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# Optimization and validation of a LIBS method for the determination of macro and micronutrients in sugar cane leaves

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Laser induced breakdown spectrometry (LIBS) was applied for the determination of macro (P, K, Ca, Mg) and micronutrients (B, Cu, Fe, Mn and Zn) in sugar cane leaves, which is one of the most economically important crops in Brazil. Operational conditions were previously optimized by a neuro-genetic approach, by using a laser Nd:YAG at 1064 nm with 110 mJ per pulse focused on a pellet surface prepared with ground plant samples. Emission intensities were measured after 2.0  $\mu$ s delay time, with 4.5  $\mu$ s integration time gate and 25 accumulated laser pulses. Measurements of LIBS spectra were based on triplicate and each replicate consisted of an average of ten spectra collected in different sites (craters) of the pellet. Quantitative determinations were carried out by using univariate calibration and chemometric methods, such as PLSR and iPLS. The calibration models were obtained by using 26 laboratory samples and the validation was carried out by using 15 test samples. For comparative purpose, these samples were also microwave-assisted digested and further analyzed by ICP OES. In general, most results obtained by LIBS did not differ significantly from ICP OES data by applying a *t*-test at 95% confidence level. Both LIBS multivariate and univariate calibration methods produced similar results, except for Fe where better results were achieved by the multivariate approach. Repeatability precision varied from 0.7 to 15% and 1.3 to 20% from measurements obtained by multivariate and univariate calibration, respectively. It is demonstrated that LIBS is a powerful tool for analysis of pellets of plant materials for determination of macro and micronutrients by choosing calibration and validation samples with similar matrix composition.

## 1 Introduction

The high production of sugar cane and its subproducts (*e.g.* sugar and ethanol fuel) make this crop one of the most important for the Brazilian agro-industrial economy. Brazil has the highest sugar cane growing area (*i.e.* 8.1 million hectares) and is the world's highest producer of sugar cane (*i.e.* 563 million tonnes in 2008/2009). The growing production and the improvement in technology make the country the world's largest exporter of sugar and ethanol fuel. The Brazilian production of sugar and ethanol fuel represents 45 and 37% of world consumption, respectively.<sup>1–3</sup> Because of the importance of sugar cane in the Brazilian economy, researchers are looking for ways to improve the sugar cane culture, making it more productive and resistant.<sup>3</sup>

The concentration of nutrients in sugar cane leaves can change according to the plant variety, age and soil type. The determination of macro (P, K, Ca, Mg) and micronutrients (B, Cu, Fe, Mn and Zn) in crops of economical interest is commonly used for the evaluation of nutritional status of plants, correction of nutrients deficiencies, optimization of crop production and evaluation of fertilizer requirements.<sup>4</sup>

A variety of analytical techniques have been applied to determine the elements concentrations in plants samples such as inductively coupled plasma optical emission spectrometry (ICP OES),<sup>5–9</sup> inductively coupled plasma mass spectrometry (ICP-MS),<sup>10</sup> and flame atomic absorption spectrometry (FAAS).<sup>11</sup> In general, these techniques require that samples should be dissolved prior to elements determination. Dry ashing and mainly acid digestion are the most recommended procedures to this task, but they are time and/or reagent consuming.

LIBS analysis of plant materials, aiming the determination of macro and micronutrients, have been recently evaluated in our laboratory by using univariate<sup>12,13</sup> and multivariate<sup>14</sup> calibration with certified reference materials (CRMs). Pellets (test samples) prepared from dried and ground laboratory samples were fixed in an ablation chamber and the plasmas were induced in the pellet surface by ns Nd:YAG laser pulses. Although there was a clear indication that LIBS could be an alternative to the existing recommended methods (*e.g.* ICP OES, FAAS), some discrepant

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results were observed when different plants were analyzed simultaneously. On the other hand, some applications of LIBS for direct analysis of plant materials have been demonstrated.<sup>15–17</sup>

The reliability of LIBS as an analytical technique in various applications involves the development and optimization of robust statistical methods for analyzing complex spectra. Multivariate calibration is a powerful approach for extracting chemical information from analytical data and for performing simultaneous determination of analytes. The most commonly used multivariate methods for chemical analysis are partial least squares (PLS)<sup>18</sup> and principal component regression (PCR),<sup>19</sup> where the factors related to variation in the response measurements are regressed against the properties of interest. Ideally, each factor added to the model would describe variation relevant for predicting property values.<sup>20</sup> An overview of multivariate calibration methodologies is found elsewhere.<sup>21–24</sup>

The theoretical basis of the PLS algorithm has been presented in relevant ref. 18,21,25–28 and some applications in LIBS analysis of plant materials were mentioned in a previous publication.<sup>14</sup> The application of multivariate analysis on LIBS data has proven its usefulness in the discrimination capability of wood furnish and wood polymer lignin,<sup>29</sup> slag samples,<sup>30</sup> qualitative and quantitative analysis of soils,<sup>31</sup> on-line monitoring of steel processes.<sup>32</sup> In this context, LIBS data management with suitable chemometric methods has been a valuable combination for analysis of different types of samples.

In LIBS analysis, the interaction between laser beam and sample depends strongly on laser parameters and on physical and chemical characteristics of the sample. In the case of ground materials, large differences in particle size distribution and particle composition between samples may also produce differences in the laser-sample interaction and can affect the quality of the results. Differences in particle size distribution are generally related to sample composition and it was frequently observed in our laboratory after grinding sugar cane leaves, when compared to other plant species.

In the multivariate approach applied for micronutrients determination,<sup>14</sup> for example, the calibration set was composed by samples with different chemical compositions. Due to the occurrence of inaccurate results, it was concluded that the development of a specific calibration method for plant species with similar matrix composition should be carried out.

In this work, the influence of some parameters for the simultaneous optimization of LIBS operational conditions for the determination of macronutrients (Ca, Mg, K and P) and micronutrients (B, Cu, Fe, Mn and Zn) in pellets of sugar cane leaves was evaluated. Interval-PLS (iPLS) was used in order to find the best spectral ranges for the determination of elements, and partial least square regression as the chemometric strategy to develop both calibration and prediction steps.

## 2 Experimental setup

### 2.1 LIBS instrumentation

Experiments were carried out using a Q-switched Nd:YAG laser (Brilliant, Quantel, France) at 1064 nm, generating 5 ns pulses up to  $(365 \pm 3)$  mJ, in a 6 mm diameter beam with quality factor  $M^2$  smaller than 2, at 10 Hz repetition rate. The pulse energy was

measured with a pyroelectric sensor (Coherent, model Energy Max J-50MB-YAG) coupled to a digital energy meter (Coherent, model Field Max II-P).

Individual samples (*i.e.* 15 mm diameter pellet) were placed in a manually controlled two-axes translation stage that was moved in the plane orthogonal to the laser propagation direction. Argon flowing at  $1.0 \text{ L min}^{-1}$  was continuously fed into the ablation chamber by one entrance inlet positioned in the sample holder. The laser pulse was focused on the sample pellet by a convergent lens with 2.54 cm diameter and 20 cm focal length (Edmund Optics, USA). The plasma emission was collected by a telescope composed of 50 mm and 80 mm focal length fused silica lenses (LLA Instruments GmbH, Germany) and coupled to the spectrometer optical fiber (1.5 m, 600  $\mu\text{m}$  core). The telescope collection angle with respect to the laser optical axis was 25 degrees.

It was used a model ESA3000 spectrometer (LLA Instruments GmbH, Germany) equipped with Echelle optics of focal length of 25 cm with 1 : 10 numerical aperture, and a  $24.5 \times 24.5 \text{ mm}^2$  flat image plane. This system assemblage is a compromise that offers maximum resolution in the wavelength range between 200 and 780 nm with resolving power ranging from 10.000 to 20.000. The linear dispersion per pixel ranges from 5 pm at 200 nm to 19 pm at 780 nm. The detector is an Intensified CCD-Array Kodak KAF 1001 CCD array of  $1024 \times 1024$  pixels full frame ( $24 \times 24 \mu\text{m}^2$ ) and a microchannel plate image intensifier of 25 mm diameter coupled to a UV-enhanced photocathode. The image signals are digitalized in dynamic range of 16 bits and further processed by an industrial computer using resident software. The dark current of the ICCD was automatically subtracted from the measured spectral data.

### 2.2 Samples and certified reference materials

A set of 6 CRMs and 41 samples of sugar cane leaves were used. The experiments were made with sugar cane varieties (*i.e.* RB 855536, RB 855035, RB 855036, RB 855113, RB 845486, SP 813250) collected in São Paulo State.

Dried sugar cane leaves were ground without the central veins as recommended for plant nutrition diagnosis.<sup>33</sup> In this way, the samples were homogenized by using a cryogenic mill (Spex model 6800) with a 5 min pre-cooling step followed by 20 grinding cycles of 2 min each one. Four laboratory samples can be independently ground and homogenized simultaneously. After grinding, pellets were prepared in a Spex model 3624B X-Press by transferring 0.5 g of powdered material to a 15 mm die set and applying  $8.0 \text{ ton cm}^{-2}$  during 5 min. Pellets were approximately 2 mm thick.

**Table 1** Concentration range of analytes covered by the calibration samples

Element	Concentration intervals ( $\text{mg kg}^{-1}$ )
P	50–1740
K	1380–14700
Ca	1580–5220
Mg	390–2040
Mn	20–200
Fe	70–210
Zn	4–19
B	6–38

Samples and CRMs were microwave-assisted acid digested in triplicate. A closed vessel microwave oven (ETHOS 1600, Milestone, Italy) was used according to the following procedure: 250 mg of ground material was accurately weighed in the TFM® vessels and then 6.0 mL of 65% v/v HNO<sub>3</sub> and 1.0 mL of 30% v/v H<sub>2</sub>O<sub>2</sub> were added. Thereafter, the residual solutions were transferred to 25 mL volumetric flasks and the volume made up with high purity de-ionized water (resistivity 18.2 MΩ cm). The final solutions were analyzed by a radially viewed ICP OES (Vista RL, Varian, Australia).

The following CRMs were used for validation of the acid decomposition procedure and ICP OES analysis: bush branches and leaves (GBW 07603), spinach leaves (NIST 1570a), apple leaves (NIST 1515), tomato leaves (NIST 1573), peach leaves (NIST 1547), and pine needles (NIST 1575a).

The development of multivariate calibration models and its validation were carried out by using sets of 26 and 15 samples of sugar cane leaves, respectively. The samples chosen for calibration and validation were the same for univariate models. The concentration range of each analyte covered by the calibration samples is presented in Table 1.

LIBS measurements were performed at 30 different sites on the pellet surface in order to minimize drawbacks related to sample micro-heterogeneity. The number of accumulated laser pulses of each site was optimized.

### 2.3 Simultaneous optimization of the laser parameters

The development of a LIBS method for multi-elemental analysis of plant materials involved the optimization of delay time ( $t_{\text{delay}}$ ), integration time gate ( $t_{\text{int}}$ ), pulse energy ( $e_p$ ), number of accumulated laser pulses ( $n_p$ ), and lens-to-sample distance (LTSD). The LTSD is defined as the distance between the focalization lens and the sample surface. As pointed out by Multari *et al.*,<sup>34</sup> the LTSD parameter affects the laser fluence, the spot size, the plasma volume, mass ablated, line emission intensities, signal to background ratio, signal to noise ratio, and consequently the method sensitivity.

The experimental levels chosen for the four studied factors are presented in Table 2. Spinach leaves (NIST 1570a) were employed as laboratory sample for optimization and Doehlert design was applied for evaluation of the variables to each element.

A Doehlert matrix consisting of a set of 31 experiments was designed. The central point was performed in five replicates. These replicates at the central point permit the model validation by estimating of the experimental error variance.

Average spectra in the VUV region (200 to 780 nm) were recorded for each experimental data point according to the

Doehlert design. The analytical responses of each experimental point of the Doehlert matrix were the emission peak areas of P I 214.914 nm, K I 404.414 nm, Ca I 315.895 nm, Mg I 277.983 nm, Mn II 257.611 nm, Fe II 259.940 nm, Zn I 213.861 nm, B I 249.773 nm, and Cu I 324.755 nm.

Results obtained by Doehlert design were employed for a quadratic polynomial model in the Bayesian Regularized Artificial Neural Network (BRANN) training. The BRANN training was performed with a tangent-sigmoidal transfer function in hidden layer and linear function in the output layer, five neurons in the input layer (corresponding to the five operational variables studied), and eleven neurons in the output layer equivalent to elements evaluated. The BRANN architecture optimization was performed by varying the number of neurons in the intermediate layer from 1 to 20 and proceeding five replicates to each network architecture generated.

The quality of the quadratic polynomial response surfaces was evaluated through ANOVA tables. The BRANN model was evaluated considering the Root Mean Square Error (RMSE) in the net estimation of each element.

The neuro-genetic optimization was built by combining BRANN and genetic algorithm (GA). The weight factors of the loss-minimization function for each element utilized in GA were defined as the ratio between the peak areas and the larger peak in the set of the Doehlert design. More details on the simultaneous optimization strategy based on a neuro-genetic approach can be found in a previous publication.<sup>35</sup>

### 2.4 Univariate and multivariate calibration

Each site (*i.e.* crater) was obtained after 25 consecutive laser pulses on pellet surface (test sample). Each test portion corresponded to an average of 10 sites (*i.e.* 250 pulses). Three test portions were analyzed (*i.e.* total of 750 pulses) and, in most cases, this approach resulted in better precision repeatability.

For background (BG) correction, averaged emission signals of independent spectral regions in the surroundings of the emission line were estimated for each analyte. Corrected emission intensities were obtained after subtraction of the calculated BG from the maximum intensity of each selected emission line. Univariate models were obtained from the regression of the corrected maximum intensities of the analyte emission line against the reference concentrations of the calibration samples by using classical least squares regression model.<sup>36</sup> P I 214.914 nm, K I 404.414 nm, Ca I 422.673 nm, Mg I 277.983 nm, Mn II 257.611 nm, Fe II 259.940 nm, Zn II 206.200 nm and B I 249.773 nm emission lines were chosen for calibration taking into account spectral selectivity and sensitivity.

When multivariate calibration is applied, a higher number of calibration samples are generally required in order to contemplate the differences on matrix samples. The addition of new samples makes the model more robust to new measurement conditions and can often leads to better prediction results. For the development of the calibration models 41 sugar cane leaves samples were separated in two sets containing 26 and 15 samples, which were used for calibration and validation, respectively. These two sets were established based on the correlations obtained by principal components analysis on LIBS spectra. The quantitative determinations were carried out by using

**Table 2** Experimental domain for the determination of B, Ca, Cu, Fe, P, Mn, Mg, and Zn in pellets of plant materials by LIBS

Factor	Unit	Low level	Upper level
lens-to-sample distance	cm	16	18.5
number of laser pulses	—	10	30
pulse energy	mJ	50	130
delay time	μs	1.5	4
integration time gate	μs	3	8

**Table 3** Number of intervals for iPLSR models and latent variables for PLSR models

Element	Intervals	Latent variables
P	10	6
K	10	6
Ca	20	5
Mg	40	6
Mn	30	6
Fe	10	8
Zn	20	4
B	10	9

chemometric tools, such as PLSR and iPLSR.<sup>37</sup> iPLSR was used to determine the important co-varying spectral region and to test the possibility of developing an optimized local PLSR model built on fewer variables for each element. A different number of intervals were selected for each element in order to obtain the best results with iPLSR.

The number of latent variables (Table 3) used in the PLSR models was chosen based on the root mean square error of cross-validation (RMSECV) values obtained for a trial number of latent variables, where the lowest value indicates the correct choice for the number of latent variables.<sup>38</sup> It was performed a RMSECV leave-one-out cross-validation, where the spectrum of one sample of the calibration set was deleted and the PLSR model was built with the remaining samples. The left-out sample was predicted with this model, and the procedure was repeated, leaving out each of the samples of the calibration set in turn. Thereafter, the RMSECV was calculated as:

$$\text{RMSECV} = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}} \quad (1)$$

where  $n$  is the number of calibration samples,  $y_i$  is the reference concentration value of the  $i_{\text{th}}$  sample and  $\hat{y}_i$  the estimative for the  $i_{\text{th}}$  sample given by leave-one-out cross-validation.

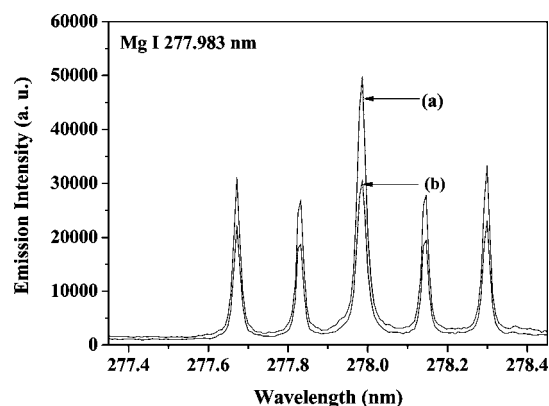
The PLSR models were evaluated by taking into account the agreement between the reference concentrations obtained by ICP OES and the estimated values by PLSR. The root mean square error of prediction (RMSEP), the correlation coefficient ( $R$ ) in the prediction set, the precision, and the limit of detection of the method were determined according to previous work.<sup>14</sup>

PLSR calculations were carried out by using home made routines in Matlab 6.5® environment.<sup>39</sup> The iPLSR calculations were accomplished with a program provided by L. Nørgaard<sup>37</sup> and the limits of detection were estimated by home made routines.

### 3 Results and discussion

#### 3.1 Optimization of the laser parameters

An approach based on a neuro-genetic procedure was used to find the best conditions for simultaneous determination of B, Ca, Cu, Fe, K, Mg, Mn, P, and Zn in plant samples. The best BRANN architecture consisted of 15 neurons at the intermediate layer. The RMSE values and the behavior of residuals were considered in the evaluation of the networks. After BRANN



**Fig. 1** Fragment of spectrum with the selected region used in the optimization for the determination of Mg in plant material: (a) by using the conditions optimized by the neuro-genetic approach (17.5 cm lens-to-sample distance, 25 accumulated laser pulses, 110 mJ pulse energy, 2.0  $\mu$ s delay time, and 4.5  $\mu$ s integration time gate), (b) best experimental point of the Doehlert design (17.3 cm lens-to-sample distance, 20 accumulated laser pulses, 120 mJ pulse energy, 2.0  $\mu$ s delay time and 5.5  $\mu$ s integration time gate).

modeling, a genetic algorithm routine was used to find out the conditions that increase simultaneously the peak areas for all elements. A single LIBS working condition pointed out by GA was obtained with the following optimized parameters: 17.5 cm lens-to-sample distance, 25 accumulated laser pulses, 110 mJ pulse energy, 2.0  $\mu$ s delay time and 4.5  $\mu$ s integration time gate. An experiment was carried out following the neuro-genetic optimized conditions and the results were compared with the best experimental point of the Doehlert design. Fig. 1 shows that better results were obtained for magnesium by using BRANN and GA. Similar results were observed for the remaining above mentioned elements.

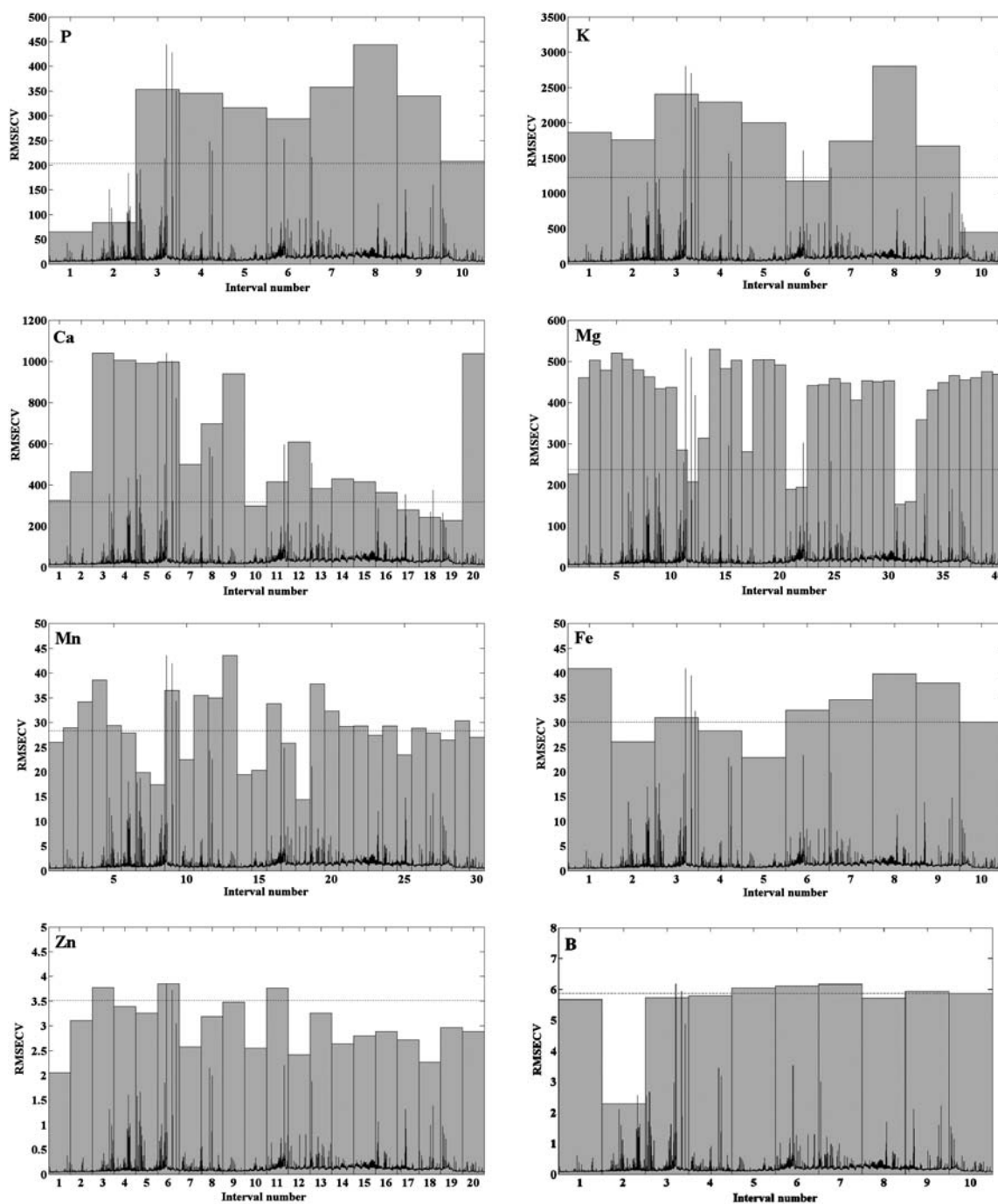
#### 3.2 Univariate and multivariate calibration

In order to obtain the best results for each element, different calibration models were constructed and evaluated for elements determination, by using univariate calibration based on peak areas at different wavelengths and multivariate calibration based on iPLSR with leave-one-out cross validation at different spectral ranges. The quantitative determinations were performed for P, K, Ca, Mg, Mn, Fe, Zn, and B. Copper concentrations were below the limit of detection of ICP OES (2 mg kg<sup>-1</sup>) in the test samples.

Before iPLSR modeling, the emission spectra were previously mean centered. Fig. 2 shows the RMSECV results (bars) estimated for the iPLSR models with equidistant subintervals and the full-spectrum model (dashed line) plotted together with a normalized mean spectrum of the 26 calibration samples. For all elements, an interval with a significant lower prediction error was obtained when compared to the global model.

It can also be observed in Fig. 2 that different numbers of intervals were tested for all elements (Table 3). Best results were obtained by using the spectrum subdivided in 10 intervals for B, Fe, K and P, 20 intervals for Ca and Zn, 30 intervals for Mn, and 40 intervals for Mg (Fig. 2). It is possible to observe that the





**Fig. 2** Observed RMSECV values for iPLSR models for P, K, Ca, Mg, Mn, Fe, Zn and B divided in different subintervals, where the dotted line is the RMSECV of the global model.

iPLSR models with the lowest RMSECV were obtained with the interval numbers 1, 10, 19, 31, 18, 5, 1 and 2 for P, K, Ca, Mg, Mn, Fe, Zn and B, respectively.

The numbers of latent variables selected in each spectral interval for iPLSR modeling were 6, 6, 5, 6, 6, 8, 4 and 9 for P, K, Ca, Mg, Mn, Fe, Zn and B, respectively. For Fe and B a relatively high number of latent variables was necessary, which can be justified by the high number of emission lines and the background variations to be modeled in the selected intervals.

Table 4 presents the average results of some figures of merit for LIBS with PLSR and univariate regression. The standard deviation of blank for each element, required for limits of detection estimation, was assumed to be the standard deviation of a spectral region that did not present emission signals and that was in the selected interval for model development. Taking into account the data presented in Table 1, it can be concluded that the limits of detection obtained by univariate or PLSR model (Table 4) are appropriate for P, K, Ca, Mg, Mn, Fe, Zn, and B in sugar cane

**Table 4** Average results of some figures of merit from univariate and PLSR calibrations

Element	Limit of detection (LOD)		RMSEP <sup>a</sup>		Mean Precision <sup>b</sup>	
	PLSR	Univariate	PLSR	Univariate	PLSR	Univariate
P (g/kg)	0.03	0.02	0.07	0.07	0.05	0.08
K (g/kg)	0.21	0.35	0.98	0.78	0.46	0.55
Ca (g/kg)	0.08	0.08	0.30	0.39	0.16	0.22
Mg (g/kg)	0.12	0.03	0.17	0.25	0.06	0.17
Mn (mg/kg)	6.6	0.5	13	12	6.8	6.5
Fe (mg/kg)	9.5	1.3	24	77	10	28
Zn (mg/kg)	1.2	1.9	1.9	2.3	0.8	1.5
B (mg/kg)	0.8	0.5	1.7	2.0	1.2	1.3

<sup>a</sup> Determined in analogy to the RMSECV (eqn (1)) but with the validation samples. <sup>b</sup> Estimated as the pooled standard deviation of the replicates in the validation samples.

leaves. The RMSEP values showed that PLSR presented better results than univariate regression for LIBS, with exception for K and Mn. The most significant improvement in the results obtained by PLSR was observed for Fe determination, where better correlation between LIBS and ICP OES was obtained by employing multivariate calibration (Fig. 3).

Table 4 shows that the mean precision results obtained by PLSR model for LIBS were generally better than the univariate model. When applying a F-test with 99% of confidence and 30 degrees of freedom, it was observed that the differences were significant just for P, Mg, Fe and Zn, where PLSR was the most precise method. It is important to note that, considering the concentration ranges presented in Table 1 and the PLSR results, the mean precision was lower than 5% for elements, excepting for Fe and Zn.

The comparison of the prediction results of the validation samples with LIBS and the reference values obtained by ICP OES is shown in Fig. 4, where only the PLSR results for the LIBS method are presented. In general, it can be observed that there was a good agreement between PLSR results and ICP OES data. Applying a paired t-test with all samples the calculated t-values were 0.21 (P), 0.13 (K), 0.39 (Ca), 2.53 (Mg), 0.21 (Mn), 0.04 (Fe), 1.05 (Zn), and 0.94 (B). The critical values for the t-Student distribution with 95 and 99% of confidence is 2.13 and 2.94, respectively. Therefore, only for Mg there was a statistical

difference between ICP OES and LIBS results at 95% confidence level, but not at 99%.

It can be concluded that the success of LIBS for plant analysis depends on the strategy chosen for appropriate calibration. It was also evident that these results represent a significant improvement on accuracy of LIBS over our recent contributions.<sup>12–14</sup>

## 4 Conclusions

There was a significant improvement over our previous contributions, including sample preparation, optical setup optimization (LTSD), development of global or matrix dependent calibration models, choice of the appropriate regression model, selection of variables and outliers identification.

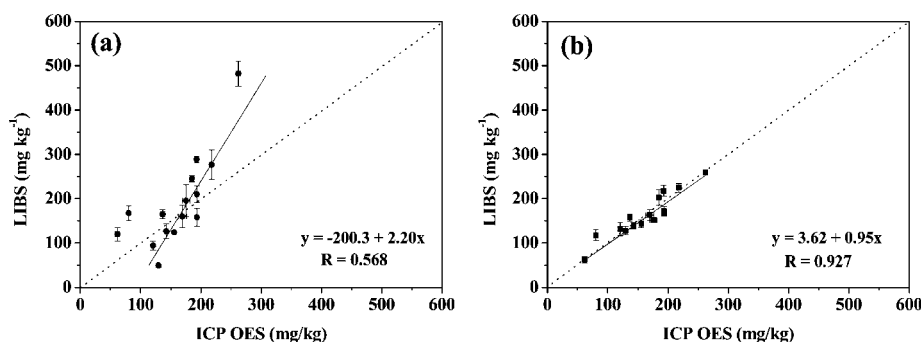
The neuro-genetic optimization was efficient to maximize peak areas of macro and micronutrients for simultaneous determinations with appropriate sensitivities. In this context, integration time gate, delay time, number of accumulated laser pulses, pulse energy and lens-to-sample distance affected significantly the results.

Data obtained confirm the good predictive ability of the PLSR models based on calibration and validation samples composed by the same plant specie, for avoiding or minimizing the occurrence of matrix effects suggested in previous work.<sup>14</sup> The present strategy makes possible the selection of the variables based on the application of iPLSR with the full spectra, instead of the manual selection of specific analyte lines.

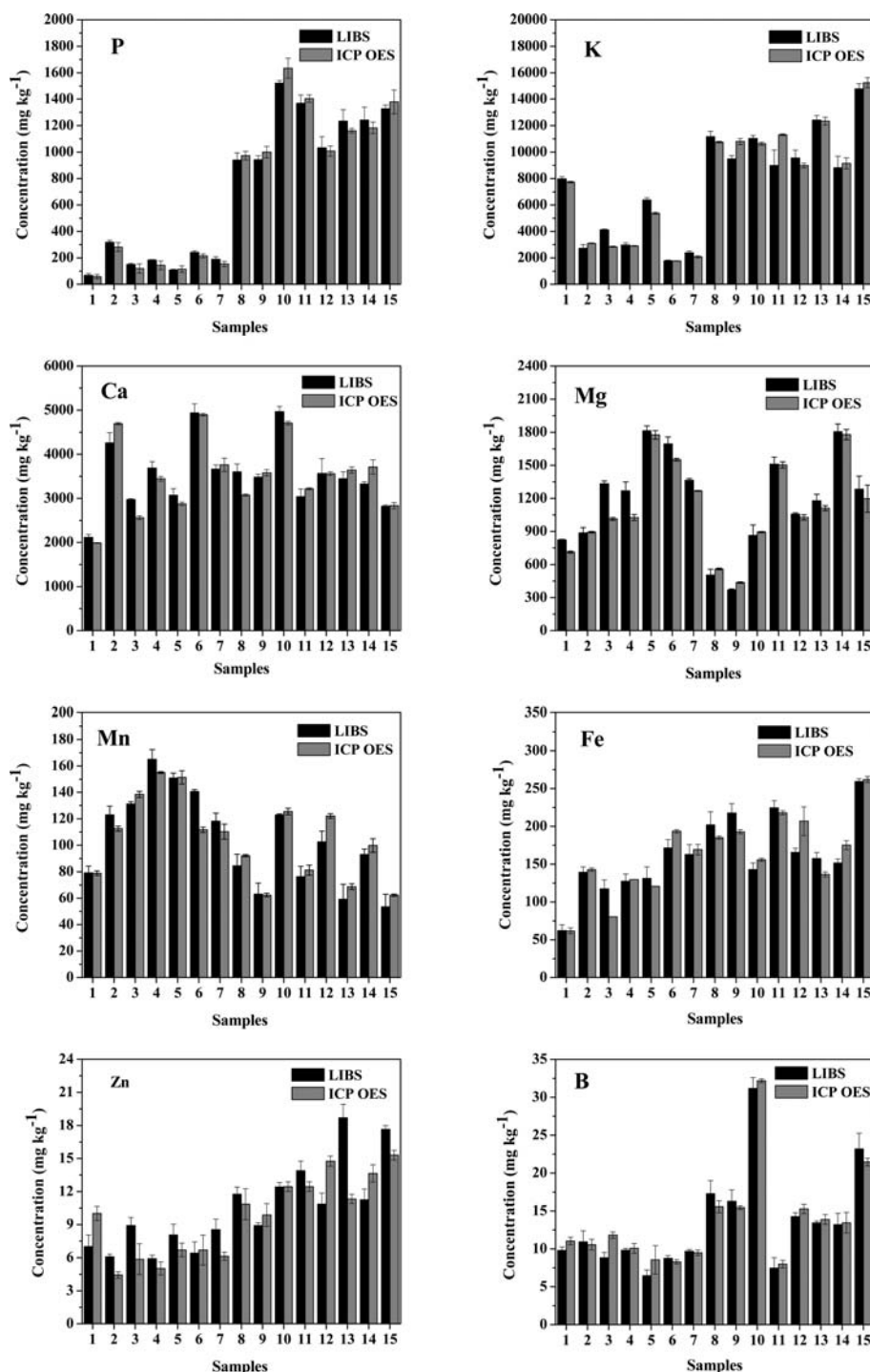
By choosing calibration and validation samples with similar chemical and physical matrix composition, good correlation between LIBS and ICP OES results was obtained, which is a good indication of better fitness for plant analysis and nutrient diagnosis purposes.

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**Fig. 3** Comparison of Fe results in sugar cane leaves obtained by ICP OES and (a) LIBS univariate at Fe II 259.940 nm and (b) LIBS multivariate PLSR calibrations. Dotted lines represent the best theoretical correlations between LIBS and ICP OES.



**Fig. 4** Determination of P, K, Ca, Mg, Mn, Fe, Zn and B in pellets of sugar cane leaves after analysis of acid digests by ICP OES and LIBS multivariate PLSR. Error bars represent  $\pm 1$  estimated standard deviation ( $n = 3$ ).

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