Determination of Metals in Brazilian Ready-to-Drink Beverages (Ice Drinks) by Inductively Coupled Plasma Optical Emission Spectrometry

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INTRODUCTION

Alcoholic ready-to-drink beverages (RTDs) are also known as FABs (flavored alcoholic beverages). These drinks were introduced into the world market in the mid-1990s. They usually consist of some kind of distilled beverage (vodka, rum, spirits, alcohol of cereals) and fruit juices or soft drinks. Their alcoholic content ranges from 4 to 7% and are available in several flavors and brands (1). The most common RTDs are the alcopops or alcoholic sodas (2). Research has shown that the consumption of drinks such as alcopops is the fastest growing market in the world (3) with the consumers usually ranging in age from 18 to 25 years.

Many analytical methods have been used for determining inorganic elements in alcoholic beverages. Anodic stripping voltammetry was used by Barbeira et al. (4) to determine trace amounts of Zn, Pb, and Cu in sugar cane spirits. Canuto et al. (5) employed electrothermal atomic absorption spectrometry (ETAAS) and ruthenium as the permanent modifier to determine Sb and Cr in 53 samples of sugar cane spirits. Flame atomic absorption spectrometry and hydride generation (FAAS and HG-FAAS) were used to determine As, Cd, Pb, Cu, Zn, Fe, and Mn in 31 Serbian old plum brandies (6). Inductively coupled plasma optical emission spectrometry (ICP-OES) was employed (7) for the analysis

ABSTRACT

The consumption of ready-to-drink (RTD) beverages has been constantly increasing. However, the control of toxic and essential elements for this type of beverage requires the employment of adequate analytical methods. Inductively coupled plasma optical emission spectrometry (ICP-OES) was employed to investigate toxic and essential elements in RTD beverages. This kind of beverage has an alcoholic composition ranging from 4 to 7% and, therefore, optimization of the plasma conditions is necessary for sample analysis. Robust conditions were obtained when the power applied was maintained at 1.4 kW. Sample and nebulization gas flow rates used were 1.0 mL min⁻¹ and 0.6 L min⁻¹, respectively. A calibration curve was plotted at 6% (v/v) ethanol and 2% (v/v) HNO₃. Satisfactory LOD and LOQ values were obtained for Al, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sn, and Zn. The recoveries were acceptable and ranged from 80 to 120%. Exploratory analysis was performed by utilizing a Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) which enabled classification of the samples based on the brand and type of distilled beverage contained in the RTD.

of several alcoholic beverages with an alcoholic content ranging from 40 and 55%. They employed two statistical methods (Canonical Discriminant Analysis – CDA and Classification Binary Trees – CBT) to evaluate the parameters for differ-

entiating the beverage samples. Fernandes et al. (8) used ICP-OES to determine 15 elements in sugar cane spirits through exploratory analysis. They only verified the differentiation between industrial and homemade spirits. Jurado et al. (9), who employed ICP-OES and several pattern recognition methods for sample classification, investigated the chemical composition of Spanish aniseed drinks. Souza et al. (10) used direct infusion electrospray ionization mass spectrometry in the negative ion mode to differentiate spirit samples. They found four groups which were characterized by the type of wood casks used.

RTD analysis employing multi-elemental techniques such as ICP-OES allows the control of toxic and essential elements in such beverages. Adjustment of the ICP conditions permits direct analysis of the alcoholic matrix and thus prevents extinction of the plasma.

The data obtained using this method can be interpreted by means of the chemometric methods of exploratory analysis such as Hierarchical Clusters Analysis (HCA) and Principal Components Analysis (PCA). These methods allow the classification and separation of the samples and indicate the elements responsible for sample distinction (11,12). This work presents the optimization of the experimental conditions and parameters of merit for RTD analysis by ICP-OES for the quantification of 15 elements in 30 samples. The exploratory analysis of the data obtained provided a better visualization and therefore allowed sample separation into different groups.

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EXPERIMENTAL

Instrumentation

A PerkinElmer Optima™ 2000 DV ICP-OES with radial and axial configuration was employed for all measurements (PerkinElmer, Inc., Shelton, CT, USA). The instrument was equipped with a 40 MHz radio frequency source, Echelle grating (79 lines nm⁻¹), solid state detector, plasma torch with a 2.0 mm internal diameter injection, and a cross-flow nebulizer coupled to a double-pass Scott-type spray chamber. An interface (shear gas) introducing a high air flow perpendicular to the torch was used to remove the low temperature at the end of the plasma. An autosampler 90 Plus (PerkinElmer) system was used for sample introduction. The instrumental operating conditions, elements, and the respective wavelengths used are listed in Table I.

Solutions, Reagents, and Samples

Solutions containing 6% (v/v) ethanol were prepared in order to simulate the original alcoholic concentration found in commercial RTDs. Reference solutions of Al, Ba,

Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sn, and Zn (1000 mg L⁻¹) of high analytical purity (CertiPUR® ICP Standard, Merck, Germany) were used to prepare the multielemental solutions containing 100 mg L⁻¹ of the analytes in 2% (v/v) HNO₃ (VETEC, São Paulo, Brazil) 70%). These solutions were employed for the construction of matrix matched calibration curves and test solutions in (6%, v/v) of deionized water obtained with a Milli-Q® system (Millipore Corporation, Bedford, MA, USA) was used to prepare all solutions. The commercial samples of RTDs were diluted (1:1, v/v) with 2% HNO₃ (v/v) and introduced into the autosampler. For Na, Ca, and K determination, a dilution of (1:10, v/v) was used for all samples. Table II lists the RTD samples analyzed and their specifications.

Procedure

Optimization of ICP-OES Conditions

A univariate optimization for the ICP-OES conditions was realized by measurement of the Mg(II)/Mg(I) ratio as the robustness criterion.

The applied power was fixed at 1.4 kW and the nebulization gas flow rate was varied from 0.4 to 1.1 L min⁻¹. All measurements were made using axial and radial view.

Analytical Figures of Merit

The analytical figures of merit were based on experiments as proposed in the literature (13,14). The short-term stability was determined by performing 15 consecutive measurements of the intensity emission at the Mg(II) 280.265 nm line. Long-term stability was evaluated by monitoring the emission line for a period of two hours where 8-minute measurements with an interval of 15 minutes were made.

The signal-to-background ratio (SBR), background equivalent concentration (BEC), limit of detection (LOD), and limit of quantification (LOQ) were calculated according to the IUPAC recommendations as follows (14):

$$\text{SBR} = \frac{(I_{rs} - I_{\text{Blank}})}{I_{\text{Blank}}} \quad \text{BEC} = \frac{C_{rs}}{\text{SBR}}$$

TABLE I
ICP-OES Operating Parameters

Parameters	
RF Power	1.4 kW
Nebulization gas flow	0.4 - 1.1 L min ^{-1a}
Sample flow	1.0 mL min ⁻¹
Auxiliary gas flow	0.2 L min ⁻¹
Plasma gas flow	15 L min ⁻¹
Injector tube diameter	2.0 nm
Interface	Shear gas
Emission lines (λ / nm)	Al (I=396.15), Ba (II=455.403), Ca (I=317.93), Cu (I=324.75), Fe (II=238.2), K (I=766.49), Na (I=589.59), Mg (I=285.21), Mn (II=257.61), Sn (II=189.99)

^a Optimized values during the experiments.

I = Atomic line II = Ionic line

TABLE II
Description of Analyzed Ready-To-Drink Samples

Brand	Description	Brand	Description
A1	Alcohol of cereals	D1	vodka
A2	Alcohol of cereals	D2	vodka
B1	Vodka	E1	Sugar cane spirits
B2	Vodka	E2	Sugar cane spirits
B3	Vodka	E3	Sugar cane spirits
B4	Vodka	E4	Sugar cane spirits
B5	Vodka	F1	vodka
B6	Vodka	F2	vodka
B7	Vodka	F3	vodka
B8	Vodka	F4	vodka
C1	Vodka	G1	Rum
C2	Vodka	G2	Rum
C3	Vodka	H1	Sugar cane spirits
C4	Vodka	H2	Sugar cane spirits
C5	Vodka	I1	Vodka

$$\text{LOD} = \frac{3 \times \text{RSD}_{\text{Blank}} \times \text{BEC}}{100}$$

$$\text{LOQ} = \frac{10 \times \text{RSD}_{\text{Blank}} \times \text{BEC}}{100}$$

where I_{rs} = Intensity of reference solution; I_{Blank} = Intensity of blank solution; C_{rs} = Concentration of reference solution; RSD = relative standard deviation of 10 measurements of the blank solution.

The accuracy of the method was evaluated with an RTD solution of 0.5 and 2.0 mg L⁻¹ of metals added. The exploratory analyses by HCA and PCA were performed by running the PLS Toolbox 3.0 for Matlab® (Mathworks, Inc., USA) (15). These methods were used to evaluate the similarity between the RTD samples by forming different groups according to types.

RESULTS AND DISCUSSION

Optimization of ICP-OES Conditions

The influence of the nebulization gas flow rate over plasma robustness was evaluated using the

Mg(II)/Mg(I) ratio. The term robustness represents the efficiency of energy transfer when the excitation and ionization conditions of the plasma are changed (14,16). In this experiment, the applied power was fixed at 1.4 kW and the nebulization gas flow varied from 0.4 – 1.1 L min⁻¹. The highest value of the Mg(II)/Mg(I) ratio was obtained with a 0.6 L min⁻¹ gas flow rate at axial view (Figure 1). With radial view, the best value for Mg(II)/Mg(I) was obtained at the 0.9 L min⁻¹ gas flow rate. The use of low gas flow values increases the plasma robustness, but values of less than 0.5 L min⁻¹ must be avoided because the residence time of the analyte is high and self-absorption can occur (17,18). The robustness condition (sample flow rate 1.0 mL min⁻¹; RF power 1.4 kW; nebulization gas flow rate 0.6 L min⁻¹; axial view) was adopted in later studies.

Analytical Figures of Merit

For axial and radial view, the short-term stability afforded an RSD of 1.03 and 1.10%, respectively; the long-term stability was 0.91% and 1.7%. Considering that in an aqueous matrix other authors (14)

obtained an RSD of 0.7 and 1.5 for short- and long-term stability, respectively, the RSD values obtained in this study were satisfactory.

The analytical figures of merit and the quantification results for 30 RTD samples (SBR, BEC, LOD, LOQ, concentration range, and recovery) are listed in Table III. Good values for the SBR, BEC, LOD, and LOQ were obtained for most of the investigated analytical lines. The recoveries observed were satisfactory with a maximum recovery value between 80 to 120% of the expected value (19). The concentration range of the samples was less than the LOQ for Cr and Mn. For Na (which is one of the elements whose levels can become dangerous for the consumer), the values obtained ranged from 11.58 to 276.8 mg L⁻¹. The elevated values obtained for Na can be associated with the addition of conservators and/or sweeteners with sodium.

Exploratory Analysis

Hierarchical Cluster Analysis (HCA)

HCA was used in order to establish possible similarities between the samples. The samples were categorized into four groups and are presented in Figure 2. The first two groups include samples with a vodka base (C4, C1, B8, B7, B3, B6, B5) and a sugar cane spirits base (E2, E1, E4); the third group contains samples with an alcohol of cereals base (A2, A1, I1); and the fourth group are samples with a sugar cane spirits base (H2, H1). The rum samples (G1 and G2) are included in the first group due similarities in their composition. Brand C are samples contained in different packaging: C1 and C4 in glass bottles and C2, C3, and C5 in cans.

Fig. 1. Effect of nebulization gas flow rate on Mg(II)/Mg(I) ratio (RF power 1.4 kW).

TABLE III
Analytical Figures of Merit for Ready-to-Drink Analysis by ICP-OES:
BEC, SBR, LOD, LOQ, Concentration Range, and Recovery

Element	SBR	BEC	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	Concentration Range ($\mu\text{g L}^{-1}$)	Recovery (%) (n=3)
Al (I)	603.6	0.0083	6.8	22.8	40-230	104.2
Ba (II)	79.9	0.03	4.3	14.3	<LOQ - 60	110.3
Ca (I)	17	0.29	120.3	401.1	610-20600	103.9
Cd (II)	25566.5	0.0002	0.4	1.3	2-8	103.1
Cr (II)	15273.4	0.0003	0.2	0.5	<LOQ	116.2
Cu (I)	4165.1	0.001	0.2	0.7	4-48	111.3
Fe (II)	340.6	0.015	0.6	1.9	40-510	108.6
K (I)	758.6	0.0066	2.0	6.9	1200-112000	93.6
Mg (I)	351.8	0.014	6.3	21.0	40-4700	109.8
Mn (II)	869.1	0.0058	0.3	0.96	<LOQ	105.6
Na (I)	13165.8	0.0004	3.7	12.3	11580-276800	91.6
Ni (II)	489.1	0.01	1.7	5.6	20-120	99.4
Pb (II)	3042.6	0.002	3.6	12.4	<LOQ-24	115.6
Sn (II)	262.3	0.019	11.7	39.1	40-500	96
Zn (II)	1893.1	0.003	0.3	1.03	3-10	110.5

SBR = Signal-to-background ratio

BEC = Background equivalent concentration

LOD = Limit of detection

LOQ = Limit of quantification

Fig. 2. Dendrogram of ready-to-drink beverages..

Principal Components Analysis (PCA)

PCA involved the same separation procedure as HCA. A model using three principal components (PC) was built and explains 99.97% of the total variance. The discriminant elements for PC1 and PC2 were K and Na (Figures 3a and 3b), while for PC3, it was Ca and Mg (Figure 3c). These discriminant elements explain the separation obtained in Figure 4, which shows the same separation as the dendrogram in Figure 2. The samples of sugar cane spirits were separated from the other beverages due to a higher K concentration and a lower Na concentration. On the other hand, a lower K concentration and a higher Na concentration differentiates the cereal alcohol samples from the other beverages. The vodka samples were at intermediary concentrations of K and Na. In each class, the samples of the inferior region possess low concentrations of Ca and Mg and higher values of the same elements in the superior region.

CONCLUSION

Adjustment of the ICP-OES operating conditions allows for a working method for the direct analysis of ready-to-drink (RTD) samples. Good analytical figures of merit were obtained under these conditions and the analysis of 30 real RTD samples was performed. Evaluating the data with Hierarchical Cluster Analysis (HCA) and Principal Components Analysis (PCA) made it possible to identify the samples into groups and the discriminant elements for each group. ICP-OES in conjunction with the exploratory statistical methods of PCA and HCA has shown to be a useful tool in providing a simple, rapid, and accurate methodology for the differentiation of RTD samples. As the preliminary results also point out, this technique can therefore be used with confidence for effective quality control of beverages.

Fig. 3(a). Variables plot of the ready-to-drink beverage samples labelled: PC1

Fig. 3(b). Variables plot of the ready-to-drink beverage samples labelled: PC2

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Fig. 3(c). Variables plot of the ready-to-drink beverage samples labelled: PC3

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Fig. 4. Scores plot of the ready-to-drink beverage samples in the space of the first three PCs.