

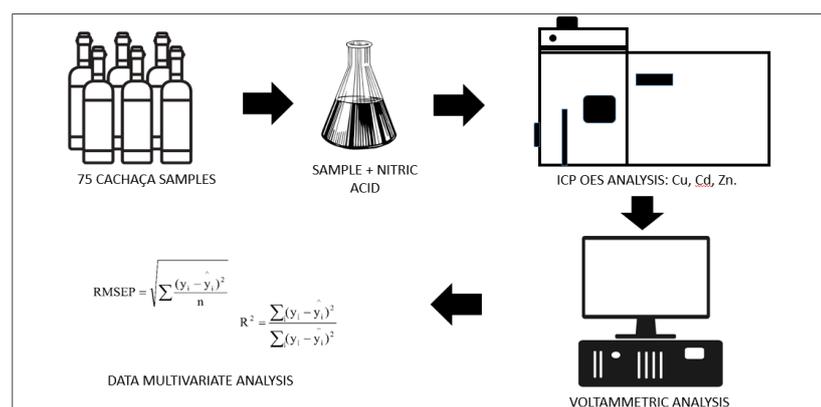
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Simultaneous Determination of Metals in Cachaça: A Study on Comparison of Multivariate Methods and Quality Control

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This study aims to compare multivariate calibration methods developed from data obtained by square wave anodic stripping voltammetry using a hanging mercury drop electrode for simultaneous determination of metals in cachaça, the following metals were studied: copper, zinc and cadmium. Multivariate calibration, partial least squares (PLS) and artificial neural network (ANN) methods were used in previous studies using other electrodes for this determination. In this new study, besides ANN and PLS, a hybrid model that combines PLS and NN, namely PLS-Neural was used. Also, samples of industrial cachaças were incorporated into the study in addition to artisanal samples. The quality of the methods was evaluated in terms of coefficient of determination (R^2) and root mean square error of prediction (RMSEP). F test was used for comparing methods at confidence level of 95%. Based on these studies, it was found that although all methods show good results, the method employing neural networks stands out in the determination of copper in samples of cachaça. All methods proved to be fast and relatively low-cost, and they can be used for such analyses.

Graphical abstract



Keywords

Cachaça
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1. Introduction

Cachaça is an alcoholic distillate obtained from the fermentation of sugar cane, with alcoholic contents ranging from 38% to 54% by volume, at a temperature of 20 °C [1]. The

Brazilian production of this drink, divided between the forms handmade (artisanal) in stills and industrial, has been growing and promoting a large influence in several sectors such as

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economic, social and health [2-4].

Cachaça has considerable complexity, with numerous organic and inorganic constituents. In this study, the following inorganic constituents, metals, were studied: copper, zinc and cadmium. Copper and zinc are essential elements required for numerous cellular functions. However, excessive intake of these metals is toxic and may cause serious health damages [2, 5-7]. Cadmium is a highly hazardous environmental pollutant, with significant toxicity in living beings [8]. The ingestion of this element can occur directly or through the process of bioaccumulation in a food chain. In view of the above, it is essential to develop methods for the analysis of metals that aim mainly to verify the quality of the beverages produced.

Atomic absorption spectrometry is commonly used for metal determination in beverages, which is the recommended method for the specific determination of copper [2, 9]. In addition to that method, metals can be determined using X-Ray fluorescence spectrometry and inductively coupled plasma optical emission spectrometry (ICP-OES) [8, 10-12].

Besides those above, the relatively low cost, non-destructive and fast techniques corresponding to voltammetric techniques, specifically stripping techniques, usually employing mercury electrodes, have shown good results. The mercury electrode is very versatile and has considerable advantages, such as a smooth, uniform and renewable surface, with excellent reproducibility. Although there are countless studies, including in our group [13], which aim to search for alternative electrodes with the same performance, it should be noted that in modern voltammetric instruments, the amount of waste generated is quite reduced, minimizing the negative aspects of the use of mercury. Also, the mercury used can be recovered with a high degree of purity and finally reused, which still makes it an interesting choice in some analytical applications, making it the target of various studies, even today [14, 15].

In terms of voltammetry, for the determination of metals using the mercury electrode, there is an extreme overlap of some peaks that undergo reduction or oxidation in near potentials. In order to overcome these difficulties, associating multivariate calibration methods and electrochemical techniques has become important for analytical determination, which can be found in several studies in the relevant literature [15-19].

In this study, investigations continued to develop methods for the simultaneous determination of metals in beverages, specifically cachaça, being used in this study, in addition to artisanal cachaças, cachaças made industrially, expanding the investigations on the quality of the products sold. Also in this study, the PLS - Neural method was introduced, and compared with PLS and artificial neural networks. The PLS-Neural method corresponds to a hybrid method between the PLS method and artificial neural networks. Thus, this study seeks to establish and verify which of these methods is suitable for analytical purposes in terms of simultaneous determination of these metals

2. Material and Methods

2.1 Reagents and Solutions

Analytical-grade nitric acid (Neon®) was double distilled. All the other reagents were of analytical grade and used without prior purification. Ultrapure water (resistivity of 18 Mohm.cm -25 °C) was obtained from a reverse osmosis

purification system (Quimis®, model Q-342, SP, Brazil) and used throughout. The standard solutions of Cu²⁺, Zn²⁺ and Cd²⁺ (1000 mg L⁻¹) (Qhemis®) were properly diluted to build the calibration curves for ICP OES analysis. Sodium acetate and acetic acid (Merck®) were also employed to prepare a solution 1.00 mol L⁻¹ and pH 4.7.

2.2 Apparatus

The experiments by ICP OES were carried out on Optima 7000 equipment, Perkin Elmer®, equipped with shear gas (removal system of the cold plasma zone), solid state detector (CCD), double vision, and an optical system with pre-purging.

The electrochemical square wave anodic stripping experiments were performed using a computer-controlled Metrohm® 797 Computrace Voltammetric Analyzer using software 797 VA computrace version 1.2 for controlling the experiment and for acquiring data.

2.3 Samples

Handmade and industrialized cachaça samples coming from several municipalities in the Brazilian States of Espírito Santo, São Paulo and Minas Gerais were used in this study. The percentage of alcohol found in the cachaça samples ranged from 38 to 50%. A total of 75 samples was analyzed. The samples, which come from different manufacturers, were obtained exclusively at commercial establishments.

2.4 Spectrometric procedure

2.4.1 Preparation of the sample for analysis in ICP OES

The same procedure performed in the previous study [13] was adopted here. Thus, all the materials were washed with detergent and decontaminated in a vessel containing 5% nitric acid solution for 24 hours. For determining the metals in cachaça, the volume of the cachaça samples was reduced by 50%. Then, 25.00 mL of the sample obtained was acidified with 0.015 mol L⁻¹ nitric acid previously distilled, completing the volume up to 50.00 mL solution with osmosis water. Next, the samples underwent analysis by ICP OES.

2.4.2 ICP OES analysis

The operational parameters used in the analyses with ICP OES are listed in Table 1.

Table 1. Operational parameters utilized in ICP OES analysis.

Parameters	Conditions
Vision	Axial
Nebulizer	Gemcone
Nebulization chamber	Cyclonic for aqueous
Injector	Alumina
Purge of the pre-optical system	High
Sample introduction Flow (ml/min)	1,57 mL.min ⁻¹
Plasma power (W)	1257 W
Nebulization gas flow rate (l/ min)	1.25 L min ⁻¹
Spectral line	
Cu	327.393 nm
Cd	226.502 nm
Zn	206.200 nm

Some parameters have been optimized [20]: introduction of the sample, power and nebulization flow. The measures found in the ICP OES had their responses associated with the intensity in area mode for the metals analyzed. Calibration curves for each metal were constructed separately using the

standards mentioned in 2.1 to later find the concentrations of the analyzed metals. The concentrations of the curves (working range) are: 0.1 to 10.0 mg L⁻¹ for copper, 0.05 to 1.50 mg L⁻¹ for zinc, and 0.005 to 0.5 mg L⁻¹ for cadmium. The quality of this method [21, 22], in which the calibration curve linearity and matrix effect; determination of the limit of quantification and detection; accuracy and precision were analyzed, was thoroughly assessed in a previous study [13].

2.5 Procedure for voltammetric analysis

For the construction of a chemometric model, the square-wave anodic redissolution voltammetry technique was used, correlating the data obtained by this technique (voltammograms) with the concentrations determined by ICP OES (reference method). As the working electrode, a hanging mercury drop electrode was adopted. The study was carried out in a conventional 25-mL electrochemical cell, also having an auxiliary platinum wire electrode with a geometric area of 1 cm² and an Ag /AgCl reference electrode (KCl 3.0 mol L⁻¹). For the analyses, the samples were inserted "in situ" about 20.00 mL in acetate solution of 1.00 mol L⁻¹ (supporting electrolyte). The square-wave anodic redissolution voltammetry analysis conditions were: electrodeposition time of 50s, amplitude of 0.05 V, velocity of 0.1 V s⁻¹ and frequency of 100 Hz. The potential range was from -1400 mV to 200 mV. These parameters were set after previous studies under controlled conditions. Every measurement was performed at room temperature (25 °C).

2.6 Data Multivariate Analysis

The different chemometric models: Partial least squares (PLS), artificial neural networks (ANN) and PLS-Neural that corresponds to a hybrid between PLS and the neural network used here have their principles found in several examples of the literature [23,24,25,26].

Root mean squared error of prediction – RMSEP and coefficient of determination - R², were used in order to evaluate the performance of the proposed multivariate methods.

$$\text{RMSEP} = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n}} \quad (1) \quad R^2 = \frac{\sum (y_i - \bar{y}_i)^2}{\sum (y_i - \bar{y}_i)^2} \quad (2)$$

Wherein y_i is the true concentration of the analyte in the sample i ; \hat{y}_i represents the estimated concentration of the analyte in the sample i ; \bar{y}_i is sample i 's average value; and n is the total number of samples used in the prediction sets

3. Results and Discussion

3.1 Analysis by using the reference method – ICP OES

The quality of the reference method based on ICP OES was verified in a previous study [13] and shows that the method is suitable for the determination of metals, specifically copper, zinc and cadmium in the analyzed drink: cachaça. Briefly: it was observed that the method had no matrix effect. Linear calibration curves were obtained in the working range used for each metal, with Cu range of 0.1 to 10.0 mg L⁻¹; for zinc the range of 0.05 to 1.5 mg L⁻¹ and for cadmium 0.005 to 0.5 mg L⁻¹, with a coefficient of determination of 0.999. In addition,

the recovery tests showed values as expected and the quantification limits for metals correspond to 0.001 mg L⁻¹ for cadmium, 0.020 mg L⁻¹ for copper and 0.011 mg L⁻¹ for zinc.

Table 2 shows the concentration range found for the samples analyzed in terms of elements copper, zinc and cadmium. For this study, industrial and artisanal samples were used. The specified quality limit allowed by Brazilian law for copper is up to 5.0 mg L⁻¹, 0.2 mg L⁻¹ is the maximum limit allowed for the cadmium element and, even though there is no specification for zinc, the limit of 5.0 mg L⁻¹, used for drinking water was used here. The quality of artisanal cachaças has already been reported in a previous study, but it can be briefly said that this type of sample presents a problem in relation to copper contamination due to its production method. For industrial samples, this type of contamination is less significant but still present. Of the 75 samples analyzed, about 20% presents level of copper above the 5.0 mg L⁻¹. It was found that all concentrations of the other metals (cadmium and zinc) were below the limits permitted.

Table 2. Concentrations values of metals (mg L⁻¹) obtained by ICP OES.

Metal	Concentration range value (Cachaça Samples) (mg L ⁻¹)
Cu	0.32 (±0.02) - 9.86 (± 0.40)
Zn	0.073 (± 0.004) - 1.22 (± 0.06)
Cd	0.030 (±0.008) - 0.17 (± 0.04)

3.2 Multivariate method (Data)

3.2.1 Data used in the construction of the chemometric models (PLS, ANN and NNPLS)

Independent variable – x : current at different potentials, obtained in the square wave anodic stripping voltammetry and the metal concentration values obtained by ICP OES (dependent variable – y). The data obtained were arranged in matrix form: matrix X and matrix y , respectively. Three independent models for zinc, cadmium and copper were obtained.

3.2.2 Multivariate methods

In the calibration phase, anomalous samples were identified and removed and after adequate models were constructed. About 70% of the samples were used in the calibration phase.

PLS model: The data arranged as a matrix went through pretreatment. The pretreatments used for data from independent variables (matrix X) were: smoothing and second derivative of the voltammogram current values, applying Savitzky-Golay algorithm and after that these data were scaled. The data from dependent variables (matrix y) were scaled. Five (5) latent variables corresponding to about 90% of the total necessary information were used to build the PLS model for copper and zinc; and eight (8) latent variables that are responsible for 85% of the total information used for the construction of the PLS model corresponding to cadmium in samples of cachaças. The number of latent variables used in these models was chosen using complete leave-one-out cross-validation.

ANN model – In order for constructing the model, the data from independent variables were submitted to mathematical pretreatments: The data were reduced by applying principal component analysis. Six major components (scores) were used in each model, which represents more than 90% of the

explained variance for all metals analyzed. The scores were normalized between -1 and 1. The data from dependent variables were also normalized between -1 and 1. All the ANN models were based on three-layered multi-layer perceptron (MLP) architecture by using Marquardt-Levenberg algorithm that adapts very well to small neural networks. The normalized scores of the main components are the data input of neural network, and then, an input layer with six neurons was used. A hidden layer with eleven neurons and an output layer with one neuron (metal concentrations) compose the neural network architecture. For training the neural network, a tan-sigmoidal function was used in the hidden layer as a transfer function. In turn, a linear function was used the output layer. A maximum number of one thousand iterations, and the error value used as criteria for stopping the training of the neural network was 1×10^{-2} . Criteria like number of neurons in hidden layer, activation functions used, and iteration number were chosen by using a trial-and-error process that took into account the nature of the data to be modeled until their best combination was found. The correct fit of the neural model was evaluated. To this end, the outputs were compared to the expected concentration values. The weights of the connections between neurons were modified as per the training algorithm rules until a perfect fit is found. By successfully completing training, cases in which the mean square error expected is found were understood. In this case, the neural network is considered trained.

PLS - Neural – This model employed second derivative and scaled for the independent variables and the data for the dependent variables were scaled, and five (5) latent variables were used to determine copper, zinc and seven (7) to determine cadmium, above 87% of the data variance was obtained for all cases. PLS focuses on extracting main components or more specifically latent variables by maximizing the correlations between in- and output data (scores called *u* and *t*, respectively). In PLS- Neural, the best relationship between *u* and *t* was set by a neural network. This study utilized an input layer with one neuron (one score at a time), a hidden layer with three neurons and an output layer with one neuron. The transfer function used between the hidden and output layers was sigmoidal and for the output layer the transfer function used was linear. In PLS-Neural, a multiple input-multiple output (MIMO) network regression is reduced to numerous single input-single output (SISO) regressions. In other words, each neural network model within the PLS framework needs to use much fewer weights. Thus, the error surface consists of fewer local minima.

3.2.3 Evaluation of the models built

It was evaluated the relative performance of the PLS, ANN

and PLS Neural models in terms of root-mean square error in calibration (RMSEC) and coefficient of determination (R^2). Adequate models were obtained as verified by the values of R^2 and RMSEC. The coefficient of determination R^2 for all metals analyzed has value around of 0.9. RMSEC has a low and adequate value. This denotes that the predicted and expected values are in compliance. These data are shown in Table 3.

3.3 Prediction phase

These models were used to determine the concentration of copper, zinc and cadmium metals in the cachaças, using other samples which constitute the prediction phase. At this stage, about 30% of the samples were used. The data used in the prediction underwent the same pretreatment used before in the calibration phase for each model analyzed. The root-mean square error in prediction (RMSEP) and coefficient of determination (R^2) for the several models are also shown in Table 4. The coefficient of determination R^2 for all metals analyzed has value around of 0.9, which shows that the predicted and expected values are consistent. It can be seen that RMSEC (table 3) and RMSEP (table 4) present similar values for the models constructed, which denotes no overfitting of these models.

Table 3. Parameters of the models (calibration) using hanging mercury drop electrode

	PLS			ANN			PLS - Neural		
	Cu	Zn	Cd	Cu	Zn	Cd	Cu	Zn	Cd
R^2	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
RM	77	87	93	90	73	02	69	96	06
SEC	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
SEP	35	68	035	42	76	028	10	67	037

Table 4. Parameters of the models (validation) using hanging mercury drop electrode

	PLS			ANN			PLS - Neural		
	Cu	Zn	Cd	Cu	Zn	Cd	Cu	Z	Cd
R^2	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.9
RM	84	77	12	92	81	95	78	90	07
SEP	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
SEP	40	64	040	43	78	032	27	60	045

Fig. 1-3 shows the comparative graphs of predicted versus expected concentrations for the considered metal for testing subsets using the three calibration models.

It is possible to observe that the association of chemometric methods with voltammetric data produced good results for all the metals analyzed in prediction for cachaças samples.

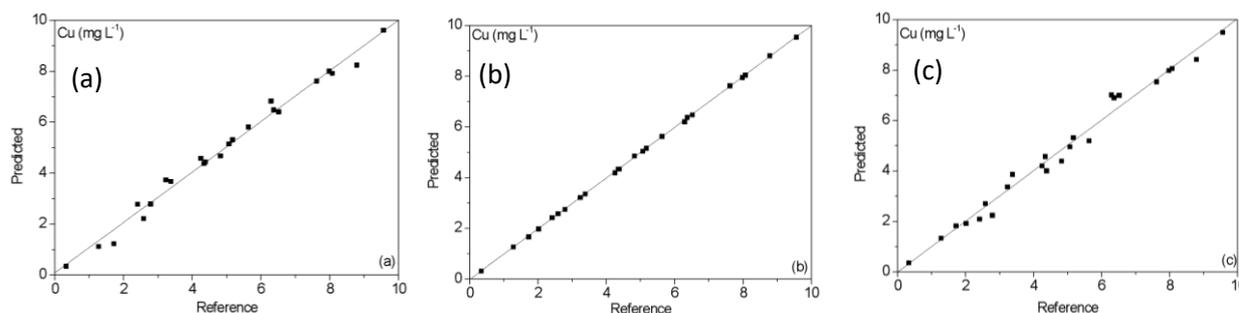


Fig. 1. Predicted versus expected concentrations for copper. (a) PLS model (b) ANN Model and (c) PLS-Neural.

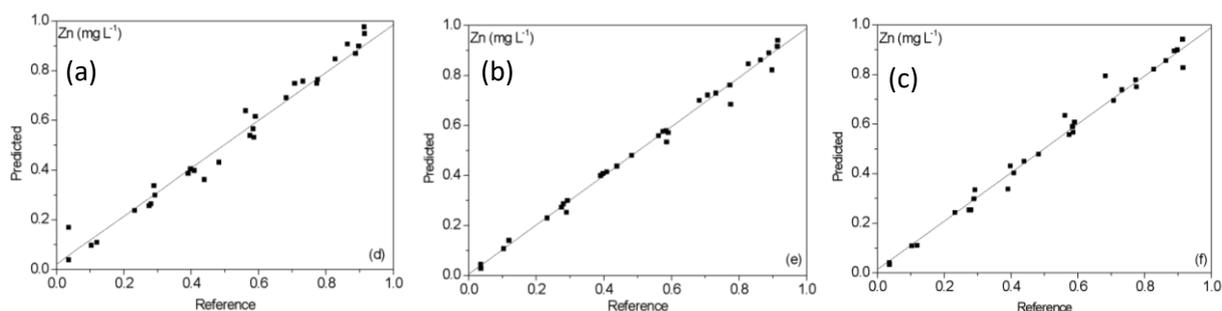


Fig. 2. Predicted versus expected concentrations for zinc (a) PLS model (b) ANN Model and (c) PLS-Neural

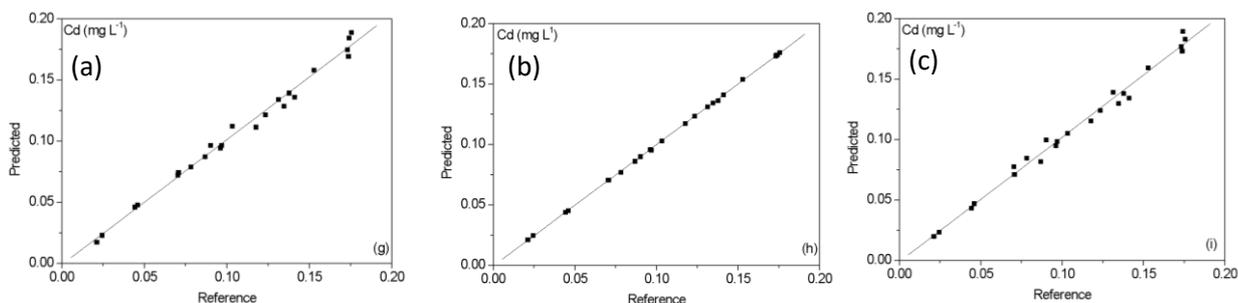


Fig. 3. Predicted versus expected concentrations for cadmium (a) PLS model (b) ANN Model and (c) PLS-Neural.

3. 4 Comparison of methods: PLS, ANN and PLS_Neural

In order for comparing the methods, the F test was carried out based on RMSEP values. This test, at the 95% confidence level presents a value for $F = 1.94$ (24 samples). By applying the test, it was verified that there is no statistical difference among methods PLS and PLS-Neural for zinc and cadmium. After that, PLS was compared with ANN, and no difference was also found for these methods in relation to metal determination. The calculated F values were lower than the theoretical values for these metals when the methods were compared in both situations. In relation to copper, there are no statistical difference for this metal by using PLS and PLS-Neural, based on F test. However, it was observed that the determination of copper using neural networks shows better results for the determination of copper and it differs statistically from other methods, being the former more suitable for this determination. However, all methods have a relative error within what is statistically adequate. Overall, the methods presented good results and any of them can be used for the determinations.

4. Conclusions

The simultaneous quantification of copper, zinc and cadmium in *cachaças* using a hanging mercury drop electrode and considering the combination of the square wave voltammetry and the chemometric methods presented satisfactory results. Therefore, the proposed methodologies can be used for determining metals in *cachaças*. An upside to these methods is that they can replace costly maintenance and performance methodologies such as ICP OES. Moreover, these methodologies are simple and fast and can be an effective tool for routine analysis for the quality control of these products.

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Author Contributions

Rosângela C. Barthus: Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing - review & editing, Supervision, Project administration. Romário Junior Ferreira and Thalles Ramon Rosa: Conceptualization, Methodology, Validation, Formal analysis, Software, Investigation, Data curation, Writing - original draft, Visualization. Alveriana Tagarro Tomaz and Josimar Ribeiro: Conceptualization, Methodology, Validation, Formal analysis, Resources.

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