



## Analytical Methods

## Fast and direct analysis of Cr, Cd and Pb in brown sugar by GF AAS

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## ABSTRACT

A simple and fast analytical method for the determination of Cr, Pb and Cd in brown sugar by GF AAS using slurry sampling was developed and *in house* validated for the first time. Analytical curves were prepared by external standardization for Cr, and by matrix simulation for Pb and Cd and they were linear. Low limits of quantification for Cr ( $32.8 \text{ ng g}^{-1}$ ), Pb ( $49.3 \text{ ng g}^{-1}$ ) and Cd ( $4.5 \text{ ng g}^{-1}$ ) were found. Repeatability and intermediate precision estimates ( $< 10\%$  and  $< 15\%$ , respectively) and recovery rates (95–103%) demonstrated a good precision and accuracy. The levels in brown sugar samples ranged from  $< 32.8$  to  $160 \text{ ng g}^{-1}$  for Cr, from  $< 49.3$  to  $211.0 \text{ ng g}^{-1}$  for Pb and from  $< 4.5$  to  $7.0 \text{ ng g}^{-1}$  for Cd and they may be assigned to anthropogenic activities and the adoption of inadequate practices of production and processing.

## 1. Introduction

Brown sugar is a product derived from sugar cane, normally obtained in an artisanal scale, from the evaporation and concentration of the newly stripped juice of sugar cane, up to the crystallization point of the sucrose (Asikin et al., 2016). In literature, there can be found several designations for this kind of sugar such as “jaggery”, “panela”, “non-centrifugal sugar”, and these names vary according to geographical location, and small distinctions in the production of this food.

Recently, the concern of the population about ingesting organic and minimally processed foods with higher nutritional value has led to an increase in the consumption of brown sugar, especially because it contains a higher concentration of minerals and vitamins, if compared to refined sugar (Ducat, Felsner, Da Costa Neto, & Quináia, 2015; Jaffé, 2012). In view of this, several beneficial effects are assigned to the consumption of brown sugar, such as cytoprotector and anticarcinogenic effects, strengthening of the immune system and decrease in the incidence of diabetes and hypertension (Jaffé, 2015). A considerable number of the benefits is related to high content of minerals of the brown sugar. In spite of that, few studies in literature have been devoted to the development of new analytical methodologies applied to determination of elemental and physicochemical composition of this food (Ducat et al., 2015; Jaffé, 2015; Quináia & Nóbrega, 1999).

Brown sugar is basically composed of sucrose, glucose and fructose, and other components such as proteins, insoluble solids and a group of minerals (K, Ca, P, Mg, Na, Fe, Mn, Zn and Cu). Most of these components come from a natural origin, deriving from sugar cane itself. However, the presence of elements such as Cr, Pb and Cd may be

assigned to anthropogenic activities, such as agricultural practices, industrial processes, exploitation of minerals, and dumping of toxic residues or of soil composition (Segura-Muñoz et al., 2006). Besides that, it is reported, in literature, that fertilizers used in plantations like sugar cane may cause plant and soil contamination by toxic metals (Kratz, Schick, & Schnug, 2016). Another factor to be considered is the lack of standardization and quality control in the preparation of an artisan product like brown sugar increasing the risk of contamination and tampering of this food.

Chromium, depending on its oxidation state, can be considered essential or toxic to living organisms. In oxidation state III it is essential, having a function in the metabolism of glucose and cholesterol, and in the action of insulin in processes of degradation of sugar in the blood (Quináia & Nóbrega, 1999). However, in oxidation state VI, this element is toxic, carcinogenic and mutagenic for the living species. The elements Pb and Cd, in turn, do not have a biological function, and, so, are extremely toxic to living organisms. These elements may cause several kinds of damage (renal, neurological, hepatic, cardiovascular, among others) and they may also cause several kinds of cancer, such as of prostate, of lungs and of testicular (Zhang, Ji, Ku, Li, & Sang, 2016). Therefore, knowing the distribution of toxic elements like Cr, Cd and Pb in brown sugar samples is indispensable to ensure safe consumption of this food. Besides this, knowing the elementary profile of brown sugar may contribute to the standardization of the practices of production and processing.

Recently, the development of analytical methods for the determination of the mineral composition of foods has aroused the interest of several sectors of the society (researchers, consumers, producers and

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regulating agencies) that have shown concern with the quality of the foods available for the population. Nevertheless, for the developed analytical methods to be attractive and easily applied, they need to present characteristics such as high sensitivity, selectivity, robustness, speed, low-cost and be environmentally-friendly. The analytical techniques that can be employed for this purpose are the inductively coupled plasma atomic emission spectroscopy (ICP OES and ICP MS) (Ohki, Nakajima, Hirakawa, Hayashi, & Takanashi, 2016; Ozdemir, Kilinc, Celik, Okumus, & Soylak, 2017), flame atomic absorption spectroscopy (F AAS) (Yildiz, Saçmaci, Kartal, & Saçmaci, 2016), anodic stripping voltammetry (ASV) (Costa et al., 2016), the instrumental neutron activation analysis (INAA) (Avigliano, Lozano, Plá, & Volpedo, 2016), capillary electrophoresis (Omar, Elbashir, & Schmitz, 2017), and graphite furnace atomic spectrometry (GF AAS) (Thongsaw, Chaiyasith, Sananmuang, Ross, & Ampiah-Bonney, 2017). However, in the majority of the techniques cited, a pre-treatment of the sample is necessary before the analysis. The procedures of sample treatment that are considered classic and the most employed in foods are the digestion using concentrated acids with heating, the microwave assisted digestion, the alkaline digestion, and the combustion of the sample with subsequent solubilization of the ashes (Mketo, Nomngongo, & Ngila, 2016). These traditional procedures of sample treatment have been recently criticized because of factors such as: lengthy analysis time, high-cost, analyte loss in the methods that involve heating, sample contamination due to lengthy handling, among others (Lejbt, Ospina-Alvarez, Miecznikowski, & Krasnodebska-Ostrega, 2016).

Slurry sampling is a pretreatment method that minimizes the possibility of contamination and analyte losses, and it is also significantly faster than the other methods of sample preparation already cited. However, it is not always an easy task to apply the slurry sampling in matrices like brown sugar. This food contains high levels of organic compounds that may form stable complexes with the investigated analytes and interfere in the quantification. In order to stabilize the sample solution and make it more homogeneous, it is necessary to add surfactants, acid solutions and diluted oxidative solutions to the suspension (Peng, Guo, Zhang, & Jin, 2015). Therefore, it is important to carry out studies of optimization of the preparation of the slurries of brown sugar before its analysis by any spectrometric or electro-analytical technique.

To make it possible the use of slurry sampling for the determination of trace metals, the GF AAS technique is a very interesting option, since it is known for being a digester analyzer, when, before the atomization stage, the sample goes through a pyrolysis stage, where most of the interferents are eliminated. The use of GF AAS requires certain precautions regarding the matrix effect. In order to minimize these effects and increase the time of use of the pyrolytic graphite tube, it is possible to use chemical modifiers and the addition of diluted solutions of acids and oxidants to make pyrolysis and atomization easier. The most common chemical modifiers used in the analysis of Cr, Pb and Cd in foods are Pd, Pd-Mg and  $\text{NH}_4\text{H}_2\text{PO}_4$  (De Andrade, Dos Anjos, Felsner, Torres, & Quinária, 2014; De Andrade et al., 2016). Bearing this in mind, in this study new methodologies were developed for the fast and simple determination of Cr, Pb and Cd in brown sugar samples by GF AAS using slurry sampling. The parameters of the preparation of the slurries of brown sugar, as well as the instrumental parameters, were optimized in a univariate way. The optimized methods were *in house* validated by the analysis of the parameters limits of detection and quantification, accuracy, precision and linearity using sophisticated statistical tools and the optimized and validated methodologies were applied in the determination of trace metals in four Brazilian brown sugar samples.

## 2. Materials and methods

### 2.1. Equipment and reagents

All the solutions were prepared by using deionized water. The

reagents used were of an analytical grade. The standard solutions of Cd, Cr and Pb were prepared from a stock solution of  $1000 \text{ mg L}^{-1}$  (Biotec, São Paulo, Brazil). Other reagents (Nitric acid 65% (w/v) (Aldrich, São Paulo, Brazil), hydrogen peroxide 35% (w/v) (Biotec, São Paulo, Brazil) and sucrose 98% (Vetec, São Paulo, Brazil)) were used in the preparation of the slurries of brown sugar and in the construction of analytical curves, and a standard solution of Palladium  $999 \text{ mg L}^{-1}$  (Aldrich, São Paulo, Brazil) was used as a modifier for Pb and Cd analysis.

The determinations of Cd, Cr, and Pb were carried out in equipment of electro-thermal atomic absorption in a Varian Graphite Furnace model AA 240Z, with automatic sampler model PSD 120, equipped with background correction by Zeeman effect. All the analyses were carried out using two hollow cathode lamps. One Varian multi-element lamp (Cd, Pb, Zn and Ag) operating in 228.8 nm with a current of 4 mA and a slit width of 0.5 nm for Cd, and in 283.3 nm with a current of 10 mA and a slit width of 0.5 nm for Pb, respectively. A Photron single element lamp operating at 357.9 nm with a current of 7 mA and a slit width of 0.2 nm was used for Cr. Except for the atomization stage, in all the stages of the determinations it was used argon gas at  $0.3 \text{ L min}^{-1}$  pressure. Graphite tubes coated with pyrolytic graphite were employed. The background correction by Zeeman effect was used in all the analyses, and absorbance was obtained from the integration of the area of the atomic absorption peak. For the preparation of the slurries of brown sugar it was used an ultrasonic bath (Spencer).

### 2.2. Brown sugar samples

Four brown sugar samples, produced in three Brazilian States (São Paulo (BS1), Rio Grande do Sul (BS3) and Paraná (BS2 and BS4)) and purchased in supermarkets, were used in the studies of optimization, validation and application of the methodologies developed. The BS4 sample was employed in the studies of optimization and validation of the analytical methodologies. After optimization, the validated methods were applied in the three remaining samples. Brown sugar samples were kept in their original packaging, at room temperature, until the analyses were carried out.

### 2.3. Graphite furnace atomic spectrometry

The direct determination of Cr, Pb and Cd was carried out by injection of a slurry of brown sugar ( $20 \text{ g L}^{-1}$ ) in the GF AAS equipment. For determination of Cr and Pb, slurries were prepared with 0.5 g of brown sugar dissolved in 25 mL of a  $\text{HNO}_3$  1% (v/v) solution. In the preparation of the slurries for the analysis of Cd, 0.5 g of brown sugar was dissolved in 25 mL of a solution composed of  $\text{HNO}_3$  1% (v/v) and  $\text{H}_2\text{O}_2$  1% (v/v). Subsequently, the slurry was subjected to sonication in ultrasonic bath for 8 min. In the optimization of the heating program of the graphite furnace, solutions of  $20 \mu\text{g L}^{-1}$  of Cr or Pb and  $2 \mu\text{g L}^{-1}$  of Cd were added to the slurries.  $10 \mu\text{L}$  of the slurries for analysis of each metal were injected into the graphite tube. No chemical modifier was added in the analysis of Cr and for quantification of Pb and Cd,  $5 \mu\text{L}$  of Pd was co-injected to the  $10 \mu\text{L}$  of the slurry of brown sugar inside the graphite tube.

### 2.4. Validation study

The validation of the methods developed was carried out through the analysis of the parameters of linearity, detection limits (LOD), quantification limits (LOQ), accuracy and precision, in accordance with recommendations in literature (AOAC International, 2012; ICH, 1996; NATA, 2013), using sophisticated statistical tests. The statistical analysis of the data was carried out with the assistance of the *Minitab for Windows* software v. 16.2.2 (Líder Software, Belo Horizonte, Brazil).

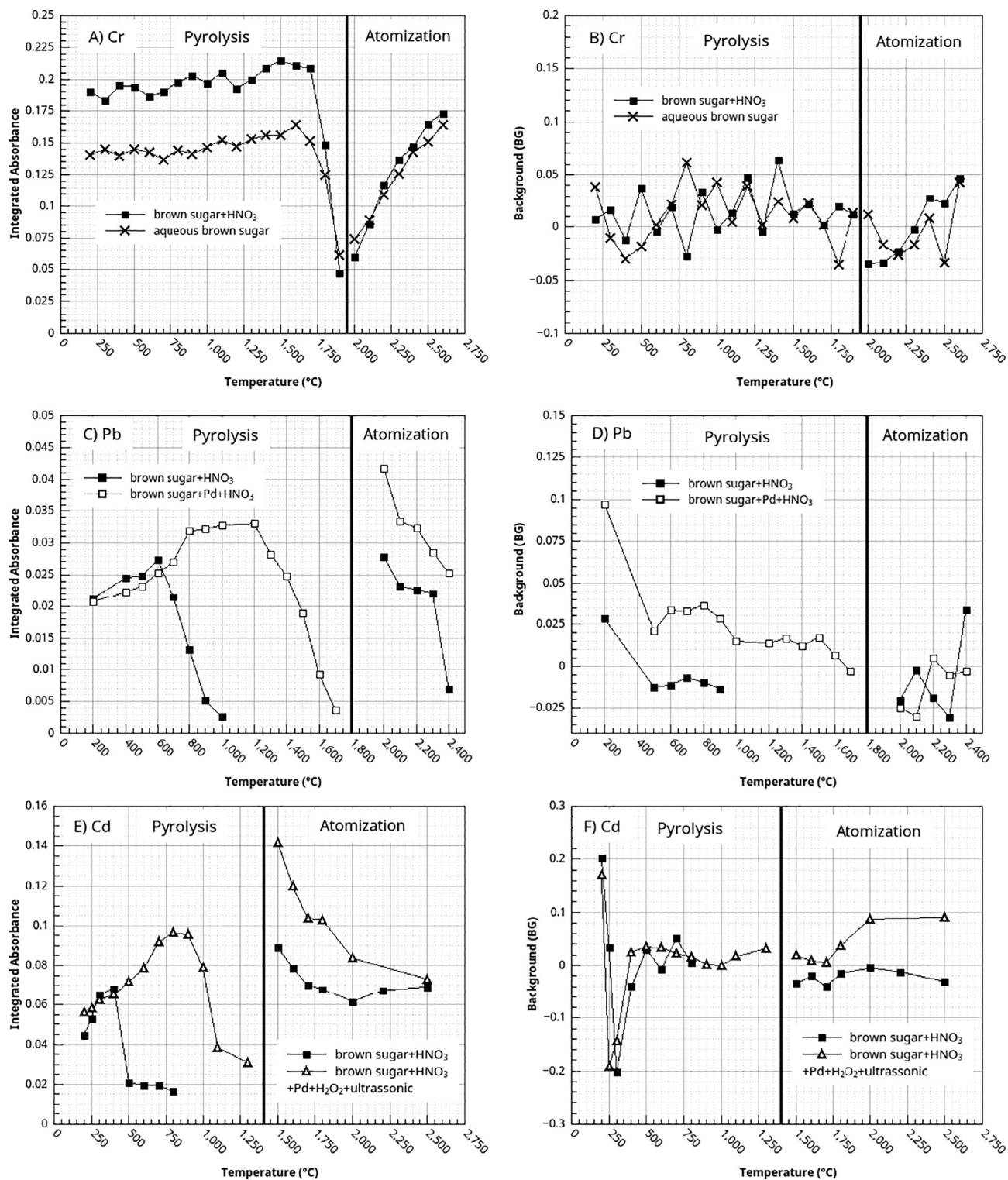


Fig. 1. Results of the optimization of pyrolysis and atomization temperatures. A, C and E represent the absorbance signs, and B, D and F, the background signs for Cr, Pb and Cd, respectively.

#### 2.4.1. Analytical curves and linearity

Analytical curves were obtained by different methods of standardization (external standardization (ES), matrix simulation (MS) and standard addition (SA)) to verify if there was matrix effect in the determinations. For Cr analysis, analytical curves ranging from 1 to  $5 \mu\text{g L}^{-1}$  in aqueous solution (ES) and in brown sugar solution ( $20 \mu\text{g L}^{-1}$ ) (SA) were built. For Pb and Cd analysis, analytical curves ranging from 1.6 to  $8 \mu\text{g L}^{-1}$  and 0.4 to  $2 \mu\text{g L}^{-1}$  in brown sugar

solution ( $20 \mu\text{g L}^{-1}$ ) (SA) and in sucrose solution ( $17 \mu\text{g L}^{-1}$ ) (MS), respectively, were built. For each metal, each point of the analytical curves was analyzed in triplicate. The linearity of the analytical curves was checked through the technique of linear regression and of the test of lack of fit at 95% confidence level.

#### 2.4.2. Detection and quantification limits

The detection limit (LOD) and the quantification limit (LOQ) were

calculated from,  $3 \times SD/b$  and  $10 \times SD/b$ , respectively, where  $b$  is the slope of the analytical curve and  $SD$  is the standard deviation of 10 consecutive measures of the analytical blank (ICH, 1996).

#### 2.4.3. Precision

Precision was assessed through a study of repeatability and intermediate precision and by the Horrat value calculated according to the AOAC International (2012). The estimates of repeatability and intermediate precision were obtained through the analysis in triplicate for the three metals (Cr, Cd and Pb) in brown sugar, in five consecutive days. For the data analysis, an analysis of variance (ANOVA) at 95% confidence level was applied. ANOVA results were used for the calculation of the relative standard deviations (RSD%) of repeatability and intermediate precision and for Horrat value (AOAC International, 2012; González, Herrador, & Asuero, 2010).

#### 2.4.4. Accuracy

The accuracy of the method was assessed through a recovery study by the standard addition in accordance with NATA (2013). The slurries of brown sugar were spiking with 100, 150 and 250  $\text{ng g}^{-1}$  of Cr, 80, 200 and 400  $\text{ng g}^{-1}$  of Pb, and 20, 50 and 100  $\text{ng g}^{-1}$  of Cd. The samples were prepared in triplicate for each metal concentration added. The generated results were also analyzed through a specific  $t$ -test at 95% confidence level, being calculated by the following equation  $t = (\text{recovery} - 100) \times (N - 1)^{1/2} / SD$ , where  $N$  is the number of measures of the recovery test, and  $SD$  is the standard deviation of the recovery test in triplicate (Van Der Voet, Van Rhijn, & Van De Wiel, 1999).

### 3. Results and discussion

For developing a direct and simplified analytical methodology for the determination of Cr, Pb and Cd in brown sugar, avoiding the numerous problems inherent to sample treatment, it was used slurry sampling, an efficient and widely used pretreatment method in studies employing GF AAS technique (Borges et al., 2014). Oxidants, like  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  and the ultrasound waves were employed in the preparation of the slurries to minimize the matrix effect and the formation of carbonaceous residues in the graphite tube (Rodríguez García, Barciela García, & Herrero Latorre, 2005).

#### 3.1. Optimization of the heating program

For the elements Cr and Pb, the addition of  $\text{HNO}_3$  1% (v/v) to the suspension of brown sugar provided and increase in the value of integrated absorbance, since the acid acts in the decomposition of the organic matter, reducing the matrix effect and minimizing the formation of carbonaceous residues in the graphite tube (Rodríguez García et al., 2005). Besides that, it also helps in the metal atomization, and for this reason the optimization study of the heating program for the two metals was carried out adding  $\text{HNO}_3$  1% (v/v) to the slurry. As Cr is a refractory metal, a chemical modifier was not used during the optimization of the methodology for this metal, while for Pb, which is known as a volatile element, the use of the chemical modifier Pd was studied (De Oliveira, Antunes, Vieira, Medina, & Ribeiro, 2016).

In the analysis of Cd, the addition of only  $\text{HNO}_3$  1% (v/v) was not enough to eliminate the matrix effects and release the metal during the pyrolysis stage, and soon it became necessary to use a second oxidant reagent, the  $\text{H}_2\text{O}_2$  combined with ultrasonic waves. It was also studied the use of the chemical modifier Pd due to the volatility of the analyte investigated (Schneider et al., 2017).

Fig. 1A–F represent the optimization of the heating program for Cr, Pb and Cd, respectively.

In Fig. 1A, the maximum absorbance for both media of slurry occurred at a temperature of 1600 °C; however, there was no a great variation of absorbance with changes in the pyrolysis temperatures.

This behavior allows us to state that Cr is not very effectively linked to the sample matrix, as low pyrolysis temperatures like 200 °C were already enough to release a great amount of the metal present in the sample. On the other hand, at higher temperatures (above 1700 °C) there was Cr loss by volatilization. The use of  $\text{HNO}_3$  1% (v/v) in the preparation of the sugar slurry triggered a considerable increase in the signs of absorbance of Cr. This element can form carbides with the material of the surface of the graphite tube during atomization. Such fact may influence in the atomization of complex samples, especially those ones with a high content of sugar (Quinúa & Nóbrega, 1999). Thus, the presence of oxidant agents such as nitric acid may help in the determination of Cr by GF AAS (De Andrade et al., 2014). In Fig. 1A, it is represented the optimization study of the atomization temperatures, where the highest absorbance values were observed at the temperature of 2600 °C. Considering these results, the temperatures of 1500 °C and 2600 °C, respectively, were chosen as optimum pyrolysis and atomization temperatures. However, this optimized condition was obtained in a brown sugar slurry which has a high content of other elements, such as Fe, Mg, Zn, Ca, among others, that can act as natural chemical modifiers. As subsequently the analytical curve will be built only in an aqueous solution (as described in item 3.2.1) without the presence of natural modifiers, the temperature of 200 °C was chosen as an optimum pyrolysis temperature, since it presented a low background sign, and it can provide an increase in the useful life of the graphite tube (Quinúa & Nóbrega 1999).

The background signs for the determination of Cr (Fig. 1B) were low in all the temperature range studied; this demonstrates that, in spite of the direct introduction of a complex sample in the graphite pyrolytic tube, like brown sugar, there was full elimination of the organic matrix and there is absence of carbonaceous residues in the atomizer. Similar behavior was observed by Barrera, Bazanella, Castro, Vale, and Dessuy (2017) in the determination of Cr in drugs, and also by Zmozinski et al. (2016) in the determination of Cr in sunscreen.

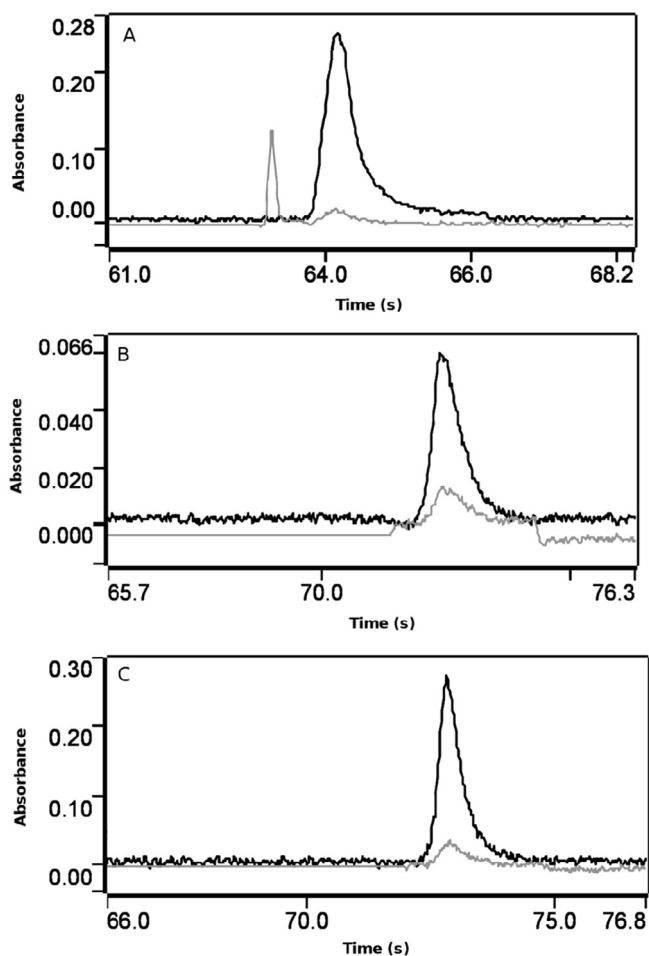
For Pb analysis (Fig. 1C), without the addition of a chemical modifier, the highest pyrolysis temperature, with no loss of the element by volatilization, was 600 °C, while with an addition of modifier, 1200 °C. This result demonstrates that the chemical modifier adopted improved the sensitivity of the measure, since there was an increase in the value of the analytical sign, and also of thermal stability of the Pb in the sample (Santos et al., 2002). In the optimization study of atomization temperature, it was observed that the lowest temperature studied (2000 °C) was the one which presented the highest level of analytical sign. Such result already was expected due to the volatile character of the Pb (Duarte et al., 2016). The background sign obtained for Pb (Fig. 1D) may be considered low, despite the direct introduction of a complex sample in the atomizer. Background absorption may be assigned to the absorption of molecular species or dispersion of incident light due to the presence of some particles (De Andrade et al., 2014). In the pyrolysis study, it can be observed that the values of BG are higher when a chemical modifier is used. However, in the optimized condition of pyrolysis (1200 °C) and atomization (2000 °C), the BG value was lower ( $< 0$ ) using the chemical modifier.

For Cd (Fig. 1E), the sign of integrated absorbance for the condition without a modifier was lower than the one using a chemical modifier. It can be noticed that without a modifier, from 400 °C onwards, there was already loss of Cd by volatilization, while with a modifier this loss started only about 800 °C. This result suggests the increase of thermal stability of the element in the sample when using the chemical modifier (Pd). In the atomization study it is again verified improvement in the chemical sensitivity when using Pd as a chemical modifier. Viitak & Volynsky (2006) also noticed that the injection of Pd as a chemical modifier improved the pyrolysis profile and atomization in the determination of As, Se, Cd and Pb in biological samples. Thus, the optimum condition of pyrolysis and atomization temperatures adopted for Cd was 800 °C and 1500 °C, respectively. The background sign for Cd is shown in Fig. 1F. High values of BG in temperatures lower than 250 °C



**Table 1**  
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Stage	Cr		Pb		Cd		Airflow (L min <sup>-1</sup> )	Sign reading
	T (°C)	Time (s) Ramp	T (°C)	Time (s) Ramp	T (°C)	Time (s) Ramp		
Drying 1	85	5.0	85	5.0	85	5.0	0.3	No
Drying 2	95	40.0	95	40.0	95	40.0	0.3	No
Drying 3	120	10.0	120	10.0	120	10.0	0.3	No
Pyrolysis	200	8.0	1200	16.4	800	16.0	0.3	No
Atomization	2600	3.2	2000	2.9	1500	2.8	0	Yes
Cleaning	2600	2.0	2000	2.0	1500	2.0	0.3	No



**Fig. 2.** Profiles of atomization for Cr (A), Pb (B) and Cd (C) in brown sugar in optimized heating conditions (Table 1). The signs regarding atomic absorption and background are shown in black and gray, respectively.

can be seen, suggesting that the use of temperatures below 250 °C do not allow the elimination of all the interferences present in the matrix of the sample. In the optimized conditions of temperatures of pyrolysis and atomization, in turn, the BG values are low. The heating programs optimized for Cr, Pb and Cd are shown in Table 1.

The profile of atomization for Cr, Pb and Cd in the optimized conditions of pyrolysis and atomization (Table 1) is shown in Fig. 2.

For Cr, it can be noticed the occurrence of a temporal separation between the signs of atomic absorption and background (BG). This result indicates that the background sign does not interfere in the integration of the area of atomic absorption peak, as already reported by Quináia & Nóbrega (1999). For Pb and Cd the BG signs were low; lower BG signs and atomic absorption peaks with better definition were identified in the optimized conditions, which demonstrates that the

optimized heating program in this study was efficient to eliminate the high content of organic matter present in a complex matrix like brown sugar.

### 3.2. Validation study

The validation study was carried out in accordance with recommendations from literature (AOAC International, 2012; NATA, 2013; ICH, 1996) through the calculation of the performance characteristics of linearity, LOD, LOQ, accuracy, precision, and by applying sophisticated statistical techniques.

#### 3.2.1. Analytical curves and linearity

The analytical curves were obtained by external standardization (ES) in an aqueous solution and by standardization by matrix simulation (MS) in a sucrose solution 85% (w/v). These methods were compared to standardization by the standard addition (SA) in a slurry of brown sugar to check the presence or absence of the matrix effects in the measures. To Cr were compared the analytical curves built by ES and SA methods, while to Pb and Cd were compared the MS and SA curves. To the results of each standardization method it was applied the technique of linear regression, a test of lack of fit, and it was calculated the confidence interval of the slope 95% confidence level. The linearity study allows to check if the range of concentration being applied is adequate to the method that is being validated, that is, if the experimental results obtained are proportional to the concentration of the analyte of interest (Ribani et al., 2004). The results are shown in Table 2.

For Cr, the standardization methods by SA and ES were statistically very similar, and the confidence interval for the slopes overlapped, which indicates that the atomization of Cr was similar in an aqueous medium and in a sugar medium, and so, there isn't matrix effect. These results are very interesting considering that the external standardization as an instrumental method of direct calibration with aqueous solution significantly simplifies the quantification of Cr in brown sugar by GF AAS.

On the others hand, for the Pb and Cd elements, it was not possible

**Table 2**  
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Calibration	Parameters of Regression	Regression <sup>*</sup>			Lack of fit <sup>**</sup>		
		Slope Confidence Interval	R <sup>2</sup>	F <sub>regression</sub>	p	F <sub>faj</sub>	p
Cr	Water (PE)	0.00852 ± 0.00092	96.0	385.3	0.000	0.34	0.844
	Sugar (AP)	0.00877 ± 0.00090	96.4	424.6	0.000	2.08	0.147
Pb	Sucrosis (SM)	0.00114 ± 0.00004	95.0	246.7	0.000	0.40	0.753
	Sugar (AP)	0.00113 ± 0.00003	97.4	486.0	0.000	0.34	0.796
Cd	Sucrosis (SM)	0.0652 ± 0.00087	99.3	1,735.3	0.000	1.95	0.185
	Sugar (SM)	0.0660 ± 0.00117	98.7	971.6	0.000	0.11	0.954

<sup>\*</sup> F<sub>critical</sub> (0.05; 1.16) = 5.32; <sup>\*\*</sup> F<sub>critical</sub> (0.05; 4.12) = 3.20.

to apply the method of external standardization, as significant differences were noticed in the slope of the analytical curves in relation to the standardization by standard addition (SA) (results not shown). The SA method demands a great amount of reagents and drastically increases the length of time to carry out the analysis. To avoid the use of standardization by SA, there was an attempt to seek alternatives of standardization for the analysis of Pb and Cd. In these situations it is convenient to use standardization by MS, which is a calibration method where there is an attempt to reproduce the matrix composition (brown sugar). The main advantage of MS over the ES is that it provides a better match with the sample composition. This method is used so that it is not necessary to use the method of standard addition (SA), which demands the construction of an analytical curve for each sample, and by this reason the calibration by standard addition is unfeasible, because of both, the cost and the length of time spent to carry out the analyses (Ribani et al., 2004).

For the elements Pb and Cr, it was employed the standardization by MS, making use of a sucrose solution 85% (w/v). This alternative standardization method was effective for the calibration of Pb and Cd, since the slopes of the analytical curves of the SA and MS methods were statistically similar, according to the confidence intervals at 95% of confidence.

The calibration methods chosen for the determination of trace elements in brown sugar in this study have several advantages, mainly with regard to the experimental work, as they improve analytical frequency, increase the useful life of the graphite tube and decrease the cost of the analyses. In similar way, De Andrade et al., (2014) verified the need of the use of standardization by matrix simulation for the calibration of Pb and Cd in samples of honey.

An analysis of variance characteristic of linear regression at 95% confidence, and a test of lack of fit ( $F_{lof}$ ) were adopted to check if the models generated were appropriate (Table 2). In the analyses of linear regression of Cr, Pb and Cd, the values of  $F_{regression}$  were higher than the value  $F_{critical} (0.05; 1.16) = 5.32$ ; thus, regression is highly significant. For the lack of fit test, the values of  $F_{lof}$  varied between 0.11 and 2.08; these values were lower than the value of  $F_{critical} (0.05; 4.12) = 3.26$  and, the values of  $p$  were higher than 0.050. Hence, there is no lack of fit of the linear models. The results indicate that the linear model is adequate to establish the relationship between the absorbance and the concentration of Cr, Pb and Cd, within the range of concentrations studied. The linear models adjusted to the analytical curves presented coefficients of determination ( $R^2$ ) ranging from 95 to 99%. This way, it was observed linearity in the range of concentrations studied for the three trace metals investigated.

### 3.2.2. Detection and quantification limits and characteristic mass

The sensitivity of the method was assessed through the detection

limits (LOD), quantification limits (LOQ) and characteristic mass ( $m_0$ ) (Table 3).

The characteristic mass ( $m_0$ ) is the analyte mass in pictograms which corresponds to 0.0044 unities of absorbance (Zmozinski et al., 2016). The values of  $m_0$ , LOD and LOQ were relatively low, indicating that the methods developed are sensitive to the direct determination of Cr, Pb and Cd in brown sugar, and are consistent with those reported in literature. Dessuy et al. (2011), Duarte, Dessuy, Vale, Welz, and De Andrade (2013) and Duarte et al. (2016) obtained values of  $m_0$  (pg) ranging from 2.5 to 4.0 for Cr, 9 to 23 for Pb, and 0.4 to 1.0 for Cd. To assess the sensitivity of the methodologies developed, the values of LOD and LOQ determined for Cr ( $9.8 \text{ ng g}^{-1}$ ), Pb ( $14.8 \text{ ng g}^{-1}$ ) e Cd ( $1.34 \text{ ng g}^{-1}$ ) were compared to studies involving other kinds of food, since, in literature, there are no reports of limits for these trace metals in this kind of sugar. Rosa et al. (2015), in the determination of these metals in honey, obtained a LOD of 140.0 and 20.0  $\text{ng g}^{-1}$  of Pb and Cd, respectively. Santos et al. (2002) obtained a value of LOD of  $75.0 \text{ ng g}^{-1}$  in the determination of Pb in foods. Considering that the values of LOD and LOQ obtained in this study were lower than the ones reported by other authors in other food matrices, it can be said that the methods developed are adequate and present good sensitivity for the determination of Cr, Pb and Cd in brown sugar.

### 3.2.3. Accuracy and precision

Precision was expressed in terms of the estimates of intermediate precision and repeatability. Accuracy was assessed by the relative standard deviations (RSD%) of repeatability (Rep) and intermediate precision (IP) (Table 3). The values obtained of Rep and IP for Cr, Pb and Cd are much lower than the maximum limit (RSD (%) < 20%) recommended by literature for the analysis of traces (Stöckl, D'Hondt, & Thienpont, 2009). The Horrat values found for the three metals confirmed the adequacy of intermediate precision, as they were lower than the ones suggested by literature (< 1,3) (González et al., 2010). This way, it can be stated that the analytical methods developed have good precision for the analysis of Cr, Pb and Cd in brown sugar by GF AAS.

Accuracy was assessed by carrying out recovery tests by standard addition at three levels of concentration (Table 3). The adequacy of the recovery rates was assessed through the application of a specific  $t$ -test. The values of the recovery rate ranged between 95 and 103%, and according to literature, the recommended interval of the concentration levels of the metals investigated in brown sugar ( $1 \mu\text{g L}^{-1}$  to  $10 \mu\text{g L}^{-1}$ ) is from 60 to 115% (AOAC International, 2012). From the analysis of the  $t$ -test it was noticed that all the recovery rates determined have  $t$  values lower than the  $t$  critical value ( $t_{critical} = 4.303$ ) at 95% confidence, indicating that there is no significant difference between the value of experimental recovery and the expected recovery value (100%). These results suggest that the proposed methods present

**Table 3**  
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	Accuracy					Precision						
	$m_0$ (pg)	LD	LQ	RSD %		Horrat value	Concentration of brown sugar ( $\text{ng g}^{-1}$ )				Recovery (%)	$t_{calculated}^*$
				Rep	IP		Initial	Added	Total	Determined		
Cr	3.2	9.8	32.8	4.47	7.18	0.34	148	100	248	246 ± 10	98	0.35
								150	298	290 ± 16	95	0.54
								250	398	393 ± 17	98	0.20
Pb	22.0	14.8	49.3	3.95	4.66	0.23	867	80	947	945 ± 30	98	0.11
								320	1.187	1.196 ± 23	103	0.22
								400	1.267	1.264 ± 18	99	0.10
Cd	0.31	1.34	4.47	9.96	15.2	0.44	18.2	20	38.2	38.4 ± 3.3	101	0.52
								50	68.2	68.5 ± 2.6	101	0.67
								100	118	117 ± 11	99	0.16

\*  $t_{critical} (0.025;2) = 4.303$ ; LD, detection limit ( $\text{ng g}^{-1}$ ); LQ, quantification limit ( $\text{ng g}^{-1}$ ); Rep, repeatability; IP, intermediate precision;  $m_0$ , characteristic mass.

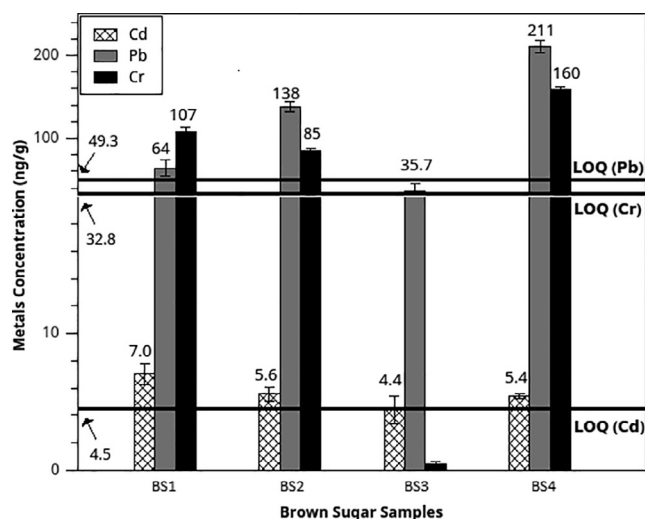


Fig. 3. Concentration of metals (ng g<sup>-1</sup>) Cr, Pb and Cd in four Brazilian samples of brown sugar, obtained in triplicate by the proposed methods, with the corresponding quantification limits (LOQ).

excellent accuracy for the analysis of Cr, Pb and Cd in brown sugar by GF AAS.

### 3.2.4. Application of the validated methods in samples of brown sugar

The optimized and validated methodologies of GF AAS for the determination of Cr, Pb and Cd in brown sugar were applied in four Brazilian samples purchased in the local commerce. The contents of each metal were determined in triplicate, making use of all the optimized conditions described in this work, and the results are shown in Fig. 3.

In general, the contents of trace metals in foods are low; however, the accumulation of toxic trace metals is normally cumulative in the body, and so the consumption of foods containing such substances must be monitored. In Brazil, it was not found a regulation that establishes maximum amounts of Cr, Pb and Cd in brown sugar. According to the African legislation enacted in 2010 (EAS, East African Standard), the maximum contents allowed for Pb and Cd in brown sugar are 500 and 50 ng g<sup>-1</sup>, respectively. The values found for Pb and Cd for the four analyzed Brazilian samples were lower than the limit specified by the African legislation. The BS4 sample presented a higher concentration of Cr and Pb, 160 and 211 ng g<sup>-1</sup> respectively, while the highest content of Cd was observed in the BS1 sample, 7.0 ng g<sup>-1</sup>.

The presence of Cr, Pb and Cd in foods such as brown sugar is derived from the anthropogenic activities, and so some findings regarding the origin of the incidence of these elements can be reported. Firstly, the use of pesticides and fertilizers in the plantations of sugar cane is responsible not only for the soil contamination, but also the plant contamination. Then this contamination may spread to the derivatives of sugar cane, like brown sugar. Some works in literature verified that the use of pesticides and fertilizers are the main sources of sugar cane contamination (Waheed, Rahman, & Gill, 2009). Another possible source of brown sugar contamination by toxic metals is in the product manufacturing. As it is produced in an artisan way, several inadequate utensils for the production of foods are employed, such as improvised wooden, iron and copper utensils, which may contain several kinds of contamination.

In Fig. 3 it is possible to observe that there is a great difference in the contents of Cr, Pb and Cd in the four samples studied. This result was already expected, since the occurrence of such metals is mainly related to anthropogenic pollution, and there is no standardization of the production of sugar cane, or the manufacturing of brown sugar. From these results, it is possible to verify that the analytical methods developed can be applied in the direct determination of Cr, Pb and Cd

in brown sugar using GF AAS.

## 4. Conclusion

Simple, fast and accurate analytical methodologies for the determination of Cr, Pb and Cd in brown sugar by GF AAS were developed and *in house* validated. The optimization study of the heating program of the graphite furnace was of major importance to determine the optimum conditions in the preparation of the slurries of brown sugar, and under which conditions the sample matrix would not cause interference in the analytical sign, making it possible, this way, direct determination of these trace elements in the brown sugar samples. From the *in house* validation study it was observed that the parameters assessed (linearity, detection and quantification limits, accuracy and precision) are within the criteria recommended by literature (NATA, AOAC and ICH), demonstrating that the methodologies developed present analytical reliability. The use of slurry sampling is very advantageous in relation to the traditional methods of sample preparation, due its simplicity, speed, low-cost and reliability, and minimization of the generation of toxic and dangerous residues, lower energy and chemical reagents consumption. Apart from that, new analytical methodologies such as the one developed in this work may contribute to the knowledge of the distribution and concentration of Cr, Pb and Cd in brown sugar, and help in the creation of limits for legislations of quality control of this kind of sugar, and in the standardization of productive and processing processes.

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