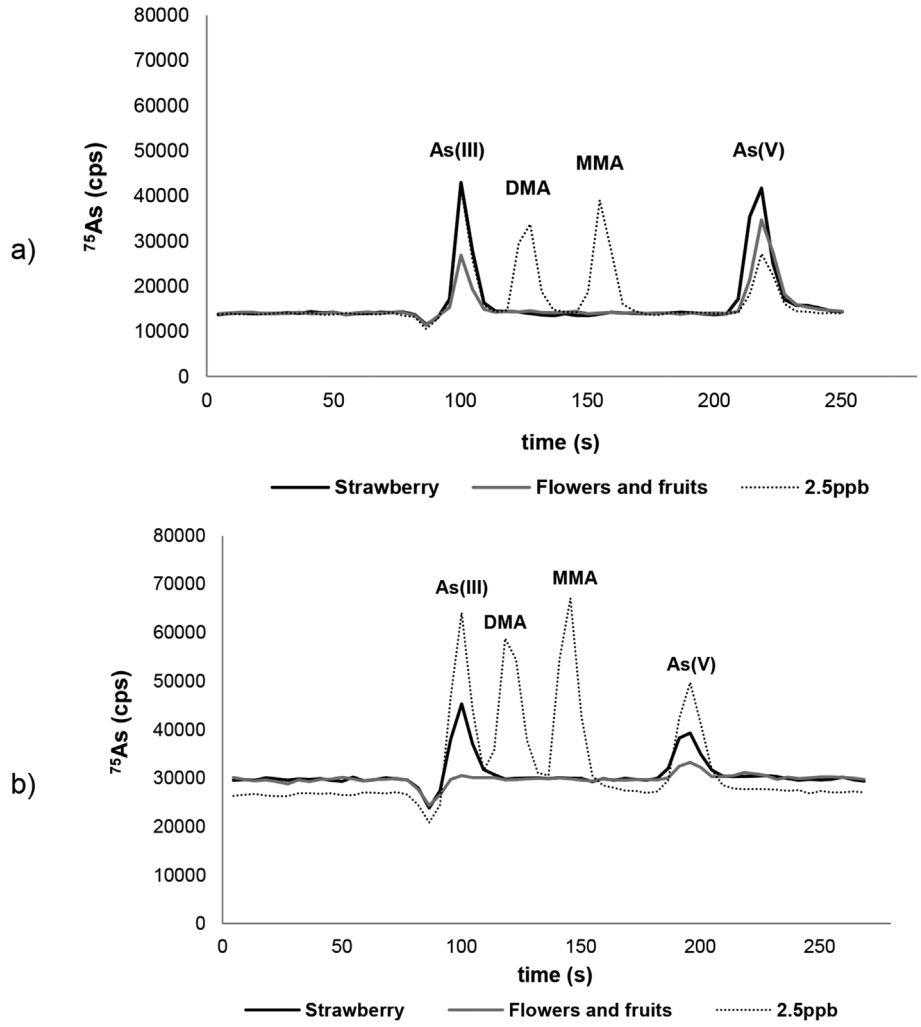

Fig. 1. Typical chromatogram for As species in herbal tea: 1a) leaves and 1b) infusions (Analytical column: Hamilton PRP X-100, 4.1 × 250 mm, 10 µm; Mobile phase: 20 mmol L^{-1} $(NH_4)_2HPO_4$ (pH 6.0), flow 1.5 mL min^{-1}, 27 °C; injection volume: 100 µL) **DMA** = dimethylarsinic acid; **MMA** = monomethylarsonic acid.

2.4. Analytical procedure

2.4.1. Determination of total arsenic in herbal tea leaves and infusions

For total arsenic determination in herbal tea leaves, samples were digested in a closed microwave digestion system (Start D, Milestone, Sorisole, Italy), using conditions described in previous work of our group (Milani et al., 2016): 0,2 g of samples homogenized in a stainless steel mill (M-20, IKA, Staufen, Germany) was added to a PTFE digestion vessel with 3 mL of water and 5 mL of purified 65% (w/v) HNO_3. Sample decomposition was performed at a maximum temperature of 170 °C for 32 min and the final solutions were transferred to 25 mL volumetric flasks using purified water.

For total arsenic determination in herbal tea infusions, the infusion were acidified with HNO_3 to obtain a 0.2% (v/v) acid concentration and directly analyzed using a discrete sampling introduction system (ISIS-DS, Agilent Technologies, Tokyo, Japan). The conditions were optimized in a previous work of our research group (Milani et al., 2015) and they are summarized in Table 1.

2.4.2. Determination of arsenic species in herbal tea leaves and infusions

For arsenic species extraction from herbal tea leaves, samples were extracted in a closed microwave digestion system (Start E, Milestone, Sorisole, Italy), using a two-step procedure: i) 0.5 g of sample was added to a PTFE digestion vessel with 10 mL of 0.05% (v/v) HNO_3 for 30 min at maximum temperature of 90 °C; ii) the supernatant was removed and the residue was re-extracted using 10 mL of purified water. The combined supernatants were transferred to 25 mL graduated flasks using purified water. The extract was centrifuged (Excelsa II 206BL, Fanem, São Paulo, Brazil) at 3600 rpm for 30 min at room temperature. Prior injection, 1.0 mL of supernatant + 1.0 mL of mobile phase was manually mixed and filtered using 0.2 µm regenerated cellulose membrane (Sartorius, Göttingen, Germany).

For arsenic species extraction from herbal tea infusions, the samples were diluted 2-fold with mobile phase and filtrated using 0.2 µm regenerated cellulose membrane.

2.5. Quality assurance and quality control

All analyses were performed in triplicate and the blank experiments followed the same procedure applied for samples. Methods were validated based on INMETRO (2011) guidelines, using certified reference materials SRM 1547 Peach leaves (NIST, Maryland, USA), INCT-TL-1 Tea leaves (Instytut Chemii I Techniki Ja˛drowej, Warszawa, Poland) and spiked samples for accuracy and precision. The limits of detection and quantification were calculated as 3s and 10s, being "s" the standard deviation of 10 blank experiments, expressed in concentration. Statistical analyses were performed using Statistica software (Stat Soft. Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Methods for arsenic species in herbal tea

Generally arsenic speciation methods are performed applying hyphenates techniques such as the HPLC-ICP-MS. For As species separation, an anionic exchange column was chosen attributable to its stationary phase composed by polystyrene-divinylbenzene copolymer with covalent bounded trimethylammonium anion exchange groups (Collins & Braga, 1987, p. 202). Mobile phase $(NH_4)_2HPO_4$ was evaluated in different concentrations (15, 20 and 25 mmol L^{-1}), pH (5.5; 6.0 and 6.5) and mobile phase flow (1.0 and 1.5 mL min^{-1}) to verify the influence on the ICP-MS measurements. The experimental conditions considered the results reported by Moreira et al. (2011), Paproski and Le (2004) and herbal tea unique characteristics such as the high Fe and Mn levels that can precipitate in alkaline pH; low time of analysis and low mobile phase concentration to prevent salt accumulation in ICP-MS and to provide a method for routine analysis. In summary, experiments demonstrated that the best resolution is achieved using mobile phase $(NH_4)_2HPO_4$ 20 mmol L^{-1}, pH = 6.0 and mobile phase flow = 1.5 mL min^{-1}. Under these conditions, arsenic species were satisfactory separated in less than 5 min (t = 250 s). A typical chromatogram is presented in Fig. 1 with retention times (t_r): As (III) = 100 s; DMA = 128 s; MMA = 159 s and As (V) = 219 s.

3.1.1. Optimization of extraction procedures

In arsenic speciation analysis, soft conditions must be applied to preserve As species and to prevent species degradation or interconversion, especially the inorganic species, As (III) and As (V). Some extraction conditions were reported for tea and vegetable samples using a closed microwave assisted system. Yuan et al. (2007) performed the arsenic species extraction from tea samples using 1:1 (v/v) ethanol:-water as extraction solution. The authors used sequential extractions combining the supernatants, evaporated and filtered for analysis. Salgado, Quijano Neto, and Simón (2008) demonstrated the applicability of deionized water as extraction solution for algae samples acquired in Spain, also using sequential extractions (3x), and the combined supernatants were diluted up to 25 mL with deionized water. Another method was reported in a study performed for edible Shiitake samples (Llorente-Mirandes, Barbero, Rubio, & López-Sánchez, 2014), employing 0.2% (w/v) HNO_3 and 1% (w/v) H_2O_2 as extraction solution, even though the extractions cannot prevent the As (III) to As (V) interconversion. Besides this, the method proposed by the authors was used for total inorganic arsenic and organic species, such as DMA and MMA determination.

To propose a simple and low-chemical consumption method for herbal tea leaves, a two-step extraction using a closed microwave assisted system were evaluated employing 0.05% (v/v) HNO_3 and 0.10% (v/v) HNO_3 in the first step and purified water in the second. To this evaluation, a blank sample (herbal tea sample with non-quantifiable total arsenic level) was spiked with 1 µg L^{-1} of each arsenic species. The results are presented in Table 2.

From Table 2, both extraction procedures presented recovery values near to 100% for organic species (DMA and MMA). Nonetheless, for inorganic species a low variation in HNO_3 concentration can influence the i-As recovery: using 0.10% (v/v) HNO_3 solution, recovery for As (V) was 71 ± 24% (coefficient of variation, CV = 34%) whilst using 0.05% (v/v) HNO_3 as extractor, recovery was 100 ± 8% (CV = 8%). Tukey's test also showed a significant difference for As (III) at 95% of confidence (p-value = 0.007).

Therefore, the extraction procedure employing 0.05% HNO_3 provided the best results for both inorganic species: As (III) and As (V), 80 ± 5 and 100 ± 8, respectively. Also in this condition no interconversion between arsenic species was observed.

These conditions were applied to herbal tea leaves samples and a typical chromatogram is presented in Fig. 1. The low values of cps (counts per second) at t_r = 95 s was proved to be mainly due to the chloride presence in herbal tea infusions and/or the NOx presence in extract solution for herbal tea leaves. This fact also demonstrates the requirement of matrix-matching curve and the collision cell - avoiding the isobaric interference of polyatomic ion $^{35}Cl^{40}Ar^+$ in monoatomic isotope $^{75}As^+$.

For beverages, the literature usually describes the sample dilution in mobile phase as sample preparation method: Moreira et al. (2011) used a 10-fold dilution for wine samples and FDA (2013) recommends a dilution of 5-fold to ready to drink juices and 30-fold for concentrated juices. In this study we opted to use a 2-fold dilution and the performance of the analytical method is described in details in item 3.1.2.

3.1.2. Analytical performance

Analytical performance for total arsenic method was verified based on the INMETRO (2011) guidance. Briefly, in the method applied for herbal tea leaves, accuracy was verified using two certified reference materials (CRM): Peach leaves (As = 60 ± 18 µg kg^{-1}; recovery = 118 ± 3%) and Tea leaves (As = 106 ± 21 µg kg^{-1}; recovery = 118 ± 13%). For precision, a coefficient of variation (CV) = 10% was observed and limits of detection and quantification were 5 and 18 µg kg^{-1} (Milani et al., 2016). For herbal tea infusions, due to the lack of certified reference material, accuracy was verified using spiking the samples with three different concentration levels (10; 25 and 50 µg L^{-1}) and recovery values ranged from 96 to 104%. For precision, CV = 4% was observed and limits of detection and quantification were 0.14 and 0.46 µg L^{-1} (Milani et al., 2015).

The methods for the determination of arsenic species were also evaluated based on the INMETRO guidelines (2011), considering the parameters accuracy, precision, limits of detection and quantification. The results are shown in Table 3.

The method applied for As speciation in herbal tea leaves presented satisfactory results for limits of detection and quantification, being lower than those established by FDA (2012) which reports LOQ = 20 µg kg^{-1} for As(III), As(V), MMA and DMA determination in rice samples. Accuracy and precision were also considered satisfactory according to AOAC (2013) recommendations: recovery for spiked experiments between 50 and 120% for concentrations below 1 ppb;

Table 2
Result of a spiked experiment for arsenic speciation in herbal tea leaves (samples spiked with 1 µg L^{-1} of each arsenic species).

As species	Extraction procedure				
	0.05% HNO_3 (v/v)		0.10% HNO_3 (v/v)		p-value[a]
	Concentration (µg kg^{-1})	Recovery (%)	Concentration (µg kg^{-1})	Recovery (%)	
As (III)	0.80 ± 0.05	80 ± 5	0.98 ± 0.04	98 ± 4	0.007
As (V)	1.00 ± 0.08	100 ± 8	0.71 ± 0.24	71 ± 24	0.725
DMA	0.98 ± 0.13	98 ± 13	0.95 ± 0.04	95 ± 4	0.577
MMA	0.88 ± 0.09	88 ± 9	0.84 ± 0.07	84 ± 7	0.127

[a] Recovery values between different extraction procedure are not significantly different at p > 0.05, according to Tukey's test.

Table 3
Analytical performance for the proposed speciation procedures for herbal tea leaves and their infusions.

Herbal tea	Parameter	As species			
		As (III)	As (V)	DMA	MMA
Leaves	**LOD (μg kg^{-1})**	8.4	11.0	7.6	8.6
	LOQ (μg kg^{-1})	28	38	25	29
	CV (%)	7	9	5	5
	Recovery L1	81 ± 3	95 ± 6	92 ± 8	77 ± 3
	Recovery L2	91 ± 12	88 ± 10	88 ± 9	89 ± 6
	Recovery L3	88 ± 2	82 ± 1	83 ± 2	83 ± 2
Infusions	**LOD (μg kg^{-1})**	0.16	0.20	0.15	0.13
	LOQ (μg kg^{-1})	0.53	0.66	0.49	0.44
	CV (%)	3	5	4	7
	Recovery L1	66 ± 4	87 ± 13	85 ± 14	109 ± 5
	Recovery L2	115 ± 6	115 ± 9	104 ± 8	107 ± 5
	Recovery L3	119 ± 3	117 ± 2	108 ± 2	110 ± 2

LOD: Limit of detection; **LOQ**: Limit of quantification; **CV**: Coefficient of variation; **Recovery levels: L1** = 0.25 μg L^{-1}; **L2** = 1.0 μg L^{-1} and **L3** = 10.0 μg L^{-1}.

70–125% for concentrations below 10 ppb and precision of 32% for concentrations near to 10 ppb.

The method applied for As speciation in herbal tea infusions presented adequate limits of detection and quantification, being near to the values reported by Moreira et al. (2011) in their study with wine samples (LOQ = 0.30; 0.69; 0.39 and 0.45 μg L^{-1} for As(III), As(V), MMA and DMA, respectively; and they were lower than those reported by FDA (2012), that is, LOQ = 2.0 μg kg^{-1} for As(III), As(V), MMA and DMA, for apple juice samples. Accuracy and precision were also satisfactory considering the AOAC (2013), ranged from 66 to 119% and 3–7%, respectively.

3.2. Total and arsenic species concentration in herbal tea and infusions commercialized in southeastern Brazil

The proposed methods were applied to determine arsenic species in 18 herbal tea samples acquired in southeastern Brazil and their infusions. The observed results as well as the total arsenic content are shown in Table 4.

Concerning the detected levels found in herbal tea leaves, ratio values (sum of arsenic species and total As content, in percentage) ranged between 93% and 107% and 62% and 104% in flower and fruits and strawberry tea samples, respectively. These values are in agreement with the results reported by Yuan et al. (2007), 60–86% in tea leaves from China. It is known that arsenic was reported in more than 20 species (Barra et al., 2000) and some of them are not extractable in soft conditions such as the ones applied in this study.

It is also possible to notice the predominance of inorganic species in both herbal tea leaves and infusions samples. Average values for As (III) and As (V) ranged between 96 and 280 μg kg^{-1} and 0.55 and 3.06 μg L^{-1} in herbal tea leaves and their infusions, respectively. This observation also agree with the study performed by Yuan et al. (2007) in which the As (V) specie predominates and just a few organic species (DMA and MMA) were found.

Total arsenic levels ranged from 30 to 1041 (μg kg^{-1}) and < 0.46–5.32 μg L^{-1} in herbal tea leaves and their infusions, respectively. This result showed poor As extraction from leaves to infusions. However, arsenic species have shown the same behavior in both the leaves and their infusions: only As(III) and As(V) were detected. Some studies verified the stability for As species in vegetal samples using model experiments with high temperature. Generally, the species interconversion was not verified (Devesa, Vélez, & Montoro, 2008), except for arsenosugars decomposition to DMA in alkaline pH at 60 °C (Gamble et al., 2002, 2003) – unusual behavior in herbal tea infusions. For cooking procedures, however, a consensus is reported. Díaz et al. (2004) analyzed vegetables and cereals from Chile after cooking the samples using distillated water and i-As contaminated water. Low As levels were found in vegetables cooked in distilled water but the values increased proportionally when contaminated water was used. Laparra, Vélez, Barberá, Farré, and Montoro (2005) also reported a similar behavior: in rice samples cooked using As (V) contaminated water, the i-As in rice increased 5–17-fold.

3.3. Estimative of inorganic arsenic daily exposure from herbal tea consumption

Brazilian and MERCOSUR regulations presents maximum level of 0.6 mg kg^{-1} for total arsenic in tea, yerba mate and other infused vegetables, and 0.05 mg kg^{-1} in non-alcoholic beverages, excluding juice (Brasil, 2013; MERCOSUR, 2011). On the topic of this values, from Table 4 is possible to notice that some herbal tea leaves presented levels above the regulation thresholds (0.6 mg kg^{-1}): 3 samples (33%) of flower and fruit samples and 2 samples (22%) of strawberry tea. Nonetheless, for infusions, the samples were found below the threshold established for non-alcoholic samples – indicating a poor arsenic extraction from herbal tea leaves to the infusions and safe levels for this contaminant in beverages.

It is already known that arsenic toxicity is associated with its chemical structure, oxidation state, physic state, absorption and elimination rates and its inorganic species are considered the most toxics: As (III) and As (V). Considering these aspects, the Joint FAO/WHO Expert Committee on food additives established the benchmark dose lower limit ($BMDL_{05}$) for inorganic arsenic in 3.0 μg kg^{-1} body weight (FAO/WHO, 2017). In this study, the estimate for inorganic arsenic exposure it was considered the daily intake of a cup of herbal tea (200 mL) by 70 kg (bw = body weight) adult and 20 kg child (WHO, 2009). The data is presented in Table 5.

The estimative of exposure to inorganic arsenic considering the daily intake of one cup of herbal tea by an adult revealed a low exposure (1.3% of $BMDL_{05}$, in average). For children, however, high values can be reached (4.6% of $BMDL_{05}$, in average). In both cases, the highest contribution was observed to strawberries tea: 2.9% for adults and 10.3% for children considering only the herbal tea daily consumption.

Regarding a daily intake of two cups of herbal tea, the estimative of exposure for adults may reach 5.8% and 2.8% of $BMDL_{05}$ for strawberry and flower and fruit tea, respectively. Nonetheless, for children an alarming value can be reached: almost 21% of $BMDL_{05}$ for strawberry tea.

4. Conclusions

The proposed method were successfully applied to determine arsenic species As (III), As (V), monomethylarsonic acid (MMA), dimethylarsonic acid (DMA) in herbal tea leaves and their infusions. The method provide high sensitivity for all As species and no interconversion between the arsenic species was observed.

The present study reveals that 28% herbal tea leaves samples present total arsenic levels above the thresholds established by Brazilian and MERCOSUR (0.6 mg kg^{-1}). Nonetheless, the arsenic extraction from the leaves to the infusions was poor and values for total As ranged between < 0.46 and 5.32 μg L^{-1}. In both samples (herbal tea leaves and infusions) only inorganic As species were quantified, mainly As (V).

An estimative of inorganic arsenic daily exposure from herbal tea consumption indicated an important contribution for children (up to 20.6% $BMDL_{05}$) if they consume two herbal tea cups per day.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Table 4
Arsenic species content in herbal tea leaves and their infusions.

Sample	Herbal tea leaves (n = 3)							Herbal tea infusions (n = 3)						
	Arsenic species (μg kg^{-1})					Total As (μg kg^{-1})	Ratio* (%)	Arsenic species (μg L^{-1})					Total As (μg L^{-1})	Ratio* (%)
	As (III)	As (V)	DMA	MMA	Sum			As (III)	As (V)	DMA	MMA	Sum		
FF1	35 ± 8	287 ± 6	ND	ND	322	345 ± 9	93	ND	0.83 ± 0.05	ND	ND	0.83	0.83 ± 0.15	100
FF2	58 ± 3	279 ± 17	ND	ND	337	314 ± 15	107	ND	0.78 ± 0.05	ND	ND	0.78	0.85 ± 0.02	92
FF3	107 ± 13	381 ± 10	ND	ND	488	519 ± 24	94	0.96 ± 0.15	0.86 ± 0.05	ND	ND	1.83	1.44 ± 0.05	127
FF4	263 ± 21	519 ± 33	ND	ND	782	816 ± 55	96	1.40 ± 0.21	1.46 ± 0.16	ND	ND	2.86	2.30 ± 0.13	124
FF5	278 ± 19	554 ± 5	ND	ND	832	834 ± 43	100	1.34 ± 0.04	1.21 ± 0.02	ND	ND	2.55	2.12 ± 0.20	120
FF6	293 ± 3	497 ± 25	ND	ND	790	853 ± 37	93	1.21 ± 0.25	1.14 ± 0.23	ND	ND	2.35	2.29 ± 0.22	102
FF7	ND	ND	ND	ND	–	30 ± 7	–	ND	ND	ND	ND	–	< 0.46	–
FF8	ND	ND	ND	ND	–	43 ± 1	–	ND	ND	ND	ND	–	< 0.46	–
FF9	ND	ND	ND	ND	–	30 ± 3	–	ND	ND	ND	ND	–	< 0.46	–
Average ± SD	**115 ± 127**	**280 ± 230**	**ND**	**ND**	**-**	**420 ± 352**	**97 ± 5**	**0.55 ± 0.66**	**0.70 ± 0.56**	**ND**	**ND**	**-**	**1.09 ± 0.99**	**111 ± 15**
Median	**58**	**287**	**ND**	**ND**	**-**	**345**	**95**	**ND**	**0.83**	**ND**	**ND**	**-**	**0.85**	**111**
Range	**(ND-293)**	**(ND-554)**	**ND**	**ND**	**-**	**(30–853)**	**(93–107)**	**(ND-1.40)**	**(ND-1.46)**	**ND**	**ND**	**-**	**(< 0.46–2.29)**	**(92–127)**
S1	ND	41 ± 2	ND	ND	41	67 ± 1	62	ND	ND	ND	ND	–	< 0.46	–
S2	ND	ND	ND	ND	–	33 ± 1	–	ND	ND	ND	ND	–	< 0.46	–
S3	ND	ND	ND	ND	–	40 ± 11	–	ND	ND	ND	ND	–	< 0.46	–
S4	ND	ND	ND	ND	–	30 ± 3	–	ND	ND	ND	ND	–	< 0.46	–
S5	ND	ND	ND	ND	–	39 ± 2	–	ND	ND	ND	ND	–	< 0.46	–
S6	ND	ND	ND	ND	–	30 ± 5	–	ND	ND	ND	ND	–	< 0.46	–
S7	401 ± 7	500 ± 119	ND	ND	901	941 ± 107	96	2.69 ± 0.24	2.12 ± 0.08	ND	ND	4.81	4.17 ± 0.18	115
S8	461 ± 45	625 ± 43	ND	ND	1086	1041 ± 33	104	3.43 ± 0.05	2.73 ± 0.21	ND	ND	6.16	5.32 ± 0.09	116
S9	ND	ND	ND	ND	–	32 ± 5	–	ND	ND	ND	ND	–	< 0.46	–
Average ± SD	**96 ± 191**	**130 ± 248**	**ND**	**ND**	**-**	**250 ± 421**	**87 ± 22**	**3.06 ± 0.52**	**2.43 ± 0.43**	**ND**	**ND**	**-**	**1.05 ± 2.11**	**116 ± 1**
Median	**ND**	**ND**	**ND**	**ND**	**-**	**39**	**96**	**ND**	**ND**	**ND**	**ND**	**-**	**< 0.46**	**115.5**
Range	**(ND-461)**	**(ND-625)**	**ND**	**ND**	**-**	**(30–1041)**	**(62–104)**	**(ND-3.43)**	**(ND-2.73)**	**ND**	**ND**	**-**	**(< 0.46–5.32)**	**(115–116)**

FF = Flower and fruits; **S** = Strawberry; **Ratio** = Ratio, in percentage, of sum of arsenic species and total As content. **SD** = Standard Deviation.
ND = Not detected for herbal tea leaves: As (III) = 28 μg kg^{-1}; As (V) = 38 μg kg^{-1}; DMA = 25 μg kg^{-1}; MMA = 29 μg kg^{-1}.
ND = Not detected for herbal tea infusions: As (III) = 0.53 μg L^{-1}; As (V) = 0.66 μg L^{-1}; DMA = 0.49 μg L^{-1}; MMA = 0.44 μg L^{-1}.

Table 5
An estimative of inorganic arsenic exposure to the daily consumption of one and two cups of herbal tea by adults (body weight = 70 kg) and children (body weight = 20 kg).

Herbal tea infusion	1 cup of herbal tea			2 cups of herbal tea		
	i-As* (μg L^{-1})	% $BMDL_{05}$		i-As* (μg L^{-1})	% $BMDL_{05}$	
		Children	Adults		Children	Adults
Flower and fruit FF1	0.83	1.4	0.4	1.66	2.8	0.8
Flower and fruit FF2	0.78	1.3	0.4	1.56	2.6	0.8
Flower and fruit FF3	1.83	3.1	0.9	3.66	6.2	1.8
Flower and fruit FF4	2.86	4.8	1.4	5.72	9.6	2.8
Flower and fruit FF5	2.55	4.3	1.2	5.10	8.6	2.4
Flower and fruit FF6	2.35	3.9	1.1	4.70	7.8	2.2
Strawberry S7	4.81	8.0	2.3	9.62	16.0	4.6
Strawberry S8	6.16	10.3	2.9	12.3	20.6	5.8
Average	3.05	4.6	1.3	5.54	9.3	2.7
Minimum	6.16	10.3	2.9	12.3	20.6	5.8
Maximum	0.78	1.3	0.4	1.56	2.6	0.8

Cup = 200 mL; **i-As** = inorganic arsenic (sum of As (III) e As (V) species).

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