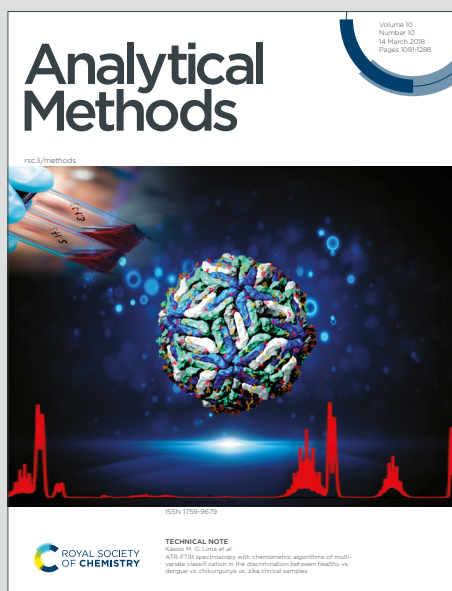


# Analytical Methods

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## A new online pre-concentration system using Hydride generation atomic fluorescence spectrometry (HG AFS) for zinc determination in mineral water and isotonic sports drinks

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Mineral water and isotonic sports drinks (hydroelectrolytic supplement) are sources of electrolytes, among them is zinc, an important microelement for several functions of body. In this study, was developed and validated a novel on-line preconcentration analytical method using atomic fluorescence spectrometry and chemical vapor generation to determine Zn, in mineral waters and isotonic sports drinks employing multivariate optimization. The optimal parameters for system were: pH = 8.00; [Na<sub>2</sub>CO<sub>3</sub>] = 6.5 × 10<sup>-3</sup> mol L<sup>-1</sup>; sample flow = 5.70 mL min<sup>-1</sup>; flow rate = 8.00 mL min<sup>-1</sup> and elution flow rate adjusted to 2.40 mL min<sup>-1</sup> to obtain analytical speed. The method to determine zinc in samples showed adequate linearity, repeatability and accuracy. The limits of detection (LOD) and quantification (LOQ) obtained for zinc were 0.03 and 0.09 µg L<sup>-1</sup>, respectively. Zinc concentration in mineral water and isotonic sports drinks ranged from 29.00 to 78.59 µg L<sup>-1</sup>. Enrichment factor (88.92) and sampling frequency/samples per hour (51) were obtained. Therefore, the results showed that the developed method is simple, fast, reliable, sensitive and selective for the determination of zinc in mineral water and isotonic sports drinks and can be applied in the control of the chemical and toxicological quality of these foods. Furthermore, the multivariate methodology using the complete factorial design and the Box-Behnken design was adequate for the optimization of the preconcentration system variables that influence the generation of the analytical signal.

### 1. Introduction

Zinc plays an important role in the body and, it is predominantly found in the intracellular environment, with catalytic and structural functions. It participates in the synthesis of proteins, carbohydrates, lipids, hormones and nucleic acids, besides being part of the structure of several enzymes. It influences the immune system and the deficiency can cause, among other factors, delay in growth and reduction in spermatogenesis.<sup>1-5</sup> Mineral water and isotonic sports drinks (hydroelectrolytic supplement) are sources of electrolytes, among which zinc stands out.<sup>6,7</sup> Determination of zinc by spectrometric techniques is a constant object of investigation, since products containing this metal require rigid quality control.<sup>8-10</sup>

In literature, there are few studies on the multi-elemental composition of isotonic sports drinks and no data on zinc determination by Hydride generation atomic fluorescence spectrometry (HG AFS). Among the detection techniques used

for determination of zinc in natural waters and beverages, the most used are atomic spectrometry (absorption and emission). HG AFS is a promising technique that has been used for elementary determination and chemical speciation studies, due to its low instrumental cost, when compared with spectrometric techniques.<sup>11,12</sup>

The literature reports several strategies for the preconcentration of trace elements.<sup>13-19</sup> Among them, solid phase extraction (SPE) is very efficient for the determination of one or more chemical species, in the most diverse samples.<sup>20-23</sup> Particular attention should be paid, not only to sorbent and analyte, but also to matrix and eluent, which must be compatible with the detection technique. Among the synthetic sorbents, polyurethane foam (EPU) has been widely used due to its chemical properties.<sup>24-26</sup> Its structure with small voids separates, pre-concentrates and extracts ions and molecules in the internal structure. Due to its porosity, it allows the use in flow systems, with high flow rates, without decreasing the efficiency in the separation of polar and nonpolar species.<sup>27,28</sup>

Flow injection systems are widely used in Analytical Chemistry due to the rapidity with which the analyses are performed, besides their various applications.<sup>29-31</sup> Among these systems, flow injection analysis (FIA) operates with laminar flow, preventing the formation of air bubbles in the system and sample interpenetration (carry-over). The low

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consumption of samples and reagents and the possibility of working with the most diverse detection techniques make FIA an attractive methodology in research laboratories, and a large number of applications is possible, to the detriment of others.<sup>32-34</sup>

In order to obtain the best results, the parameters of a flow system or any other assay can be investigated from a multivariate optimization perspective, determining how the experimental conditions and their interactions influence the process performance.<sup>35</sup> This type of optimization allows the most critical factors to be analyzed simultaneously through an experimental design. Among them, Box-Behnken Design encompasses the experimental domain at minimum, maximum and central levels, thus varying all factors at the same number of levels.<sup>36</sup>

Thus, the objective of this study was to develop and validate an on-line preconcentration analytical method using atomic fluorescence spectrometry and chemical vapor generation to determine Zn in mineral waters and isotonic sports drinks employing multivariate optimization.

## 2. Materials and methods

### 2.1 Material

All solutions were prepared using ultrapure water from the Gehaka® OS 10LX (Gehaka, São Paulo, Brazil) reverse osmosis system and all reagents used were of analytical grade. The glassware was decontaminated by immersion in 10% (v v<sup>-1</sup>) HNO<sub>3</sub> solution (Merck, Darmstadt, Germany), for a minimum of 24 hours. After this period, it was washed with ultrapure water for later use. The stock Zn<sup>2+</sup> solution (1000 µg L<sup>-1</sup>) was prepared from a standard 1000 mg L<sup>-1</sup> Specsol® (Quimlab, Jacareí, Brazil) solution, diluted in ultrapure water. A 3% (v v<sup>-1</sup>) HCl solution was prepared by dilution in ultrapure water of concentrated HCl (Merck, Darmstadt, Germany).

The 6.5x10<sup>-3</sup> mol L<sup>-1</sup> and 6.5x10<sup>-2</sup> mol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub> buffer solutions from Química Moderna® (Barueri, São Paulo, Brazil) were prepared by dilution in ultrapure water of a stock solution of 1.0 mol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub>, and the pH was adjusted with 0.1 mol L<sup>-1</sup> HCl at pH 8.00 ± 0.02. The 0.10% (w v<sup>-1</sup>) PAR solution, 4-(2-pyridylazo) resorcinol, from Sigma (Sigma-Aldrich, Germany) was prepared by dissolving 0.1 g of the reagent in 100.00 mL of absolute ethanol (Merck, Darmstadt, Germany). A 1.4% (w v<sup>-1</sup>) NaBH<sub>4</sub> solution from Vetec® (Vetec Química Fina, Duque de Caxias, Brazil), in 0.5% NaOH (w v<sup>-1</sup>), was prepared by dissolving 2.5 g of NaOH from Química Moderna® (Barueri, São Paulo, Brazil) in 500.00 mL of ultrapure water. After this preparation, about 7.0 g NaBH<sub>4</sub> from were dissolved in the prepared NaOH solution.

Mineral water and isotonic sports drinks were purchased from a local trade and supermarket in Salvador, Bahia, Brazil and were analysed, in triplicate.

### 2.2 Sample preparation

Samples were identified for further dilution. For mineral water samples, a 10-mL aliquot was removed, pH was adjusted to 8.00 with 1.00 mL of 0.1 mol L<sup>-1</sup> NaOH solution and addition of 14.00 mL of 6.5 x 10<sup>-3</sup> mol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> buffer. For isotonic sports drinks samples, a 5.00-mL aliquot was removed, the pH was adjusted to 8.00 with 5.00 mL of 0.1 mol L<sup>-1</sup> NaOH solution and addition of 15.00 mL of 6.5 x 10<sup>-3</sup> mol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> buffer. A pH meter Instrumentation mPA210 (MS Tecnopon®, Piracicaba, Brazil) was used to determine the pH of the solutions. After, the samples were submitted to the online preconcentration system, for the determination of zinc by HG AFS.

### 2.3 Preparation of the mini-column

The mini-column was prepared from a cylindrical BBraun® PVC tube, 6.0 cm in length and 4.0 mm diameter (Hessen, Germany). This tube was filled with polyurethane foam, obtained in Bettanin® (Esteio, Rio Grande do Sul, Brazil) cleaning sponges, after grinding, cleaning with alcohol, ultrapure water and drying in an oven. Subsequently, about 100 mg were measured to fill the tube. After the column was prepared, it was coupled to the flow system for impregnation with 0.10% PAR (w v<sup>-1</sup>) solution at a flow rate of 0.50 mL min<sup>-1</sup> for 6 hours; 10% (w v<sup>-1</sup>) NaOH; 5% (v v<sup>-1</sup>) HCl and ultrapure water were used, with the same flow rate used in the impregnation of the chromogenic reagent until the effluent became clear.

### 2.4 On-line preconcentration system and analytical application

The on-line preconcentration system was used according to the scheme in Fig. 1. It consists of a peristaltic pump (P) that promotes the propulsion of liquids by the polyethylene pipes at a controlled flow rate. There is also a six-way valve (V), where the mini-column is (C), besides sample and effluent inlet and outlet. Through time control, the valve was in sampling or elution position. In the sampling position (a), the sample conditioned at pH 8.00 ± 0.02 was pumped for 1.0 minute at a flow rate of 8.00 mL min<sup>-1</sup> towards the preconcentration column. Zn<sup>2+</sup> is retained by the complexation reaction with PAR, at a stoichiometric ratio of 1:2 Zn<sup>2+</sup>-PAR. The remaining solution was sent for disposal (D). Meanwhile, the 3% HCl (E) eluent passes directly into the HG AFS via another valve path, with no contact with the mini-column. After 1.0 minute, the valve is placed in the elution position (b) where, in the opposite direction to the sample flow, the eluent passes through the preconcentration column for 10 seconds, promoting the elution of the analyte and its forwarding to the HG AFS at a flow rate of 2.40 mL min<sup>-1</sup>. Meanwhile, the sample goes directly for disposal, without any contact with the mini column. In HG AFS, there is the formation of the volatile hydride ZnH<sub>2</sub>, through the reaction with NaBH<sub>4</sub>. The determination of analytical characteristics of the system were determined.<sup>37</sup>

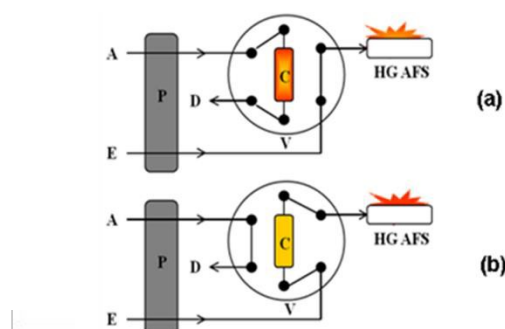


Fig.1 Adaptation of the representation of the on-line preconcentration system.<sup>38</sup> A = sample; P = peristaltic pump; E = eluent; D = disposal; C = column; V = valve; (a) System operating in sampling position; (b) System operating in elution position.

The transient signals were processed as integration of the fluorescence intensity signals emitted for 10 seconds by the equipment program. The experimental procedure was performed three times for each sample, recording the mean signal integration values at an analytical frequency of 51 samples per hour. The optimized values of pH, concentration of the  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  buffer and sample flow were determined, after the complete factorial design experiments.

### 2.5 Atomic fluorescence analysis and analytical validation

An Aurora Lumina 3300 atomic fluorescence spectrometer (Vancouver, Canada) fitted with an antimony hollow cathode lamp (HCL) of high intensity and with a quartz tube atomizer. Argon (99.996% purity degree) from (White Martins, São Paulo, Brazil) was used as the carrier gas of the generated hydride. The operating conditions are described in Table 1.

Table 1 Operational parameters of the atomic fluorescence spectrometer.

Parameter	Optimal condition
Light current / mA	140
Photomultiplier (PMT) Voltage / V	400
Spray height / mm	8.0
Transport gas flow (argon) / mL min <sup>-1</sup>	400
Protective gas flow (argon) / mL min <sup>-1</sup>	1000
Peristaltic pump speed / rpm	50

All samples and standards were analyzed in triplicate and precision was evaluated by the performance of intra-day and inter-day assays by six replicated injections of the standard solutions. The calibration curves were obtained using zinc standard solutions with concentrations ranging from 1 to 100  $\mu\text{g L}^{-1}$ . Limits of detection ( $\text{LOD} = 3 \sigma/S$ ) and quantification ( $\text{LOQ} = 10 \sigma/S$ ) were obtained, where  $\sigma$  is the standard deviation of the analytical blank ( $n = 10$ ) and  $S$  is the slope of the calibration curve.<sup>39</sup> The validation of the procedure was

carried out according to the International Conference on Harmonisation (ICH) guidelines.<sup>40</sup> DOI: 10.1039/D0AY00139B

## 3. Results and discussion

To evaluate the variables, a full two-level factorial design was performed. A standard of 4  $\mu\text{g L}^{-1}$   $\text{Zn}^{2+}$  was selected, and the independent variables to be optimized were: pH,  $\text{Na}_2\text{CO}_3$  buffer concentration and sample flow, all related to the preconcentration system. The elution flow and the preconcentration time were fixed at 5.00 mL min<sup>-1</sup> and 1 minute, respectively. The maximum and minimum levels are represented by the + and - signs, respectively.

The definition of a pH range for study is due to the fact that the complexation reactions between PAR and metal ions depend on the pH of the medium. In Table 2, shows factorial planning matrix 2<sup>3</sup> for the evaluation of the variables, with the responses (analytical signal).

Experiment	Flow	[ $\text{Na}_2\text{CO}_3$ ]	pH	Analytical signal
1	10.0 (+)	$1 \times 10^{-2}$ (+)	9.0 (+)	159.08
2	3.0 (-)	$1 \times 10^{-2}$ (+)	9.0 (+)	146.72
3	10.0 (+)	$1 \times 10^{-3}$ (-)	9.0 (+)	187.63
4	3.0 (-)	$1 \times 10^{-3}$ (-)	9.0 (+)	185.67
5	10.0 (+)	$1 \times 10^{-2}$ (+)	7.0 (-)	99.50
6	3.0 (-)	$1 \times 10^{-2}$ (+)	7.0 (-)	57.70
7	10.0 (+)	$1 \times 10^{-3}$ (-)	7.0 (-)	177.95
8	3.0 (-)	$1 \times 10^{-3}$ (-)	7.0 (-)	168.77
9	10.0 (+)	$1 \times 10^{-2}$ (+)	9.0 (+)	163.95
10	3.0 (-)	$1 \times 10^{-2}$ (+)	9.0 (+)	154.66
11	10.0 (+)	$1 \times 10^{-3}$ (-)	9.0 (+)	188.86
12	3.0 (-)	$1 \times 10^{-3}$ (-)	9.0 (+)	185.16
13	10.0 (+)	$1 \times 10^{-2}$ (+)	7.0 (-)	120.51
14	3.0 (-)	$1 \times 10^{-2}$ (+)	7.0 (-)	72.84
15	10.0 (+)	$1 \times 10^{-3}$ (-)	7.0 (-)	179.79
16	3.0 (-)	$1 \times 10^{-3}$ (-)	7.0 (-)	169.64

Table 2 Full factorial design matrix 2<sup>3</sup> for the evaluation of the variables, with the results obtained for each experiment.

Figure 2 shows the Pareto chart generated with the aid of the Statistica® 7.0 software. It was obtained from the responses of the experiments and, through it, the variables and their interactions will be evaluated. According to the chart, all studied variables have a significant effect on the system, at 95% confidence, and on interactions, only the one that evaluates the effect of the three variables simultaneously on the system is not significant. Positive values indicate that the increase in the level of a particular variable or its interactions within the experimental domain causes a positive effect on the system in order to increase the analytical response. Otherwise, negative values show that increasing levels of a particular variable or its interactions cause a negative effect on the system, reducing the analytical response.

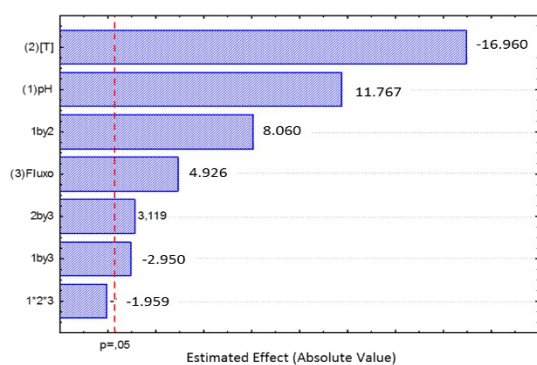


Fig. 2 Pareto chart generated from the full factorial design  $2^3$  of the Zn-EPU-PAR system.

Considering the results of the factorial design, a new domain was established, and the Box-Behnken Design was chosen to optimize the analytical method and determine the critical conditions. The elution flow was set to  $5.00 \text{ mL min}^{-1}$ . A matrix with 15 experiments was generated, since the central point was made in triplicate. The experiments were performed randomly to avoid statistical distortions. The Box-Behnken Design matrix for three variables and the responses are shown in Table 3.

Table 3. Box-Behnken Design experimental matrix for optimization of variables.

Experiment	pH	[Na <sub>2</sub> CO <sub>3</sub> ]	Flow	Analytical signal
1	7.00 (-)	$1 \times 10^{-3}$ (-)	7.00 (C)	162.76
2	9.00 (+)	$1 \times 10^{-3}$ (-)	7.00 (C)	167.47
3	7.00 (-)	$1 \times 10^{-2}$ (+)	7.00 (C)	171.26
4	9.00 (+)	$1 \times 10^{-2}$ (+)	7.00 (C)	172.73
5	7.00 (-)	$5.5 \times 10^{-3}$ (C)	4.00 (-)	180.80
6	9.00 (+)	$5.5 \times 10^{-3}$ (C)	4.00 (-)	187.93
7	7.00 (-)	$5.5 \times 10^{-3}$ (C)	10.00 (+)	199.06
8	9.00 (+)	$5.5 \times 10^{-3}$ (C)	10.00 (+)	204.15
9	8.00 (C)	$1 \times 10^{-3}$ (-)	4.00 (-)	184.76
10	8.00 (C)	$1 \times 10^{-2}$ (+)	4.00 (-)	193.34
11	8.00 (C)	$1 \times 10^{-3}$ (-)	10.00 (+)	207.78
12	8.00 (C)	$1 \times 10^{-2}$ (+)	10.00 (+)	215.57
C	8.00 (C)	$5.5 \times 10^{-3}$ (C)	7.00 (C)	177.76
C	8.00 (C)	$5.5 \times 10^{-3}$ (C)	7.00 (C)	192.04
C	8.00 (C)	$5.5 \times 10^{-3}$ (C)	7.00 (C)	223.81

C: Central point

From the results obtained in the experiments, the critical values were determined, applying the Lagrange criterion, defined by the conditions to obtain a greater analytical signal, and generated the response surfaces and the contours. In this criterion, calculations are used to determine if there is a minimum, maximum or saddle point (when there are

minimum and maximum points on the same surface), and it is based on the calculation of the Hessian determinant of the generated function. When the function has three variables, the calculations are performed according to the mathematical relationships (Equation 1)<sup>41</sup>. According to the results, the following possibilities exist:

- There will be no information of  $\Delta_2 = 0$ ;
- There will be a relative maximum if  $\Delta_1 < 0$ ,  $\Delta_2 > 0$ ,  $\Delta_3 < 0$ ;
- There will be a relative minimum if  $\Delta_1 > 0$ ,  $\Delta_2 > 0$ ,  $\Delta_3 < 0$ ;
- A saddle  $\Delta_1 = \partial^2 R / \partial A^2$  point, when none of the above situations is satisfied.

$$\text{Equation 1: } \Delta_2 = \begin{vmatrix} \partial^2 R / \partial A^2 & \partial^2 R / \partial A \partial B \\ \partial^2 R / \partial B \partial A & \partial^2 R / \partial B^2 \end{vmatrix}$$

$$\Delta_3 = \begin{vmatrix} \partial^2 R / \partial A & \partial^2 R / \partial A \partial B & \partial^2 R / \partial A \partial C \\ \partial^2 R / \partial A \partial B & \partial^2 R / \partial B^2 & \partial^2 R / \partial B \partial C \\ \partial^2 R / \partial C \partial A & \partial^2 R / \partial C \partial B & \partial^2 R / \partial C^2 \end{vmatrix}$$

The coordinates of the critical point are determined by solving the following system of equations:  $\partial R / \partial A = 0$ ;  $\partial R / \partial B = 0$ ;  $\partial R / \partial C = 0$  where, R is the response, and A, B and C are the variables. According to the authors, some information about the geometry of the response surface can be obtained from the signals and quantities of the quadratic coefficients and polynomial functions. When all the coefficients are negative, the surface has a maximum point; when all coefficients are positive, there is a minimum point; and a saddle point when there are negative coefficients (maximum point) and positive coefficients (minimum point). However, for further conclusions, it is necessary to apply the Lagrange criterion.

In view of the complexity of the calculations involved, statistical programs are used to aid in the generation of polynomial equations and response surfaces, as well as the application of the Lagrange criterion. With the aid of the Statistica® 7.0 software, the critical values for this system were: pH = 8.00; [Na<sub>2</sub>CO<sub>3</sub>] =  $6.5 \times 10^{-3} \text{ mol L}^{-1}$ ; sample flow =  $5.70 \text{ mL min}^{-1}$ . By evaluating Figure 3, it is possible to work with flow values greater than the critical point. The option was chosen for a flow rate of  $8.00 \text{ mL min}^{-1}$  to obtain analytical speed. The elution flow rate was also adjusted to  $2.40 \text{ mL min}^{-1}$ . The shape of the surfaces can also be evaluated by the equations of the quadratic model. In Fig. 3, the quadratic terms have opposite signals, which is in agreement with the surface format, of the saddle type.

Analytical signal =  $-943.6434 + 283.0942 \cdot x - 5.2128 \cdot y - 17.5025 \cdot x \cdot y - 0.1312 \cdot x^2 + 0.7706 \cdot y^2$

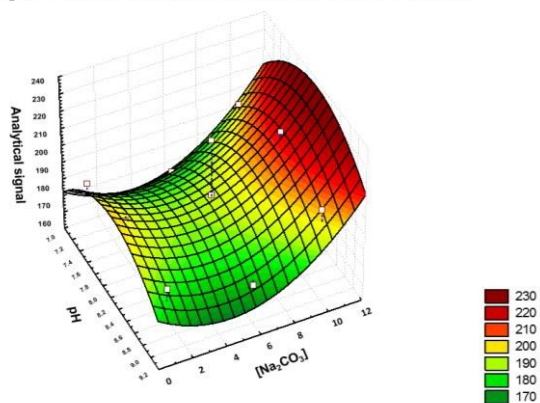


Fig. 3 Response surface flow x pH for the system Zn-EPU-PAR

In order to evaluate the quality of the adjustment, the residue analysis was performed. Figure 4 shows that the residues follow a normal distribution, since the experimental points are close to the highlighted line.

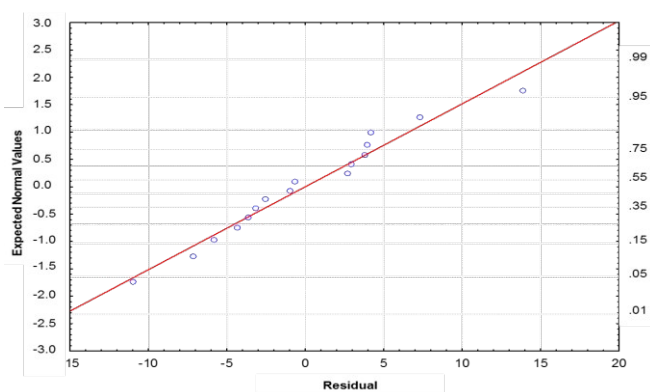


Fig. 4 Curve of residue analysis - Normal probability

The analytical characteristics of the system (Fang 1993) and validations parameters were determined and are listed in Table 4.

Table 4 Analytical characteristics and validations parameters

Analytical characteristics	Values
Enrichment factor	88.92
Consumption index / mL	0.09
Sampling frequency / samples per hour	51
Concentration efficiency / min <sup>-1</sup>	75.58
Phase transfer factor	0.59
Maximum retention capacity	1.9 $\mu\text{g Zn}^{2+}$ per gram of EPU
Figures of merit for the system Zn-EPU-PAR	Values ( $\mu\text{g L}^{-1}$ )
Linear range	0.09 to 25.00
Detection limit	0.03
Quantification limit	0.09
Precision	1 (10.2%) and 10 (4.3%)
Sensitivity	22.219

Precision was assessed and RSD values lower than 10% indicated the good precision of this method. One of the processes used to evaluate the accuracy of a method was the addition and recovery of the analyte in the samples. Zinc was also determined in certified reference material from the National Research Council of Canada (Ottawa, Ontario, Canada): CASS-4 sea water. The results obtained in Tables 5 show that the method can be applied satisfactorily in the samples proposed in this study.

Table 5 Accuracy tests: analysis in certified reference material (CRM): CASS-4 sea water / addition and recovery of zinc, in samples.

Samples and CRM	Amount of $\text{Zn}^{2+}$ found in the proposed method ( $\mu\text{g L}^{-1}$ )	$[\text{Zn}^{2+}]$ added ( $\mu\text{g L}^{-1}$ )	Recovery (%)
1	34.01 $\pm$ 0.02	30.00	113
2	40.25 $\pm$ 0.04	40.00	101
3	64.54 $\pm$ 0.07	60.00	107
CASS-4 sea water	0.319 $\pm$ 0.03	0.381 $\pm$ 0.06 (Certificate value)	84

For robustness evaluation, an experimental domain was established, considering a variation of 10% for flow and concentration of the  $\text{Na}_2\text{CO}_3$ /  $\text{NaHCO}_3$  buffer and 5% for pH around the optimized values. A full factorial design  $2^3$  was used with the variables flow, concentration of  $\text{Na}_2\text{CO}_3$ /  $\text{NaHCO}_3$  buffer and pH, giving rise to 11 experiments. Table 6 shows the factorial design matrix  $2^3$  for the robustness test, with the responses.

Table 6 Full factorial design matrix  $2^3$ , with the results obtained for each experiment.

Experiment	pH	$[\text{Na}_2\text{CO}_3]$	Flow	Analytical signal
1	7.60 (-)	$5.85 \times 10^{-3}$ (-)	7.20 (-)	36.90
2	8.40 (+)	$5.85 \times 10^{-3}$ (-)	7.20 (-)	45.00
3	7.60 (-)	$7.15 \times 10^{-3}$ (+)	7.20 (-)	40.06
4	8.40 (+)	$7.15 \times 10^{-3}$ (+)	7.20 (-)	35.03
5	7.60 (-)	$5.85 \times 10^{-3}$ (-)	8.80 (+)	31.92
6	8.40 (+)	$5.85 \times 10^{-3}$ (-)	8.80 (+)	33.35
7	7.60 (-)	$7.15 \times 10^{-3}$ (+)	8.80 (+)	30.14
8	8.40 (+)	$7.15 \times 10^{-3}$ (+)	8.80 (+)	31.70
9	8.00 (C)	$6.5 \times 10^{-3}$ (C)	8.00 (C)	30.50
10	8.00 (C)	$6.5 \times 10^{-3}$ (C)	8.00 (C)	25.00
11	8.00 (C)	$6.5 \times 10^{-3}$ (C)	8.00 (C)	14.85

Pareto chart (Fig. 5) was generated by Statistica® 7.0 software, showing that all the variations were non-significant, which guarantees that the method is robust, considering variations of 10% for flow and concentration and 5% for pH, with 95% confidence.

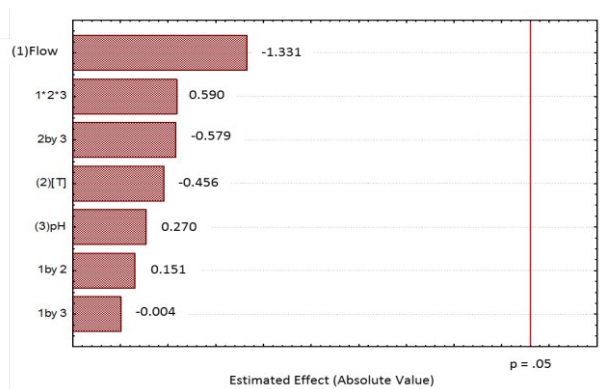


Figure 5 Pareto chart generated from full factorial design  $2^3$  (robustness test).

Two solutions containing zinc and the main ions in mineral water and isotonic sports drinks were prepared for selectivity evaluation (Table 7). As the chemical composition of mineral waters varies according to the collection source, the largest amounts of each ion present in the samples were used and multiplied by two, in order to guarantee that the amount of interferents in the solution would be sufficient for the analysis of waters originating in other sources not included in this study. The same analysis was performed in the preparation of the solution of the main ions in isotonic sports drinks.

Table 7 Concentration of ions in solution, for zinc determination using the proposed preconcentration system

Mineral water		Isotonic	
Ion	Amount (mg L <sup>-1</sup> )	Ion	Amount (mg 200 mL <sup>-1</sup> )
Ca <sup>2+</sup>	53	Cl <sup>-</sup>	168
Mg <sup>2+</sup>	24	K <sup>+</sup>	48
Na <sup>+</sup>	52	Na <sup>+</sup>	180
Zn <sup>2+</sup> (μg L <sup>-1</sup> )	15	Zn <sup>2+</sup> (μg L <sup>-1</sup> )	15
Ba <sup>2+</sup>	0.4		
Sr <sup>2+</sup>	0.6		
Li <sup>+</sup>	0.02		
K <sup>+</sup>	5		
SO <sub>4</sub> <sup>2-</sup>	14		
NO <sub>3</sub> <sup>-</sup>	24		
HCO <sub>3</sub> <sup>-</sup>	198		
F <sup>-</sup>	0.06		
HPO <sub>4</sub> <sup>2-</sup>	0.40		
Cl <sup>-</sup>	84		

The result found were  $15.16 \pm 0.04 \mu\text{g L}^{-1} \text{Zn}^{2+}$  for mineral water (recovery of 101%) and  $14.36 \pm 0.65 \mu\text{g L}^{-1}$  (recovery of

104%) for the isotonic sports drinks, showing that the proposed method is selective for zinc determination in the proposed samples. In Table 8, some parameters are shown in different preconcentration systems for zinc. From them, it is noticed that the method proposed in this study has a lower limit of detection, when compared to the others.

Table 8 Comparison of some parameters in EFS systems for Zn, with literature.

Preconcentration system	Linear range ( $\mu\text{g L}^{-1}$ )	LD ( $\mu\text{g L}^{-1}$ )	EF	Technique	Reference Article Online DOI: 10.1039/D0AY00139B
Ion polymers	5 – 9	1	100	F AAS	42
EVA	-	0.06	223	CVG-ICP OES	43
EVA	-	0.08	44	ICP OES	43
Modified magnetic nanoparticles	10-200	0.8	30	ICP OES	44
Poly (2-thiozyl methacrylamide-co-divinylbenzene-co-2-acrylamido-2-methyl-1-propanesulfonic acid)	5 – 50	2.2	40	F AAS	45
EPU/ PAR	0.09 to 25	0.03	88.92	HG AFS	This study

EVA: Ethyl vinyl acetate; EPU/PAR: polyurethane foam/4-(2-pyridylazo) resorcinol; LD: Detection limit; EF: Enrichment factor

The method developed for zinc determination was used in 10 samples (4 of mineral water and 6 of isotonic sport drinks of the same batch, purchased in local trade and supermarket in Salvador, Bahia, Brazil). The results are described in Table 9, at 95% confidence.

Table 9 Determination of  $\text{Zn}^{2+}$  in mineral water and isotonic sport drinks, at 95% confidence. Experimental conditions: HG AFS (pH = 8.00;  $[\text{Na}_2\text{CO}_3] = 6.5 \times 10^{-3} \text{ mol L}^{-1}$ ; sample flow =  $5.70 \text{ mL min}^{-1}$ )

Isotonic sport drinks		Mineral water	
Samples	$\text{Zn}^{2+}$ found ( $\mu\text{g L}^{-1}$ )	Samples	$\text{Zn}^{2+}$ found ( $\mu\text{g L}^{-1}$ )
I5	$74.50 \pm 0.16$	A1	$34.01 \pm 0.23$
I6	$78.59 \pm 0.04$	A2	$29.00 \pm 1.65$
I7	$48.75 \pm 0.41$	A3	$37.58 \pm 0.73$
I8	$64.54 \pm 2.14$	A4	$36.75 \pm 0.45$
I9	$43.60 \pm 1.16$		
I10	$41.28 \pm 0.99$		

Water samples are clinically important due to presence of macro- and microminerals. In this study, zinc concentration in mineral water ranged from 29.00 to 37.58  $\mu\text{g L}^{-1}$ . The results showed less variation in the zinc concentration, in selected water minerals samples. The investigation of zinc concentration in mineral water is poorly explored in the literature. Azlan et al.<sup>46</sup> evaluated the quality and safety of bottled drinking water and mineral water in Malaysia using flame Atomic absorption spectrophotometer (AAS) method. The contents of zinc in samples ranged from 0.4 to 24.3  $\mu\text{g L}^{-1}$ . The obtained results, in this study, were superior to those found by these authors.

Bolawa and Adelusi<sup>47</sup> determined Zn by AAS, in bottled and sachet water samples obtained from various markets in Lagos, Nigeria. The authors found a Zinc level (0.78 to 8.23  $\text{mg L}^{-1}$ ) above the World Health Organization (> 5  $\text{mg L}^{-1}$ ) permissible limit in only one bottled water sample.<sup>7</sup> Bratty et al.<sup>48</sup> evaluate drinking water quality in Jazan, province located in the South-Western region, of Kingdom of Saudi Arabia using inductively coupled plasma-mass spectrometry (ICP-MS). The concentration range of Zn were 16.16 to 98.47  $\mu\text{g L}^{-1}$ . In Brazil, Santos et al.<sup>49</sup> evaluated Zn levels in bottled mineral waters



from local markets in Curitiba, State of Paraná, Brazil by ICP OES. Zn, in three samples, was quantified at 14 to 37  $\mu\text{g L}^{-1}$ . Similar levels of zinc were found for most of the analyzed mineral water samples, in present study.

Diduch et al.<sup>50</sup> elaborated a vast literature review on inorganic constituents in bottle water. In their study, the authors presented zinc concentration levels (in  $\mu\text{g L}^{-1}$ ) employing various spectrometric techniques, in several countries: USA (0.9 to 27.0), France (8.0), Canada (27.0) and Egypt (4.85 to 64.1). Variations in zinc concentration levels can be attributed to geographical differences as the composition of natural water sources varies and contamination can occur during the bottling process.

From the data obtained in this study, all water samples were Zn concentrations below the maximum specified by Brazilian legislation ( $< 5 \text{ mg L}^{-1}$ ). ANVISA, in Brazil, through Collegiate Board Resolution (RDC) no. 274<sup>51</sup>, approved the Technical Regulation for bottled water and ice. This agency has also set the identity criteria and minimum quality characteristics for natural mineral water, natural water, bottled salt water and ice for human consumption. This Resolution defines "natural mineral water" as that obtained directly from natural sources or by groundwater extraction, being characterized by the defined and constant content of certain mineral salts, trace elements and other constituents considering natural fluctuations. The concentration of zinc in mineral waters is not established in the legislation and, like other chemical species, it may have varying concentrations, depending on the source from which it was extracted.<sup>51</sup>

Food and Drug Administration (FDA) defines the food "bottled water" or "drinking water" as a water that is intended for human consumption and that is sealed in bottles or other containers with no added ingredients except that it may optionally contain safe and suitable antimicrobial agents.<sup>52</sup> Still, "mineral water" is defined as water containing not less than 250 parts per million (ppm) total dissolved solids (TDS), coming from a source tapped at one or more bore holes or springs, originating from a geologically and physically protected underground water source. This health agency states that zinc concentration should not exceed  $5 \text{ mg L}^{-1}$ , but mineral water is exempt from allowable level. In Brazil, the Ministry of Health (MS) indicates that zinc in drinking water can cause turbidity and is also considered a micro-pollutant and its maximum allowable concentration (acceptance standard for human consumption) is  $5 \text{ mg L}^{-1}$ .<sup>53</sup>

Consumption of isotonic sports drinks, classified an ergogenic aid, is increasing worldwide. Athletes preferred sports drink instead of water, to boost their performances and compensate the dehydration (induces weight loss between 1 to 3%) and electrolyte loss.<sup>54,55</sup> Many sports drinks consist of carbohydrates, minerals, electrolytes (for example sodium, potassium, calcium magnesium) and some vitamins.<sup>56</sup> In 2018, through the National Health Surveillance Agency (ANVISA), Brazil established lists of constituents, limits of use, claims and complementary labeling of food supplements. This list describes isotonic, dietary supplements that provide carbohydrates as the main energy source and contain at least

80 and 350 Kcal/1000 mL, respectively. Still, contain at least 75% of the energy derived from metabolizable carbohydrates, contain at least 20 mmol  $\text{L}^{-1}$  ( $460 \text{ mg L}^{-1}$ ) and 50 mmol  $\text{L}^{-1}$  ( $1150 \text{ mg L}^{-1}$ ) sodium (as  $\text{Na}^+$ ) respectively, and have osmolality between 270 and 330 mOsm  $\text{Kg}^{-1}$  of water. This legislation recommends the minimum and maximum limits of nutrients, bioactive substances, enzymes and probiotics that should be provided by food supplements in the daily consumption recommendation and by population group indicated by the manufacturer. For zinc, the recommended minimum and maximum limits, in mg, are 0.3 to 2.0 for the 0 to 6 month age group; 0.45 to 2.0, 7 to 11 months; 0.45 to 4.0, from 1 to 3 years; 0.75 to 7.0, 4 to 8 years; 1.65 to 12.77, 9 to 18 years old; 1.65 to 29.59 for  $\geq 19$  years. For pregnant and lactating women, the values range from 1.8 to 23.5 and 1.95 to 24.45 mg, respectively. This legislation also indicates that zinc is an antioxidant agent that aid protect against free radical damage, vision, vitamin A metabolism, hair, skin, nail and bone maintenance, protein synthesis and cell division. It also acts on the metabolism of proteins, carbohydrates and fats and on the functioning of the immune system.<sup>2</sup>

Isotonic sports drinks represent an important supplemental and clinical alternative of carbohydrates, proteins, fats,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ .<sup>57</sup> During exercise, there is a decrease in total body water and plasma volume, causing an increase in sweating rate and skin blood flow, which can lead to increased temperature.<sup>58</sup> Zinc and other potentials toxic metals (e.g. arsenic, copper, chromium, cadmium, lead, mercury and selenium) and polyaromatic hydrocarbons (PAHs), usually, are not stated on the labels of these products.<sup>59</sup> These microelements can be salt components used for production as well as contaminants. Kregiel<sup>60</sup> informed that bottles seem to be the safest option for food packaging and storage. Different materials (glass, soft and hard PET) and different bottle colors have different effects. Under certain conditions glass bottles can contaminate bottled water during the production process. More elements leach from glass than from PET bottles and Zn was significantly higher compared to water sold in glass bottles than PET bottles.<sup>61,62</sup>

In literature, there is a gap of works that determined Zn in isotonic sport drinks. In this study, zinc concentration (in  $\mu\text{g L}^{-1}$ ) in isotonic sport drinks samples ranged from 41.28 to 78.59. Supplemental zinc improve muscle metabolism, promoting greater strength and enhanced immunity.<sup>1,63,64</sup> Szymczycha-Madeja et al.<sup>65</sup> determined Zn (mean  $0.02 \mu\text{g mL}^{-1}$ ) in energy drinks by ICP OES, in Poland. Abdelazim et al.<sup>66</sup> compared the presence of micronutrients for natural carbonated and noncarbonated soft drink using atomic absorption spectrophotometer. Zn concentrations varied of 1.40 to mg/100 mL. The authors justified the presence of Zn in samples, due to possible activity of caffeine to complex formation with this metal ion.

#### 4. Conclusions

A simple, fast, reliable, sensitive and selective HG AFS method was firstly developed and validated. The multivariate

methodology using the complete factorial design and the Box-Behnken design was adequate for the optimization of the preconcentration system variables that influence the generation of the analytical signal.

The method proved to be selective to Zn<sup>2+</sup>, through the complex isotonic matrix. It had a high enrichment factor (88.92), robust to small variations in the factors studied and had low detection and quantification limits. As disadvantages, we can mention the pre-concentration time (60s), for a higher flow can to promote an increase in the internal pressure of the mini-column, which can to generate its packaging. Considering the analytical characteristics studied, as well as the figures of merit, the developed method was adequate for the determination of Zn<sup>2+</sup> in mineral water and isotonic sport drinks samples, and can be applied in the control of the chemical and toxicological quality of these foods. The amount determined in these beverages is below the recommended by the WHO, FDA and MS (Brazil) for daily intake, and is complementary to the diet for the daily required zinc intake. This article is a pioneer in quantifying zinc in the analysed samples (mineral water and isotonic sport drinks) in Brazil, ranged from 29.00 to 78.59 µg L<sup>-1</sup>.

## Conflict of interest

There are no conflicts to declare.

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